The Potential of Bacterial Cellulose as a Sustainable Material in the Textile Industry

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Abstract

Kombucha is commonly known as a fermented tea drink with purported health-giving properties. It is brewed using a SCOBY (**S**ymbiotic **C**ulture **O**f **B**acteria and **Y**east) in a liquid medium with glucose as a carbon source. A by-product of an extended brewing process is the production of a biofilm (or pellicle) containing bacterial cellulose (BC) nanofibrils. This can be harvested and used as a textile material without the use of synthetic chemicals in its production. The aim of this study is to develop reproducible BC pellicles and investigate potential applications of these as a sustainable alternative material in the textile industry.

Optimal growth conditions were explored in the laboratory and via citizen science projects (> 200 samples). Sterile black tea with sugar inoculated with a Kombucha SCOBY liquid gave the highest yield ($p < 0.05$) albeit the most variable. There was no significant impact ($p > 0.05$) of incubation time (27 – 42 days) or temperature (room temperature vs 30 °C) observed.

Genetic community profiling revealed that the population tended towards stability after three sub-culturing events, with *Komagataeibacter xylinus* noted as the most abundant species.

Once optimal conditions had been identified the properties of the resultant BC pellicles were tested using standard apparel textile testing methods, a unique feature of this study, as this type of testing was not recorded in existing literature. The testing revealed remarkable abrasion resistance alongside very high-water absorption characteristics (in comparison to standard textiles commonly used in apparel).

Since a focus of the study was on sustainable approaches, apparel-based textile applications were not pursued further as these were likely to require synthetic intervention to ensure fitness for purpose in modern apparel products. Instead, the hydrophilic nature of the pellicles was explored as an absorbent to remove residual colour from dyehouse effluent. Pellicles successfully removed a degree of colour from dye solution, thus presenting an innovative application of the sustainably produced pellicles in the textile industry as a potential alternative to traditional coloured effluent treatments.

The methods employed using citizen science as a data collection method in this study demonstrate how the integration of creative approaches with physical science investigation foster deeper levels of enquiry and participant engagement in the research process. This methodological approach highlights the potential of interdisciplinary practice to broaden public involvement in scientific research, enhancing outcomes and diversifying applications.

Publications Arising from this Project.

Peer Reviewed Publications

- Verran, J., **Wood, J**., Redfern, J., Moravej, H., & Radclyffe-Thomas, N. (2023). Hands On Biofilm! A multidisciplinary public engagement event using Kombucha tea pellicle as an accessible example of biofilm. *Biofilm*, Volume 6.
- Verran, J., Redfern, J., Cunliffe, A., Romachney, A., **Wood, J**., (2023) Hands on Biofilm! Utilizing a public audience in a citizen science project to assess yield variability when culturing Kombucha pellicle, *FEMS Microbiology Letters*, Volume 370.
- **Wood, J.,** Redfern, J., Verran, J., (2023) Developing textile sustainability education in the curriculum: pedagogical approaches to material innovation in fashion, *International Journal of Fashion Design, Technology and Education*, Volume 16.
- **Wood, J**., Verran, J., & Redfern, J. (2023). Bacterial cellulose grown from Kombucha: Assessment of textile performance properties using fashion apparel tests. *Textile Research Journal*, Volume 93.
- **Wood, J**., van der Gast, C., Rivett, D., Verran, J. & Redfern, J. (2022) 'Reproducibility of Bacterial Cellulose Nanofibers Over Sub Cultured Generations for the Development of Novel Textiles', *Frontiers in Bioengineering and Biotechnology,* Volume 10.
- **Wood, J.** (2019). Bioinspiration in Fashion-A Review. *Biomimetics,* Volume 4.

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Chapter 1: Introduction

The environmental and social impacts of the textile industry have reached a critical point, with mounting evidence highlighting the unsustainable trajectory of current production and consumption patterns. The Ellen Macarthur Foundation estimates that every second, the equivalent of a truckload of clothes is either burned or buried in landfill (Ellen MacArthur, 2017). Many studies present similarly shocking figures; the European Parliament quotes that the average European citizen consumed 391 kg of raw textile materials in 2020 (European Parliament, 2023), whilst the United Nations environment programme estimates that people are buying 60 % more clothing but wearing these items for half as long (U.N. Environment Programme, 2023). Niinimaki et al. (2020) claim that fashion brands now produce twice the amount of clothing compared with figures calculated before 2001.

Across the whole textile supply chain there are environmentally polluting practices that lead to contaminated wastewater, including toxic dyes and finishing chemicals, and microfibre released into the environment (Singh, 2024). Current estimates suggest that 20 % of global industrial water pollution originates from the textile industry (Drew and Yehounme, 2023). A common sight near textile factories is that of dye pollution in the waterways, with reports of unnaturally brightly coloured rivers in China, India and Bangladesh, all regions of textile manufacturing activity (Regan, 2020; Singh, 2024).

Aside from the pollutants caused by textile products, the drain on natural resources by the manufacture of modern textiles can have devastating impacts on the environment. For example, it is estimated that approximately 7500 gallons of water are required to

produce one pair of jeans (Ellen MacArthur, 2017). Set on a global scale, estimates range between 79 - 93 billion cubic metres of textiles water use per year, potentially leading to water scarcity in some regions of the world (Kocabas et al., 2010; UNCTAD, 2020; Singh, 2024). The impacts can be seen on the natural landscape; the levels of groundwater have dropped dramatically in some parts of Bangladesh where textile production is a prominent industry (Islam, 2021).

Additionally, the global fashion industry produced approximately 2.1 billion tonnes of greenhouse gases in 2018, equating to 4 % of the global total (Zille & Raoux, 2021; McKinsey, 2023). Seventy percent of these emissions were associated with materials manufacture. However, this does not consider the emissions produced by the textiles that are sent to landfill (estimated in excess of 92 million tonnes per year), incinerated or exported to developing countries at the end of their perceived useful life (Niinimäki et al., 2020; Kew, 2024).

These facts are alarming, but the trends of pollution and consumption are estimated to rise; global annual textile consumption has risen from 5.9 kg (1975) to 13 kg (2018) per capita and by 2030 this is estimated to be in the region of 102 million tonnes globally (Global Fashion Agenda, 2023; Kew, 2024). This volume of consumption, waste, environmental impact, and the associated costs are unsustainable; the textile and apparel industries need to change (da Silva et al., 2021; Tessari et al., 2023).

1.1 The Definition, Communication and Education of 'Sustainability'

'Sustainability' is a frequently used word in the modern textile industry, often interchangeably, with terms such as eco-friendly, biodegradable, natural- and renewable-resources; there is no universally accepted definition and much disagreement on its true meaning (Gardetti and Torres, 2013; Henninger et al., 2016). The Brundtland Report – Our Common Future, commissioned in 1987, defined sustainability as meeting 'the needs of the present without compromising the ability of future generations to meet their own needs' (Brundtland, 1987). There is some agreement that 'sustainability' implies environmental impacts; however, others argue that 'sustainability' should be used in a wider context and describes fundamental human behaviour, being viewed as a paradigm shift in societal behaviours (Birkeland et al., 2018; Stephenson, 2023).

Whilst there is no universally agreed meaning to sustainability, the question therefore arises of wider public understanding and engagement with sustainable or environmentally responsible practices, particularly those who are not working directly in the field (Nerlich et al., 2010; De Meyer et al., 2020). This lack of clarity also affects industry action, as businesses face increasing pressure to implement change but are often uncertain about the most effective and appropriate steps to take. Although significant efforts are being made globally to address the environmentally damaging practice of the textile industry, the limited progress in the field highlights the need to improve education and communication around emerging innovations that could drive systemic change. In the fashion industry, the tendency to separate creativity from scientific investigation may represent a missed opportunity for more interdisciplinary

approaches and therefore restrict development in sustainable innovations (Brundtland, 1987; Nisbet, 2009; Fletcher & Grose, 2012; Niinimaki, 2013).

As the textile industry is under such pressure to reduce its harmful effects on the planet, in this thesis the word 'sustainability' is used to imply the use of naturally occurring products which do not cause negative consequences for the natural environment.

1.2 Traditional Textile Sources

In the textile industry, particularly in the field of apparel, there is an increased focus on the sustainability of traditional fibre sources (Fletcher, 2008; Muthu and Gardetti, 2020). Whilst the public have a perception that naturally occurring fibres are more 'environmentally friendly' than their oil-derived counterparts (such as polyesters and polyaramids), this may not be the case (Sigaard and Laitala, 2023).

For cotton, a plant cellulose-based fibre, achieving good quality high yield crops is dependent on the use of pesticides and chemicals that can be extremely harmful to the environment (Gardetti and Muthu, 2019). Organic cotton goes some way to counter this (Gardetti and Muthu, 2019; Delate et al., 2021), but the cotton plant itself still requires significant amounts of water for growth, and in some parts of the world (e.g. the Aral sea in Kazakhstan) this has led to the creation of manmade deserts with the displacement of human populations due to the devastating impact on the natural landscape (Kotlyakov, 1991; Stone, 1999; Waltham and Sholji, 2001).

The cotton fibre itself is of cellulosic base. However, a proportion of the fibre structure is made up of non- cellulosic proteins, amino acids, wax, organic acids, and sugars (Fang,

2018). Many of these non-cellulosic components have a negative impact on the properties of cotton and need to be removed via a series of cleaning and processing techniques before the fibre can be used successfully in a textile form (Sinclair, 2015).

The process of manipulating the cotton fibre into yarns and fabrics is energy-, chemical- and cost- intensive. Once the fibre is cleaned and prepared for yarn spinning, it is subjected to a variety of mechanical processes to sort the fibres into appropriate lengths and align and twist these into yarns, with the application of synthetic lubricants to assist this process and minimise fibre damage (Gordon and Hsieh, 2007). Yarns are then manipulated into either knitted or woven structures, again by being passed through a variety of mechanical processes and synthetic chemicals. These chemicals then need to be removed (with synthetic detergents) before the fabric can be processed for colour application (usually via synthetic dyes and auxiliary chemicals). Finally residual chemicals are removed via a washing process before the fabric is mechanically dried and set. In some cases, further mechanical and chemical processes are applied to impart specialist properties to the fabric, such as water repellency (Elsasser and Sharp, 2022).

In the case of cotton, it is easy to see how the textile industry is so energy - intensive and produces such high levels of pollution, even without considering the diverse range of natural and synthetic fibres and fabrics commonly found in modern textiles and apparel applications.

Whilst it is accepted that synthetic fibres (such as polyester) are unlikely to biodegrade in less than 50 years, a common misconception is that fibres from natural sources (such as wool and cotton) biodegrade quickly after synthetic treatments. The heavy synthetic processing described above severely impacts the biodegradability credentials of natural

fibres. Whilst the natural fibres still break down much more quickly than their synthetic counterparts (Blackburn, 2005), the synthetic chemicals present leach out into the soil with potentially poisonous effects on the environment.

1.3 Next Generation Materials

Many researchers in textiles and apparel are seeking novel approaches to the industry's problems. A popular approach is to look to the natural world to seek alternative fibre and textile sources. The phrase 'next generation' (or next gen) textiles is used to describe these alternative textile sources which are created with the aim of being sustainable and not environmentally harmful. Aside from the potentially positive environmental impact of these textiles, many product developers are investigating these alternatives due to consumer demand and cost. Examples of cheaper alternatives include where the raw materials are based on waste products. However, most of these investigations are early stage and some of the benefits of these products are speculative (Biofabricate, 2021; Muthu, 2024).

One such approach is to use living organisms to 'grow' materials, often referred to a 'biofabrication' (Biofabricate, 2021). In fashion, alternatives for leather are popular because in addition to the chemicals used in leather tanning, there is an increased public concern regarding animal welfare. To this end, mycelium has been explored in depth, with start-up industries producing 'mushroom leather' such as Mylo™ (Bolt Threads, 2023) and Reishi™ (MadewithReishi, 2023). Both products are grown in agricultural waste (Visjager et al., 2019; Williams et al., 2022). Mylo™ has been used by mainstream fashion designers (such as Stella McCartney) to create fashion products. However, none of these

products have been produced in volume due to perceived problems in manufacture, such as scale up from laboratory to bulk production (Kent, 2023). Additionally, these products still have some reliance on environmentally harmful production techniques (such as synthetic coatings) to ensure the product performs to consumer expectations.

Another approach is to use microbial species that produce fibres such as bacterial cellulose. Early studies suggest that this could be a viable next-gen with start-up companies such as Modern Synthesis (2023) and ScobyTec (2022) exploring applications such as shoes and handbags; to date none of these products are commercially available. However, in a similar fashion to mycelium, bacterial cellulose has been suggested as a viable alternative to animal leather and has attracted public interest.

1.4 Bacterial Cellulose – A Potential Solution?

In contrast to cotton plant cellulose, bacterial cellulose production yields cellulose in a highly pure state without contaminants (Khan and Kamal, 2022). It possesses properties such as a highly crystalline structure, high tensile strength, and considerable water retention behaviour (Dufresne, 2012; Khan and Kamal, 2022). Alongside this, the considered potential end uses range from food stuffs (such as thickening agents), paper manufacturing and pharmaceutical applications (Lin et al., 2013a). Researchers (Czaja et al, 2006; Meftahi et al, 2010; Picheth et al., 2017) have explored the application of bacterial cellulose in the medical industry as the porosity of the material lends itself well to the transfer of medicines into the wound site, 'whilst providing a barrier to external infection' (Ashjaran et al., 2012:123). Other studies suggest these properties would enable the bacterial cellulose to be used as an artificial skin (Alvarez et al., 2004).

Outside the medical industry, applications such as acoustic membranes, synthetic fibre coatings and nonwoven cloth have been investigated (Czaja et al., 2004; Shoda & Sugado, 2005; Ashjaran et al., 2012). An overview of these applications is explained later in part two of this thesis.

Bacterial cellulose is a naturally occurring biopolymer, reportedly synthesised by a variety of microorganisms, but studies suggest it is *Gluconacetobacter xylinus* that produces bacterial cellulose in the greatest abundance (Dufresne and Farnworth, 2000; Jarrell et al., 2000; Dufresne, 2012; Ovcharenko, 2013; Reva et al., 2015). There have been many studies that aimed to define the best / optimal production methods of bacterial cellulose, such as static culturing, agitating culture, and airlift reactor (Lin et al., 2013a).

Kombucha, a symbiotic culture of bacteria and yeast (or SCOBY), has been used for thousands of years to brew tea and this tea is reported to have health – giving properties (Jarrell et al.,2000; Dragana et al.,2012; Jayabalan et al.,2014). As a by-product of the extended tea brewing process, a pellicle (also referred to as a biofilm) is formed on the top of the tea broth, which can be removed, rinsed, and dried. Analysis of the Kombucha SCOBY pellicle reveals the presence of many strains of bacteria, including *Gluconacetobacter*, alongside colonies of yeasts which vary according to the geographical location of development of the Kombucha SCOBY pellicle growth (Reva et al., 2015). This pellicle has a bacterial cellulose base and can be used as an inoculum to brew further batches of tea (Teoh et al., 2004). Upon drying, the pellicle is reported to display some properties that can be compared to fine animal hide, such as flexibility, sewability and tensile strength. Additionally, the pellicle displays high water retention

(Dufresne, 2012). However, the physical and aesthetic characteristics of the pellicle substrate can vary from batch to batch.

As bacterial cellulose possesses enhanced properties in comparison to its plant-based counterpart (Khan and Kamal, 2022) and the previous research into potential end uses (Chapter five) it is surprising that there is little detailed research conducted to explore its uses in the textile industry as both a next-gen and sustainable material. Some limited work has explored its production and use in garment applications (Chan et al., 2018; da Silva et al., 2021; Lee, 2023), but none has focussed on its reproducibility or performance limitations in apparel applications. This thesis will explore both topics and investigate ways in which bacterial cellulose could help reduce the polluting nature of textiles. In an industry under pressure to become more sustainable, bacterial cellulose presents a viable option to produce a material without the use of harmful chemicals.

1.5 Project Aims and Objectives

The overall aim of this work is to establish the optimal growth conditions to create a reproducible pellicle from Kombucha or a pure culture starter and explore the versatility and suitability of this as a sustainable textile material. This thesis is divided into two parts to meet the following objectives:

Part one: An exploration of bacterial cellulose solid pellicle production.

- **Objective one:** Critically analyse previous studies to assess the range of growth conditions used to create bacterial cellulose pellicles.

- **Objective two**: Perform laboratory experiments to investigate the optimal growth conditions required for this study.
- **Objective three**: Using a Kombucha SCOBY or pure culture starter, critically evaluate the producibility and development of the bacterial cellulose pellicle.
- **Objective four:** Critically analyse the reproducibility of multiple bacterial cellulose pellicles inoculated in a public space.

Part two: An evaluation of the current uses of bacterial cellulose and its performance properties when considered as a material for use in the textile industry.

- **Objective five:** Investigate and critically review current applications of bacterial cellulose.
- **Objective six:** Evaluate the properties of the pellicle using textile testing methods to enable identification of potential uses of bacterial cellulose as a sustainable textile.
- **Objective seven:** Analyse selected properties of bacterial cellulose in a practical application using lab-based methods.

Chapter 2: Methodological Approaches

2.1 Introduction

This chapter explores the methodological approaches adopted for the studies undertaken in this thesis, followed by a discussion of the specific methods used. Whilst technical detail of methods, where relevant, is explained in each chapter, this part of the thesis aims to map the use of the approaches and highlight the route adopted in the study for the elucidation of original insights and contributions to knowledge.

2.2 What is research?

Research can be defined as a process or practice by which knowledge is extended and answers to questions are sought (Matthews and Ross, 2010; Pole and Lampard 2013), and can be considered as follows:

- Structured and purposeful: Research should always have a purpose, but the results do not always have to have a practical application. Research can serve the purpose of abstract knowledge development.
- Rigorous: Research should always be carefully planned with considerations such as ethics and experimental design considered equally to data collection and analysis
- Robust and defensible: Research needs to be strong in all its elements so our peers and critics can understand how conclusions are derived

- Systematic: Everything that is examined in the research is treated in the same way, i.e. is subjected to the same level of scrutiny

Research extends beyond the mere collection of facts; it requires a rigorous and systematic consideration of all relevant elements to ensure a comprehensive and well-founded investigation. Additionally, Bryman et al (2008) suggest that quality research should include the following features:

- Accessibility to appropriate audiences
- Design that addresses the research question
- Transparency in data collection and analysis
- Clarity of the research process
- The contribution to knowledge

The research in this thesis addresses the above using methods that involve both materials investigation and knowledge generation across different contexts including a literature review, controlled laboratory experiments, citizen science data collection and an exploration of BC applications. It is therefore essential to consider the underlying research philosophy that underpins this work.

2.3 Research philosophy

Research philosophy is the set of beliefs and assumptions that guide how research is conducted, what is considered valid evidence and how knowledge is created. It underpins the methodological choices made in a study and influences how data is collected, analysed and interpreted. It ensures there is alignment between research questions and data analysis, whilst assisting researchers in the reflections of bias and study limitations.

It can be broken down into key components:

Ontology is concerned with the nature of reality and how it is perceived. Researchers may view this as objective and independent (positivism), or socially influenced and subjective (constructivism).

Epistemology is a stance which views the perception of valid knowledge and how it is acquired. This may be viewed as either empirical observation and measurement, or construction through interpretation and experience.

Different research philosophies align with specific paradigms that further shape methodological choices, as illustrated in (Table 1).

Table 1. Research Philosophies, Paradigms and Methodological Choices

Paradigm	Ontology	Epistemology	Associated Methods
Positivism	Objective reality exists independently of perception	Knowledge is gained through empirical observation and measurement.	Experiments, surveys, quantitative analysis.
Interpretivism	Reality is socially constructed and context dependent.	Knowledge is subjective and shaped by experience.	Interviews, case studies, qualitative analysis.
Pragmatism	Reality is both objective and subjective, depending on the research context.	Knowledge is acquired through multiple methods that address real-world problems.	Mixed methods, experimental and contextual research.

2.4 Research philosophy in this study

This study involves both materials investigation and knowledge generation across different contexts, including a literature review, controlled laboratory experiments, citizen science data collection, and an exploration of BC applications.

The ontological stance in this study can be considered realist**,** as it assumes that bacterial cellulose (BC) exists as a material entity with definable properties, regardless of the context in which it is studied. However, there is also a recognition that the conditions of production (e.g., controlled lab vs. citizen science) may influence its characteristics, leading to context-dependent variations**.**

The epistemological approach is pragmatist which acknowledges that different methods provide complementary insights into BC production and applications. By adopting a pragmatic approach, an allowance is made for methodological flexibility by integrating multiple methods to address complex research questions. Pragmatism focusses on what works in practice rather than a strict adherence to a philosophical stance, suiting a mixed methods approach that includes fundamental research and real-world applications.

In this study, the three complementary approaches that are used to investigate BC production and its potential applications can be considered as follows:

1. **Laboratory – based research (Positivist stance)**

   Positivism assumes objective measurement and quantification, aligning with the lab-controlled study of BC reproducible generation.

2. **Citizen science as an empirical data collection method**

   Citizen science often aligns with constructivism, providing insights into the social and creative dimensions of scientific research. In this thesis, citizen science was used primarily as an alternative method of data collection, rather than a participatory knowledge production process. This enables comparisons of BC production in different settings, expanding the scope of the investigation.

3. **Investigation into BC Applications (Applied pragmatism)**

Pragmatism is also relevant to the exploration of BC applications as it highlights practical outcomes of the relevance of these in a real-world setting. The understanding of BC in different contexts requires an understanding of its material properties (by scientific testing) to determine its functionality in various applications (by practical evaluation).

Pragmatism values methodological flexibility and practical application of knowledge across different research settings. While lab-controlled experiments ensure scientific rigour, the citizen science method of data collection provides insights into variations in BC production and the investigation into applications ensures relevance outside a lab environment. Throughout this study, these methods are used in a complementary manner (rather than opposing) to develop a contribution to knowledge in the understanding of BC production and its potential uses. Thus, whilst pragmatic in methodological choices, this research is empirically grounded and ensures findings are scientifically rigorous with practical relevance.

Table 2. Data Collection Methods Mapped Against the Aims of the Research

Aim	Objectives	Methods
A critical evaluation of bacterial cellulose solid pellicle production	Critically analyse previous studies to assess the range of growth conditions used to create bacterial cellulose pellicles	Literature review
	Perform laboratory experiments to investigate the optimal growth conditions required for this study	Laboratory based experimentation
	Using a Kombucha SCOBY or pure starter culture, evaluate the producibility and development of the bacterial cellulose pellicle	Laboratory based experimentation
	Analyse the reproducibility of multiple bacterial cellulose pellicles inoculated in a public space	Laboratory based experimentation 'Citizen science' – mass experimentation in a public space
An evaluation of the current uses of bacterial cellulose and its performance properties when considered as a material for use in the textile industry	Investigate and critically review current applications of bacterial cellulose	Literature review
	Evaluate the properties of the pellicle using textile testing methods to synthesise potential uses of bacterial cellulose as a sustainable textile	Laboratory based experimentation
	Critically analyse selected properties of bacterial cellulose in a practical application using lab-based methods	Literature review Laboratory based experimentation

However, whilst a pragmatic approach offers methodological flexibility and an ability to integrate a variety of perspectives, there are drawbacks to consider by taking this stance. The main drawback is that of data reliability, consistency and comparability between

modes of measurement (lab-based vs citizen science). The influence of environmental and procedural variability cannot be ignored, and this is considered throughout the thesis when evaluating data collected via the various methods presented.

Part One

Chapter 3: Bacterial Cellulose and its Production

3.1 Introduction

This chapter provides an overview of the molecular structure of bacterial cellulose and relevant techniques currently used in its production. Two types of starter culture are considered: a pure bacterial culture and a Kombucha SCOBY. The conditions to support these two inocula in the production of bacterial cellulose pellicles are evaluated via a survey of current literature and laboratory-based experiments. 'Optimal' growth conditions of BC pellicles will be defined to produce bacterial cellulose pellicles for further exploration.

3.1.1 The Cellulose Polymer

In 1838 Anselme Payan discovered a fibrous component in plant material, naming it cellulose (Dima et al., 2017). However, the basic formula of the cellulose polymer, $(C_6H_{10}O_5)_n$, (Figure 1), was not established until 1913 by Willstatter and Zechmeister (Kalia et al., 2011). It was later discovered that cellulose is also present in the cell walls of algae and some fungi, whilst some bacteria can secrete cellulose as a by-product of their own metabolic processes (Dufresne, 2012).

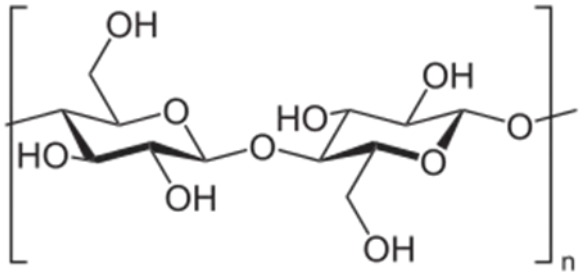


Figure 1 Basic Molecular Structural Unit of Cellulose.

The hydroxyl groups in the structure are a key feature of cellulose. The ability of these groups to form both inter- and intra- chain hydrogen bonds enable highly crystalline structures to be created (Dufresne, 2012). However, the levels of crystallinity can vary depending on the packing of the polymeric chains and therefore the numbers of hydrogen bonds, resulting in the existence of cellulose in different polymorphs, known as cellulose I, II, III and IV. Table 1 illustrates how these polymorphs are related and converted. Cellulose II is the focus of this study.

3.1.2 Microfibrillar Structure

In addition to the occurrence of the inter- and intra- hydrogen bonds, van der Waals bonds have also been observed between the layers of cellulosic chains (Schenzel and Fischer, 2001). This leads to highly ordered, crystalline regions and the arrangement of chains parallel to each other, forming structures referred to as ‘microfibrils’ (Dufresne, 2012). Yamanaka et al. (2000) state that these microfibrils vary in dimension and crystallinity according to the origin of the cellulose, but Nishiyama (2010) observed that any crystallinity is diminished if there are twists in the microfibrillar structure.

Table 3. Inter Relationships of Cellulose Polymorphs.

Adapted from Dufresne (2012) and Ashjaran et al. (2013)

Polymorphs		Comments
Cellulose I	α	Found in cell walls of structures such as algae and bacteria.
	β	Found in cell walls of more complex structures such as plants and trees.
Cellulose II		Created from the treatment of Cellulose I with NaOH, or from the dissolution of Cellulose I in a solvent to create regenerated fibre such as viscose. Also formed by bacteria such as *Gluconacetobacter xylinus*.
Cellulose III	i	Created by the treatment of cellulose I α with an amine. The least crystalline of all the cellulose structures.
	ii	Created by the treatment of cellulose I β with an amine. The least crystalline of all the cellulose structures.
Cellulose IV		Produced by the treatment of Cellulose III in high temperature glycerol. Considered a 'disordered' / less crystalline version of Cellulose I

3.1.3 Bacterial Cellulose

'Bacterial (or microbial / nano-) cellulose is a form of cellulose that is produced by bacteria' (Dufresne, 2012: 24). It is a product of a microbial metabolic process and can be thought of as a generated protective layer for that microbe because it has reported protective properties against ultraviolet light, and promotes retention of moisture (Williams and Cannon, 1989). It is generated as micro- (or nano-) fibrils via pores on the surface of the microbial cell, generally at the interface of liquid and air (Brown et al., 1976). Glucose molecules are polymerized, and several polymer chains are held together via hydrogen and van der Waals bonds to form the nano-fibrils (Iguchi et al.,

2000) forming a dense 3D network (Dufresne, 2012; Dima et al., 2017). This network is formed as a mat, or pellicle, generally on the surface of the liquid in which the microbes are active.

There have been many studies investigating the production of bacterial cellulose (BC), ranging from highly controlled laboratory-based conditions (Schramm and Hestrin, 1954b; Yamanaka and Sugiyama, 2000) to those using artisanal techniques (Chakravorty et al., 2016; Domskiene et al., 2019). This thesis will explore both approaches.

3.2 Production of Bacterial Cellulose from a Single Strain Inoculum

3.2.1 Single strain inocula

Whilst several genera of bacteria can produce bacterial cellulose (BC), it is the *Acetobacteraceae* family that is the most prolific (Table 2). More specifically, the *Komagataeibacter xylinus* (reclassified from *Acetobacter xylinus* and *Gluconacetobacter xylinus*) species have been identified as the most abundant producer (Chen and Liu, 2000; Dutta and Gachhui, 2006; Abaci et al., 2022). Studies agree that a liquid medium, an oxygen supply, and a carbon source provide ideal conditions for the bacteria to create the BC microfibrillar mat (Schramm et al., 1957; Iguchi et al., 2000; Yamanaka and Sugiyama, 2000; Krystynowicz et al., 2002; Dufresne, 2012; Lin et al., 2013a). Further studies suggest that the presence of a nitrogen source promotes more vigorous BC production and thus nitrogen sources such as yeast extract and peptone are also added to growth media to improve BC pellicle yield (Mohammadkazemi et al., 2015;

Yim et al., 2017). The types of growth media commonly used to support BC development with a single strain inoculum will now be discussed.

Table 4. An Overview of Microbial Species Producing Bacterial Cellulose.

Genus / Species	Source
Acetobacter / Gluconacetobacter / Komagaeibacter xylinus	Cakar et al. (2014), Fijalkowski et al., (2016), Hestrin and Schramm (1954), Sherif and Sameshima (2005), Kongruang (2008)
Gluconacetobacter swingsii	Castro et al. (2011)
Gluconacetobacter acetii	Dayal et al. (2013)
Gluconacetobacter hansenii	Ha et al. (2008)
Gluconacetobacter oboediens	Jahan et al. (2012)
Gluconacetobacter pasteurianus	Kumar et al. (2009)
Enterobacter sp.	Hungund and Gupta (2010)
Escherichia coli	Liu and Catchmark (2019)
Rhodococcus sp.	Tanskul et al. (2013)
Lactobacillius hilgardii	Khan et al. (2020)

3.2.2 Lab manufactured media

In microbiological studies, convention dictates the use of 'defined' media, which are prepared from highly purified ingredients of known concentrations. Using defined media, alongside distilled water to create an aqueous solution (also referred to as liquid medium, or broth), which is then sterilized, enables the exact content of the medium to be known and thus homogenous conditions created. These types of media are often

used to support the growth of microorganisms and usually contain a carbon energy source (e.g. glucose), an inorganic nitrogen source (amino acids) and other growth nutrients (e.g. vitamins, purified amino acids) (Chauhan and Jindal, 2020).

To encourage maximum BC yield, several studies have proposed various laboratory manufactured broth formulations with glucose and sucrose favoured as carbon sources, the addition of polypeptides (peptone / tryptone) and yeast extract as nitrogen based nutrient sources (Table 5) (Schramm and Hestrin, 1954a; Krystynowicz et al., 2002; Yim et al., 2017). However, the use of yeast extract as a nitrogen source automatically renders the growth broth as complex (i.e. undefined). Yeast extract is a mixture of hydrolysed yeast cells and therefore the exact chemical composition can be variable. Additionally, some broths are created with the addition of acid to lower the pH as it has been suggested acetobacter species favour acidic conditions (Schramm and Hestrin, 1954a). Good yields of BC have been achieved without artificially lowering the pH of the broth (Fijałkowski et al., 2016); *acetobacter* species generate acetic acid as part of their growing process and therefore naturally lower the pH of the growing broth medium over time.

The literature has demonstrated that across different methodological approaches, the yields of BC were variable, as were the way in which they were reported. For example, Shezad et al. (2009) reported a wet pellicle yield of 245.4 g/l, whilst Karahan et al. (2011) reported a dry yield of 2.0 g/l. Additionally, some studies reported yields as a percentage of the weight of BC pellicle produced by volume of liquid medium (w/v %). The w/v % method allows comparison of BC pellicle production regardless of size of growth vessel

volume of liquid medium and therefore is adopted throughout this thesis as a means of BC pellicle production analysis and comparison.

Table 5. Laboratory Manufactured Growth Media for Bacterial Cellulose Production: Variation in Experimental Conditions.

Authors	Liquid medium constituents	Inoculum	Incubation conditions		Drying conditions		Reported Yield (g/l)	Comments
			Days	Temp (°C)	Rinse	Dry		
Singh et.al. (2016)	2% glucose, 0.5% yeast extract, 0.5% peptone, 0.27% disodium phosphate, 0.15% citric acid	10% (v/v) *Gluconacetobacter xylinus* MTCC7795	variable	30	2 % NaOH for 90 min @ 100 °C 3 times rinse with H_2O	80 °C 12 hours	77.0	Shaker incubator (agitated)
Vazquez et.al. (2013)		*Gluconacetobacter xylinus* NRRL B-42	14	28	2 % NaOH for 90 min @ 100 °C 3 times rinse with H_2O	37 °C until constant weight	ns	Static
Karahan et al. (2011)		*Gluconacetobacter xylinus* NRRL B-759	7	28	0.1 M NaOH for 20 min @ 80 °C 3 times rinse with H_2O	80 °C 8 hours	2.0	Static
Setyawati et al. (2007)		*Acetobacter xylinum* BCRC12334	7	30	1 M NaOH for 20 min @ 95 °C Rinse with H_2O	70 °C 2 hours	ns	Static and agitated

Authors	Liquid medium constituents	Inoculum	Incubation conditions		Drying conditions		Reported Yield (g/l)	Comments
			Days	Temp (°C)	Rinse	Dry		
Wei et al. (2011)	2.5% mannitol, 0.3% tryptone, 0.5% yeast extract	*Acetobacter xylinum* (strain 1.128)	30	30	1.0 % NaOH for 2 hours @ 100 °C Washed in distilled H_2O	Freeze dried	ns	Static
Tang et. al. (2010)	2.5% glucose, 1.0% yeast extract, 0.75% peptone, 1% disodium phosphate, 1% acetic acid	*Gluconacetobacter xylinus*	5	30	0.1 M NaOH for 30 min, rinsed in H_2O	80 °C (no time stated)	6.2	Static
Shezad et.al. (2009)	1.0% glucose, 1.0% yeast extract, 0.7% peptone, 0.15% acetic acid, 0.02% succinate	*Gluconacetobacter hansenii* PJK (KCTC 10505BP)	30	30	0.3 M NaOH @ 100 °C for 20 min. Repeated and rinse with H_2O	60 °C until constant weight	245.4 (wet)	Static
Schramm & Hestrin (1954a)	2.0% glucose, 0.5% bactopeptone,	*Acetobacter xylinum*	variable	30	Distilled water for 90 min	80 °C *in vacuo* until constant weight	42.0	Static

Authors	Liquid medium constituents	Inoculum	Incubation conditions		Drying conditions		Reported Yield (g/l)	Comments
			Days	Temp (°C)	Rinse	Dry		
Fijalkowski et. al. (2016)	0.5% yeast extract	*Gluconacetobacter xylinus* ATCC53524, *Gluconacetobacter xylinus* DSM46602, *Gluconacetobacter xylinus* DSM46604	31	28	0.1 M NaOH for 30 min @ 80 °C 3 times rinse with H_2O	60 °C 12 hours	5.5	Static
Yamanaka et al. (2000)	5% sucrose 0.5% amino acid compound, 0.3% potassium phosphate, 0.24% magnesium sulphate, 0.1% ammonium sulphate	*Acetobacter aceti* (AJ12368)	30	25	Rinse with H_2O for one day. Immerse in 5% NaOH for15 hours Rinse with H_2O for three days.	Desiccator @ ambient temp for 1 day	ns	Static

Schramm and Hestrin (1954b) are widely considered the pioneers of bacterial cellulose studies, with many researchers referring to their work as a starting point for future investigations (Setyawati et al., 2007; Karahan et al., 2011; Singh et al., 2016). Schramm and Hestrin's initial examinations of growth medium formulations led to the development of a standard uniform broth, known commonly as Hestrin-Schramm (H&S), comprising glucose (as the carbon source), bactopeptone and yeast extract (as nitrogen sources) (Schramm et al., 1957). However, Kongruang (2008) criticised this complex medium as needing many ingredients and being expensive in comparison to others, suggesting that media such as naturally occurring fruit juices may be suitable to support BC pellicle development.

3.2.3 Alternative media

Liquid medium, oxygen supply and carbon source are required to support BC pellicle development, with additional nitrogen promoting yield (Schramm et al., 1957; Iguchi et al., 2000; Yamanaka and Sugiyama, 2000; Krystynowicz et al., 2002; Dufresne, 2012; Lin et al., 2013a). As such, this has led researchers to investigate alternative media to those manufactured in a laboratory to support growth.

Food wastes have been explored as inexpensive alternatives to expensive laboratory formulations (Table 6). Foodstuffs such as coconut juice (Kongruang, 2008), beer (Shezad et al., 2009) and pear juice (Kurosumi et al., 2009) have been evaluated with varying degrees of success in terms of pellicle yield. Media based on food extracts (not necessarily waste) have also been assessed, such as beets (molasses, sugar syrup), corn (starch, glucose syrup), cactus pear juice (Ayed and Hamdi, 2015), pineapple juice

(Neera et al., 2015) and potatoes (Kongruang, 2008). Reported yields vary from 750 g/l wet pellicle (Shezad et al., 2009) to 1.56 g/l dry pellicle weight (Zeng et al., 2011). Green and black tea (Teoh et al., 2004) have also been investigated as potential liquid media to support bacterial growth and cellulose development, with pure strain types as a starter culture. Whilst tea can be considered a source of nitrogen and therefore a promoter of BC growth (Yim et al., 2017), it is widely accepted that, in some cases, tea has an antibacterial effect, due to polyphenol content (Antolak et al., 2021). Nguyen et al. (2008) suggest that too high a concentration of tea (and therefore polyphenols) could inhibit growth of some bacterial strains, therefore limiting bacterial cellulose yield.

Table 6. Food Waste Growth Media for Bacterial Cellulose Production: Variations in Experimental Conditions.

Authors	Liquid medium constituents	Inoculum	Incubation conditions		Drying conditions		Reported Yield (g/l)	Comments
			Days	Temp (°C)	Rinse	Dry		
Rajwade et al. (2015)	Pineapple / Watermelon with 55 mL sucrose and 0.7% ammonium sulphate	*Komagataeibacter hanseii* (ATCC 23770)	7	Room temp	ns	ns	Pineapple 12.5 Watermelon 10.0	static
Kongruang et al. (2008)	Coconut juice, 1 % yeast extract, 14mL of 9 5% ethanol	*Gluconacetobacter xylinus* (TISTR 998)	6	30	1 % NaOH @ 90 °C for 30 min 2 x rinse with distilled water	ns	553.33 (wet)	Static
Ha et al. (2008)	Waste from beer culture broth 1 % glucose	*Gluconacetobacter hansenii* PJK (KCTC 10505BP)	5	30	0.3 M NaOH @ 100 °C for 5min. Rinse with water until neutral pH	Freeze dry @ -50 ˚C	4.52	Static
Shezad et al. (2009)	Waste from beer culture broth 1 % glucose	*Gluconacetobacter hansenii* PJK (KCTC 10505BP)	30	30	0.3 M NaOH @ 100 °C for 20 min. Rinse with distilled water	ns	750 (wet)	Static

Authors	Liquid medium constituents	Inoculum	Incubation conditions		Drying conditions		Reported Yield (g/l)	Comments
			Days	Temp (°C)	Rinse	Dry		
Kurosumi et al. (2009)	Orange / Apple / Pineapple / Grape / Japanese pear juice, 2.0 % peptone, 0.5 % yeast extract 0.12% citric acid	*Gluconacetobacter xylinus* (NBRC 13693)	ns	ns	Alternate rinses with water, 2 % NaOH solution, 2 % acetic acid solution	80 °C until constant weight	5.9	H&S broth added to fruit juice
Rani & Appiah (2013)	Coffee cherry husk	*Gluconacetobacter hansenii* UAC09	ns	ns	1 M NaOH for 1 day at room temp. Rinse with water until neutral pH	60 °C between filter papers until constant weight	5.6	Static
Zeng et al. (2011)	Maple syrup 20 g fructose 20 g yeast extract 1 g ammonium sulphate 0.122 g magnesium sulphate	*Gluconacetobacter xylinus* BPR 2001 (ATCC 700178)	ns	28	15 mL 1%w/v NaOH @ 90 °C for 30 min. Rinse with distilled water	60 °C for 24 hours	1.56	Agitated

Authors	Liquid medium constituents	Inoculum	Incubation conditions		Drying conditions		Reported Yield (g/l)	Comments
			Days	Temp (°C)	Rinse	Dry		
Jahan et al. (2012)	Rotten vegetables & fruits (apple, pineapple, orange, lime, pomegranate, carrot, cabbage, tomato, spinach)	*Gluconacetobacter* sp. F6	7	30	1.0 M NaOH @ 80 ˚C for 20 min. Neutralise with 5% acetic acid solution. 2 x rinse with water	Ambient temperature until constant weight	4.5	Static
Nguyen et al. (2008)	Whey, sucrose, black tea	*Komagataeibacter xylinus*	15	30	0.5 % NaOH for 60 min. Rinse with distilled water until neutral pH.	Freeze dried at -110 °C for 24 hr	12.59 g/l (dry)	Static
	Whey, apple, brewed spent grain extract, sucrose, black tea						12.81 g/l (dry)	
Castro et al. (2011)	Pineapple peel juice and sugar cane juice	*Gluconacetobacter swingsii*	13	28	ns	ns	2.8	Static
Song et.al. (2009)	Saccharified food waste	*Gluconacetobacter xylinus* (KJ1)	3	30	ns	ns	5.6	Agitated

3.2.4 Temperature and Time Effects

There is no consensus on the optimal temperature required to facilitate efficient BC pellicle production. In experiments using either laboratory - manufactured formulations or food waste to support pellicle growth (Table 5, Table 6), temperatures of between 25 – 30 °C were used. Only the Yamanaka et al. (2000) study used the lower temperature of 25 °C; the majority used 28 – 30 °C as an incubation temperature.

Views on the optimal incubation time for pellicle development are diverse. Lengths of between 7 – 30 days are reported for laboratory media, whilst pellicle development is seen at an average of 14 days in food - derived media (Table 5, Table 6).

Inconsistencies in previous research make it impossible to determine optimal time and temperature for cellulosic pellicle growth based on a search of literature alone. It is therefore imperative that these two variables are explored in more detail, via laboratory-based trials, to establish the optimal conditions required to efficiently develop pellicles that are suitable for further evaluation as a textile.

3.2.5 Static vs Agitated Incubation

The formation of the pellicle yields can depend on the method of incubation. Under static conditions, the BC begins to form as a solid pellicle on the surface of the supporting media. Under agitated conditions, the BC forms as smaller pellets within the growth medium (Schramm et al., 1957), affecting the potential applications. In the food industry, where BC is used as an additive, smaller pellets are desirable. However, in the medical or textile industries where films of bacterial cellulose are more useful (for example, in the treatment of burns), a static method of manufacture is more desirable (Lin et al., 2013a).

Static methods were therefore chosen for study in this thesis because a flat film is more comparable to traditional sheet – like textile materials.

3.2.6 Summary

- A detailed review of the literature revealed several factors that influence the development and yield of a BC pellicle: liquid media, culture type, aerobic conditions, incubation time and temperature.
- Media to support pellicle growth fell broadly into two categories: laboratory - prepared and those media relating to liquid foodstuffs. Liquid foodstuffs appear to support the greatest yield by weight of BC pellicle.
- Whilst the literature agrees that optimal incubation temperatures are in the range of 28 – 30 °C, there is debate around optimal incubation times for BC growth, with times ranging from 3 to 31 days.
- Due to the vague and often incomplete information, it is not possible to determine the best conditions in which to grow BC pellicles to achieve the greatest and most consistently reproducible yield by literature review alone. Therefore, a necessary first step for this study is to establish the most effective conditions in which to develop BC pellicles to conduct further investigations.

3.3 Production of Bacterial Cellulose from a Kombucha SCOBY Inoculum

3.3.1 The History of Kombucha Fermentation

Kombucha, also traditionally known as Haipao, Kargasok Tea, Tea Fungus, and Manchurian Mushroom (Kurtzman et al., 2001; Ovcharenko, 2013), has been used in the fermentation of drinks dating back several thousand years. Evidence shows that the practice originated in the East and the fermented tea drink is purported to have detoxifying and energising properties (Četojević-Simin et al., 2012; Jayabalan et al., 2014). In modern society such perceived health benefits to drinks are increasingly popular and the Kombucha tea fungus has been used for fermentation in various formulations (Malbaša et al., 2011) such as milk (Kruk et al., 2021) and coffee (Bueno et al., 2021). However, tea remains the most popular medium for the fermentation and consumption of Kombucha. Whilst green tea is widely used in health drink preparation with studies documenting high bacterial activity in this broth (Nguyen et al., 2008), other studies disagree, reporting black tea to be the 'finest' substrate to support bacterial growth (Jayabalan et al., 2014). There is still much ongoing research investigating the benefits of the biological activity of Kombucha, which claims health benefits such as anti-ageing, anti-hypersensitivity, and anti-hypertensive activity (Abaci et al., 2022).

3.3.2 Kombucha Fermentation Conditions

The drink is prepared by placing the tea fungus inoculum (also referred to in literature as the starter / mother culture / pellicle / biofilm) into a tea broth, which can be prepared in various ways, but most commonly by steeping tea bags in a quantity of hot water for 10 minutes. The tea bags are removed, and the liquid allowed to cool before the inoculum

and sugar are added. The mixture is allowed to ferment over a period of days at room temperature. The type of tea, amount of sugar and fermentation time varies. The resultant drink is reported to have a 'pleasantly fruity sour-like sparkling flavour' (Jayabalan et al., 2014: 539).

During the fermentation process, the starter / mother culture / pellicle inoculum sinks to the bottom of the tea broth, whilst a new culture, often referred to as the 'daughter' culture, forms on the surface of the liquid tea (Jayabalan et al., 2014). After a period of fermentation, the daughter culture has developed to such a degree that it is similar in size and appearance to that of the mother. Both the mother and daughter cultures can then be removed and used as starter cultures for fresh fermentations, albeit some methods recommend using a small amount (1% w/v) of the original tea broth alongside the solid cultures (Jarrell et al., 2000; Jayabalan et al., 2014).

The practice described above is in reference to 'home' brewing of a Kombucha based drink. Commercially, Kombucha is available to buy as a bottled drink in various liquids such as lemonade and passionfruit. However, in these cases, the liquid drink has been sterilized prior to bottling rendering the potential of further fermentation redundant.

There have been many studies which attempt to assess the optimal conditions for Kombucha fermentation. Some researchers consider that temperature is critical in the fermentation of the tea (Petrovic et al., 1995). However, others consider it is the length of the fermentation cycle that has the largest impact on bacterial activity (Battikh et al., 2013). Antolak et al (2021) further suggest that variances in the composition of the starter cultures or pellicles also influence the optimal conditions for fermentation. Table 7

provides an overview of conditions used in various studies to ferment Kombucha tea for analysis.

Table 7. Kombucha Tea Fermentation Conditions.

Authors	Liquid Medium	Carbon Source	Inoculum		Incubation conditions	
			Kombucha Pellicle (wet w/v %)	Kombucha broth	Days	Temperature (°C)
Ayed & Hamdi (2015)	4.8 g Russian black tea in 400 mL boiling water, steeped for 5min	40 g saccharose	0.03	10 % tea	15	30
Battikh et al. (2013)	10 g Les jardins green tea in 1000 mL boiling water steeped for 15 min	20 g sucrose	0.1	-	21	not stated
Chen & Liu (2000)	2 bags black Lipton tea in 1000 mL boiling water, steeped for 5 min	100 g sucrose	0.2	2.5 % tea	14	24 +/- 3
Cetojevic-Simin et al. (2012)	5 g lemon balm leaves in 1000 mL boiling water, steeped for 15 min	70 g sucrose	ns	10 % lemon balm	variable	28
Jarrell et al. (2000)	3 bags black Lipton tea in 1000 mL boiling water, steeped for 5 min	62 g sucrose	0.075	20 % tea	7	28
Marsh et al. (2014)	0.49 % Barrys original blend black tea in 2000 mL boiling water, steeped for 15 min	10 % sucrose	whole pellicle (size not stated)	10 % tea	10	23
Teoh et al. (2004)	5.4 g black Lipton tea in 1000 mL boiling water, steeped for 15 min	100 g white sugar	whole pellicle (size not stated)	-	14	22
Wang et al. (2013)	5.0 g Yunnan Dianhong black tea in 1000 mL boiling water, steeped for 15 min	100 g glucose	ns	20 % tea	8	30

3.3.3 Kombucha Pellicle Composition

As discussed in section 1.4, a Kombucha tea fungus, or pellicle, can be considered a consortium of yeasts and bacteria (Jarrell et al., 2000; Teoh et al., 2004; Reva et al., 2015), leading to it often being referred to as a SCOBY (**S**ymbiotic **C**ommunity of **B**acteria and **Y**easts). The exact composition of the pellicle is determined by the geographic and climatic conditions of cultivation (Jarrell et al., 2000; Antolak et al., 2021; Abaci et al., 2022), but it is suggested that a Kombucha pellicle consists of a 'core' consortium of bacteria and yeasts (Reva et al., 2015) which always possess cellulose–forming properties (Teoh et al., 2004). Furthermore, it is thought that additional geographically local bacteria and yeasts can affect the growth behaviour of the overall pellicle community, typically enhancing the ability of the active bacteria to produce a Kombucha 'daughter' pellicle (Reva et al., 2015). Studies have attempted to evaluate the composition of the Kombucha cultures (Abaci et al., 2022), both in the tea liquid and in the mother / daughter solid tea fungus using genetic sequencing analysis techniques (Marsh et al., 2014; Reva et al., 2015; Chakravorty et al., 2016).

The physical structure of the Kombucha pellicle is a mat of intermeshed bacterial cellulose microfibrils, created by acetic acid bacterial species present (Iguchi et al., 2000; Marsh et al., 2014; Antolak et al., 2021), to which other bacterial cells and yeasts are attached (Budhiono et al., 1999; Jayabalan et al., 2014). The composition of the Kombucha SCOBY has therefore led to exploration of this being used as a starter to produce BC sheets, as illustrated in Table 8.

Table 8. Conditions Used to Cultivate Kombucha Pellicle as a BC Mat.

Authors	Liquid Medium	Carbon Source	Inoculum		Incubation time (days)	Temperature	Reported Yield
			Kombucha Pellicle (wet w/v%)	Kombucha broth			
Domskiene et al. (2019)	1 L water, 4 g green tea, 100 mL 6 % yeast extract	100 g sucrose	Weight not stated	-	7	20 – 24 °C	ns
Yim et al. (2017)	Green / Black / Rooibos / Corn silk tea (0.3 % w/v),	Sucrose / fructose / corn syrup (5 % w/v)	1 % w/v	5 % v/v	12	26 °C	0.8 – 9.3 % w/v Dried for 24 hr @ 25 °C
Priyadharshini et al. (2022)	Black & Green tea 18 g/l	105 g/l	18 g/l	89 g/l	13.5	27	55 g/l (wet)
Tran et al. (2021)	Black tea 1 % w/v	60 g /l	-	12 % v/v	14	26 °C	9.3 – 11.2 g (dry) 178.7 – 462.9 g/l (wet)

3.3.4 Bacteria Species Present in Tea and Pellicle

Several studies have described the bacterial composition of both the Kombucha pellicle and the tea medium in which it ferments (Table 7, Table 9). The most frequently observed genus is *Acetobacter* (reclassified as *Gluconacetobacter* or *Komagataeibacter*) (Chen and Liu, 2000; Dufresne and Farnworth, 2000; Jarrell et al., 2000; Dutta and Gachhui, 2006; Abaci et al., 2022), with *Lactobacillius* often identified (Diez-Ozaeta and Astiazaran, 2022; Mas et al., 2022).

3.3.5 Yeast Species Present in Tea and Pellicle

Researchers have attempted to identify all yeast species found in the Kombucha brewing cycle. Generally, the yeast populations are found to be varied from sample to sample (Marsh et al., 2014), with species such as *Zygosaccharomyces, Torulaspora, Saccharomyces* and *Pichia Brettanomyces* being discovered (Jayabalan et al., 2014; Marsh et al., 2014; Reva et al., 2015) (Table 9). It is thought that the desirable yeast characteristics required to promote efficient fermentation are those displaying high levels of osmotolerance, acid tolerance and the production of alcohol from the conversion of sucrose, such as *Schizosaccharomyces pombe* and *Brettanomyces bruxellensis* (Marsh et al., 2014; Teoh et al., 2004). The production of alcohol (specifically ethanol) from sugars by yeasts present in the Kombucha SCOBY community is considered key to support the activity of the bacterial species responsible for the production of BC (Harrison and Curtin, 2021).

Table 9. Bacteria Commonly Isolated from Kombucha Tea Liquid and Pellicle Samples.

Bacteria	Source
Acetobacter xylinum	Chen and Liu (2000), Jarrell et al. (2000), Wang and Baoping (2013)
Acetobacter xylinoides	Wang and Baoping (2013)
Gluconacetobacter	Jarrell et al. (2000), Ovcharenko (2013), Marsh et al. (2014),
Lactobacillius	Reva et al. (2015)
Komagataeibacter Kombuchae	Dutta and Gachui (2006)
Gluconacetobacter sacchari	Trovatti et al. (2011)
Acetobacter aceti	Abaci et al. (2022)
Acetobacter nitrogenificans	Dutta and Gachui (2006)
Acetobacter pasteurianus	Chen and Liu (2000), Wang and Baoping (2013)
Acetobacter liquefaciens	Wang and Baoping (2013)

Table 10. Yeast Commonly Isolated from Kombucha Tea Liquid and Pellicle Samples.

Yeast	Source
Schizosaccharomyces pombe	Chen and Liu (2000), Jarrell et al. (2000), Wang and Baoping (2013)
Saccharomyces ludwigii	Chen and Liu (2000), Jarrell et al. (2000), Wang and Baoping (2013)
Saccharomyces inconspicus	Wang and Baoping (2013)
Saccharomyces cerevisiae	Wang and Baoping (2013)
Pichia sp.	Chen and Liu (2000), Jarrell et al. (2000), Marsh et al. (2014), Reva et al. (2015)
Torula sp.	Jarrell et al. (2000), Teoh et al. (2004),
Brettanomyces bruxellensis	Teoh et al. (2004), Reva et al. (2015)
Zygosaccharomyces sp.	Chen and Liu (2000), Marsh et al. (2014)
Candida tropicalis	Wang and Baoping (2013)
Candida stellate	Chen and Liu (2000), Teoh et al. (2004), Wang and Baoping (2013)

3.4 Production Of Bacterial Cellulose: Establishing Optimal Growth Conditions

The next stage of this thesis is a practical investigation of experimental variables found in literature to identify the most effective method of reproducibly generating BC pellicles and specify these conditions for further exploration.

The detailed review of existing literature revealed that several factors influence the yield of BC pellicles. Different types of inocula produced a comparable range of yields of dry pellicle; 0.8 – 11.2 g/l (0.08 – 1.12 w/v %) for Kombucha and 1.56 – 12.81 g (0.16 – 1.28 w/v %) for pure

strain culture (Table 5, Table 6, Table 6). It is worth noting that these yields consider the pellicle mass as a whole and do not consider the absolute mass (or proportion) of bacterial cellulose microfibrils within the structure. For the purposes of this study, this is not a concern because the application of the whole pellicle is being considered, not that of the pure bacterial cellulose.

The literature search did not reveal a 'recommended' method for pellicle development from either inoculum (whether single strain or Kombucha SCOBY) but because both gave very similar yield range results, preliminary work was based on a Kombucha SCOBY inoculum due to ease of access, maintenance, and use. The methods and findings discussed in this section are part of a wider peer - reviewed study (Wood et al., 2022) (appendix 1).

Existing literature suggested that various factors, aside from inoculum type, could impact pellicle development such as aerobic conditions, incubation time, temperature, and culture media. To establish optimal growing conditions to further this study, these factors were considered in more detail.

3.4.1 Growth Media

Literature describing media that supported the growth of BC fell into two categories; methods using liquid foodstuffs or food waste (Table 4) and those using laboratory prepared 'standard' media (Table 3).

Liquid foodstuffs often supported the development of a BC pellicle most effectively (Table 6) and thus three food - based liquids were chosen for preliminary study.

- Coconut water due to its 'natural' origin (Vitacoco, 2020),
- Beer contains yeast extract. It is suggested that enhanced yeast extract content helps support pellicle development as it is considered a source of nitrogen (Ha et al., 2008),
- Sweetened black tea is extensively documented as a medium in which to brew Kombucha (and therefore support pellicle development) (Jayabalan et al., 2014).

As a comparison to these, a medium prepared in a laboratory was also used in preliminary explorations, to support the development of BC pellicles (Table 3). The two 'standard' complex media, referred to most frequently in literature are Hestrin and Schramm (H&S) (Schramm et al., 1957) and Yamanaka (Iguchi et al., 2000). However, as discussed in section 3.2.2 , there is no agreement in the current literature as to which is superior in terms of BC yield. Hestrin and Schramm medium was chosen for this study because it was the most frequently referred to in the literature reviewed.

3.4.2 Incubation Time

There is some disagreement in the literature with suggestions of 'optimal' incubation times, ranging between 3 (Song et al., 2009) and 31 days (Fijałkowski et al., 2016) (Table 5). However, the greatest pellicle yields (expressed by either wet or dry weight) were reported at >20 days incubation (Schramm and Hestrin, 1954a ; Shezad et al., 2009). Thus, for this thesis, pellicles were sampled at 27-, 34- and 42-days incubation (in accordance with laboratory access) and the wet and dry yields compared.

3.4.3 Incubation Temperature

Previous studies indicated that temperatures of between 25 °C and 30 °C had been investigated for the effects on BC yield. As most studies (Kongruang, 2008; Ha et al., 2008; Nguyen et al., 2008; Shezad et al., 2009; Song et al., 2009; Jahan et al., 2012) used 30 °C and this was readily available as a walk-in incubator in the laboratory, this temperature was chosen for the study.

3.4.4 Sterile vs Nonsterile Media

The literature encompasses both sterile and non-sterile conditions (Schramm and Hestrin, 1954a; Yamanaka and Sugiyama, 2000) for the development of pellicles. For preliminary work, the effect on pellicle development and yield in both sterile and non-sterile conditions was evaluated.

3.4.5 Aerobic Conditions

It is well documented that the bacteria responsible for the production of BC are aerobic; that is, they need oxygen for survival (Schramm and Hestrin, 1954a; Chao et al., 2000; Dufresne, 2012; Gullo et al., 2014; Zheng et al., 2018; Aswini et al., 2020). As this is an already proven requirement for BC forming bacteria, this condition was not explored, and all experiments described in this thesis were conducted under aerobic conditions.

3.5 Experimental Details

3.5.1 Inoculum Preparation

A commercially available 200 g Kombucha pellicle in 100 mL of green tea was purchased from a web-based company Happy Kombucha (2022) producers of Kombucha starter culture for the brewing of tea as a health drink. This supplier was chosen as previous Kombucha SCOBY had been purchased from here and used successfully to create further Kombucha pellicles. However, it should be noted that literature states there is potential for variation between sources of Kombucha SCOBY depending on storage and fermentation conditions and geographical location (Jarrell et al., 2000; Marsh et al., 2014).

This pellicle was stored in its original tea broth, in ambient conditions as advised by the supplier (room temperature 22 °C + / - 2 °C, on a bench top). The exact composition of this tea broth was not known; however, it was assumed to have a base of green tea (according to the supplier's website). To provide a standardised inoculum, 100 g of the starter culture pellicle was placed in a sterile pot containing 250 mL Hestrin and Schramm (H&S) medium. A lid was loosely placed on the pot. This pot was stored in an incubator (30 °C) for 15 days to enable the production of fresh inoculum. After 15 days, any solid biofilm (pellicle) was removed from the pot by filtration, and the remaining filtrate (broth) was used immediately as the stock inoculum for the study.

Table 11. Media Used for Pellicle / Biofilm Growth.

Type of Medium	Preparation	Comments
Coconut water	Pre-packaged coconut water (Vitacoco, 2020) was purchased from a local supermarket. The liquid was centrifuged to extract any residual solids.	After preparation / purchase of the media, each liquid was decanted into separate sterile 500 mL Duran bottles and sterilised in an autoclave for 10 min @ 115 °C (the lower temperature was used to reduce risk of glucose caramelisation).
Beer - Bitter	Own brand bitter - Morrisons supermarket (Morissons, 2020)	
Black Tea	Tea broth was prepared by steeping 1 tea bag (Yorkshire brand black tea) in 1 L boiling water for 15 min (approx. 3 g / l tea). The bag was removed, and 100 g glucose added (10 %).	
Hestrin & Schramm	2 % Glucose, 0.5 % Bactopeptone,0.5 % Yeast extract added to 1 L distilled, deionised water	

3.5.2 Sample Preparation

To determine whether sterile conditions were necessary to produce BC pellicles, equal volumes were either autoclaved (10 min @ 115 °C) and cooled to ambient temperature, then 10 mL aseptically transferred into 30 sterile 25 mL containers; or 10 mL aliquots with no autoclave treatment were dispensed into 30 sterile 25 mL containers.

Each container was then inoculated with 1mL of Kombucha pellicle tea broth (prepared as described in section 3.5.1) with the lid placed loosely on top, incubated at 30 °C with environmental conditions monitored using an USB datalogger (model: Easylog USB, manufacturer: Lascar Electronics, Wiltshire, UK).

Sampling took place 27, 34 and 42 days after inoculation due to accessibility of the laboratory (10 pots per medium per sample date).

Any pots which failed to develop a pellicle were noted. Visual observations of pellicle appearance on the surface of the medium were observed and recorded by photography. Pellicle thickness (to the nearest mm) was measured using a ruler on the outside of the pot before pellicle removal.

Pellicles on the surface of the liquid media were removed with sterile forceps and placed on dry filter paper to remove any excess surface moisture. The pellicles were then individually placed in petri dishes and weighed twice – (i) in the wet state, (ii) after drying for 24 hours in a fan oven at 60°C (to constant weight).

The weight of BC produced per volume of liquid (% w/v) was calculated, in concurrence with the method used by several other studies (Schramm and Hestrin, 1954a; Shezad et al., 2009; Karahan et al., 2011; Singh et al., 2016). Mean and standard deviations of the weights and thicknesses of pellicles were calculated (any pots which failed to develop a pellicle were excluded from the calculation).

3.5.3 Results And Discussion

3.5.3.1 Pellicle Yield: Thickness

In all sterile media, every pot developed a pellicle. In unsterile media, all pots containing H&S or coconut developed pellicles, whilst one pot of unsterile beer medium (27 days) and

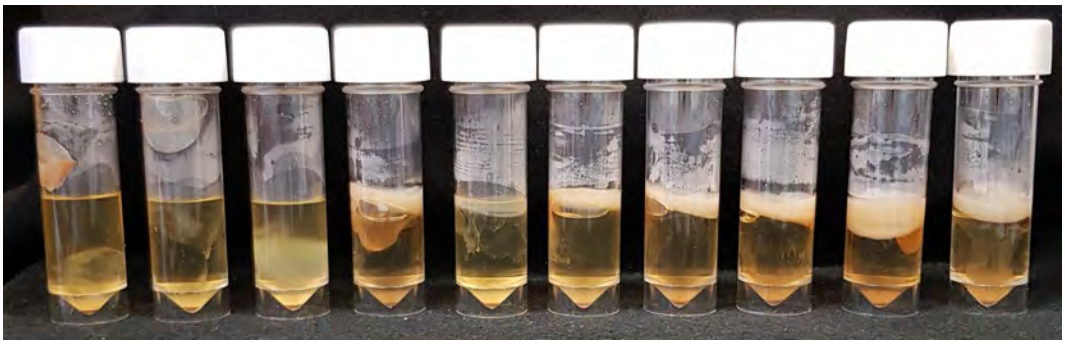
seven pots (three (27 days); four (34 days)) of unsterile tea medium failed to produce a pellicle.

Variability in thickness of pellicles was visually noted throughout all the pots at any given incubation time (Figure 1).

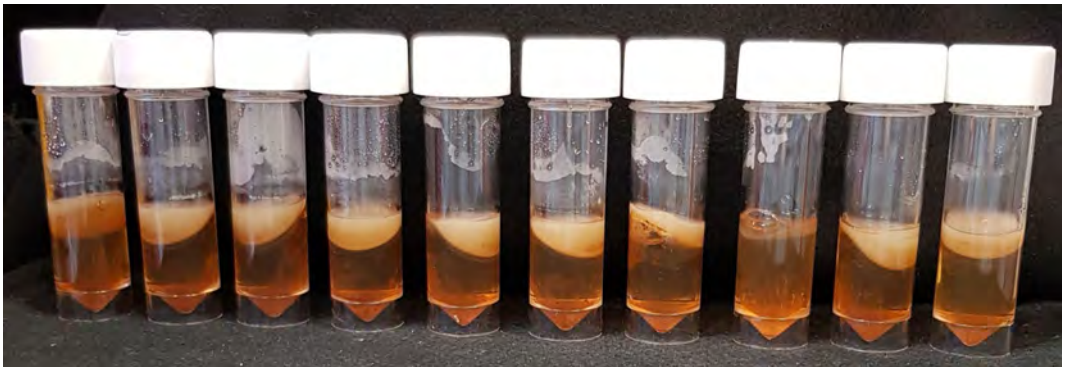
In unsterile media, no clear trends of pellicle thickness were observed, either across incubation times or growth media (Figure 2). Some significant differences were noted at 27 days (coconut / H&S and H&S / Beer ($p < 0.05$)) and some observed at 42 days incubation time, but no clear trends could be elucidated from this data.

In sterile media, some significant differences in pellicle thicknesses were observed, most notably, the mean thickness of pellicles grown in tea and coconut were significantly greater than beer at 27, 34 and 42 days ($p < 0.05 – 0.0001$) (Figure 3).

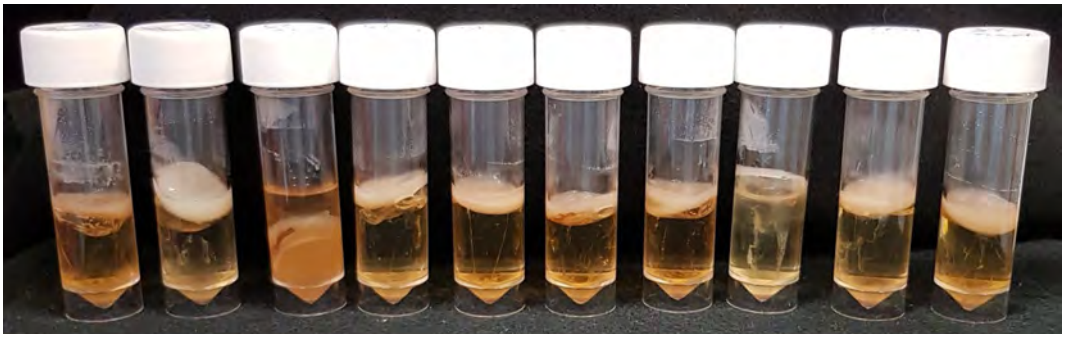
(a)



(b)



(c)



(d)

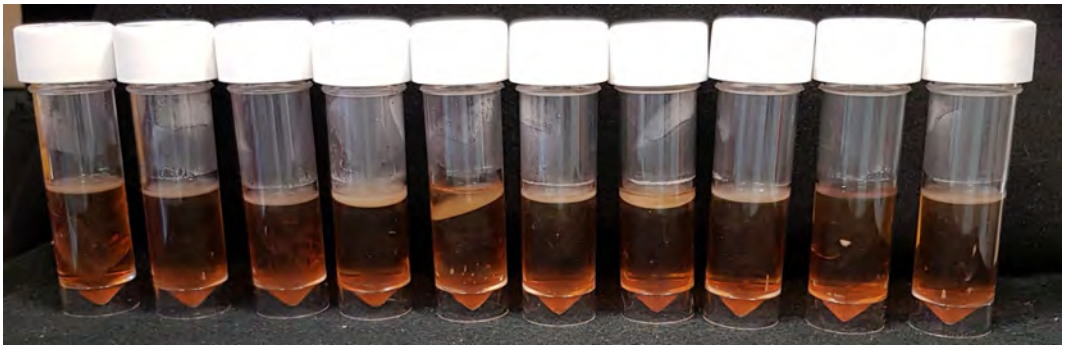


Figure 1. Variability in Thickness of Pellicles Developed After 27 Days in Sterile Broths.

(a) Coconut, (b) Tea, (c) H&S, (d) Beer
(universal pot actual size: diameter = 25mm, height = 90mm)

(a) (b) (c)

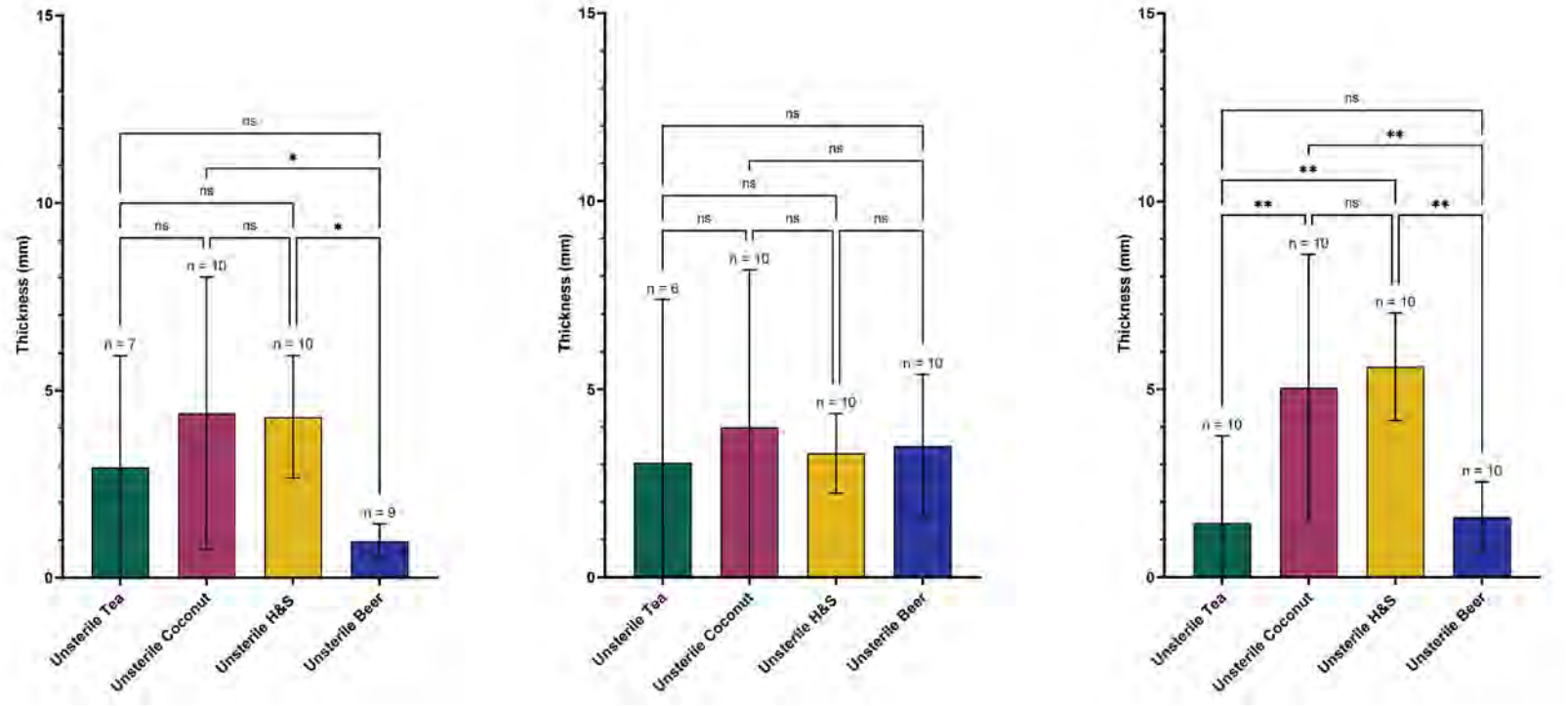


Figure 2. Mean Pellicle Thickness in Unsterile Media Incubated for (a) 27 Days, (b) 34 days, (c) 42 days.

(error bars = standard deviation (σ), ns = not significant, * = $p < 0.05$, ** = $p < 0.01$)

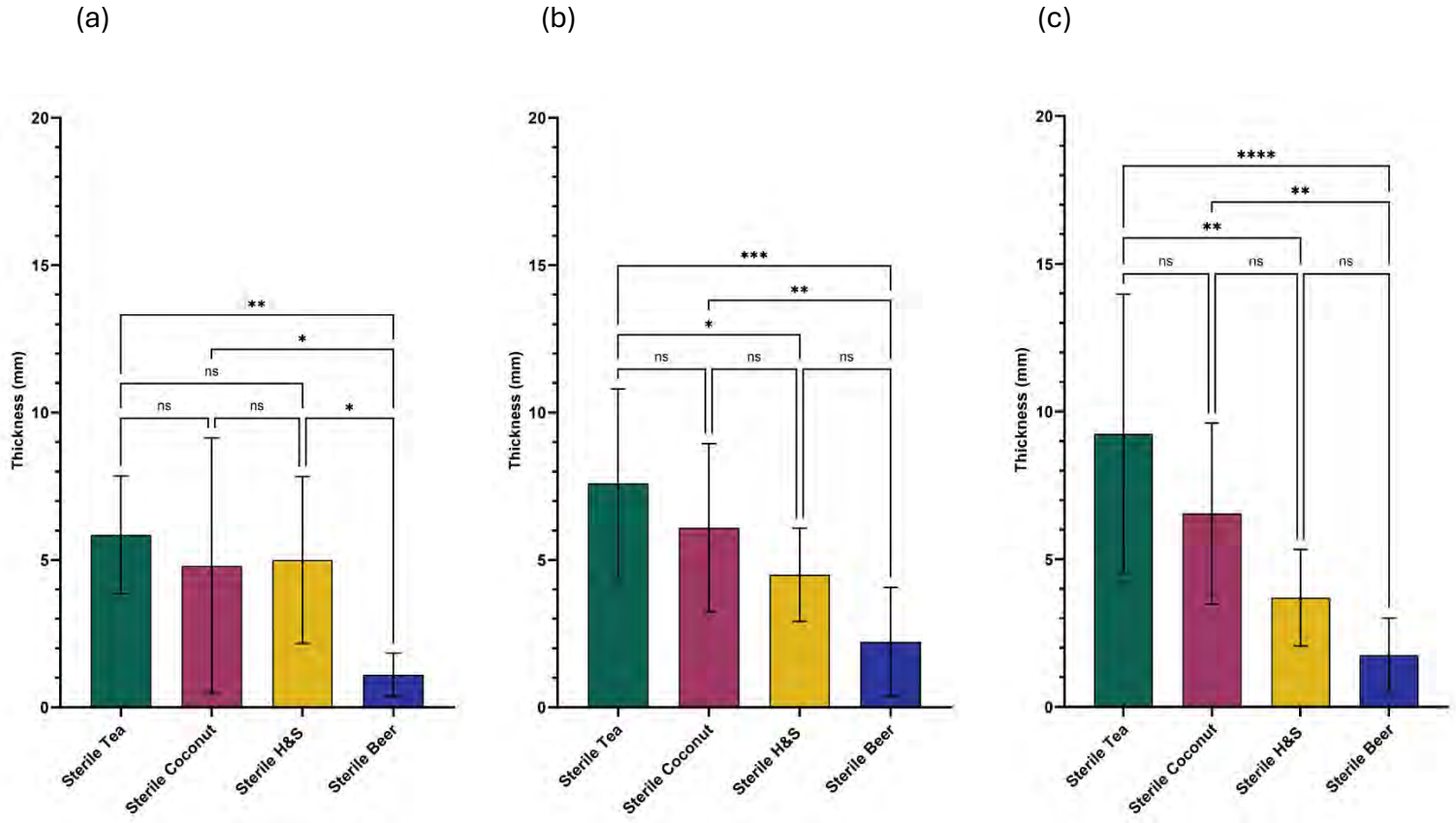


Figure 3. Mean Pellicle Thickness in Sterile Media Incubated for (a) 27 days, (b) 34 days, (c) 42 days.

(n = 10 for all media, error bars = standard deviation (σ), ns = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$)

Whilst increased mean pellicle thickness over incubation time was observed in sterile tea, with the thickest pellicles produced after 42 days (tea mean = 9.3 cm) (Figure 4), the trend of thicker pellicles over incubation time was not statistically significant ($p > 0.05$).

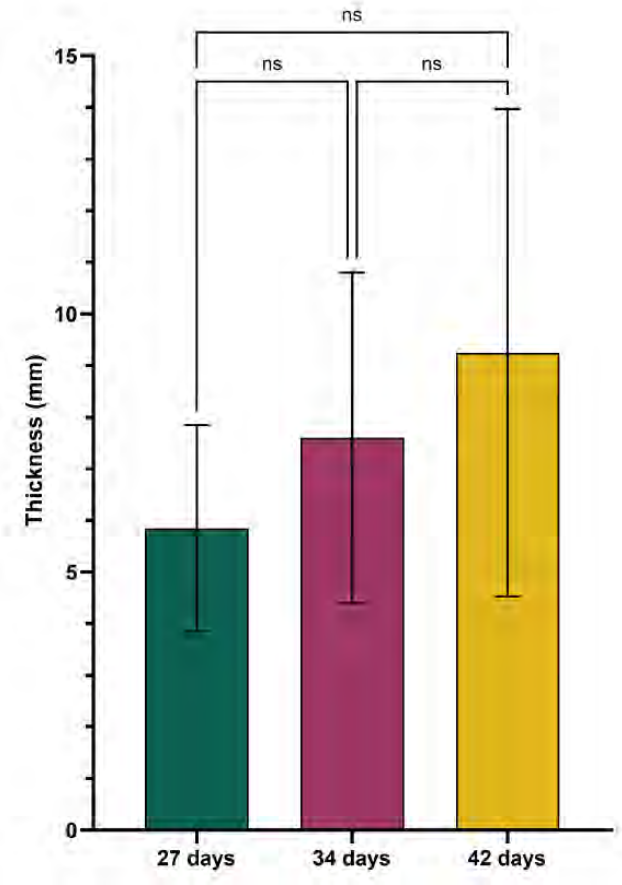


Figure 4. Mean Pellicle Thickness in Sterile Tea Medium Over Incubation Time.

(n = 10 for all timepoints, error bars = standard deviation (σ), ns = not significant)

3.5.3.2 Pellicle Yield: Weight

In unsterile media, no significant trends ($p > 0.05$) were observed in pellicle wet or dry weight across the different media or incubation times.

Mean wet weight pellicle yields ($p < 0.0001$) were significantly higher in sterile than those in unsterile media. Sterile media observations also revealed that tea produced the largest significant yield ($p < 0.05 – 0.0001$) by wet (mean = > 20 % w/v) and dry (mean = > 1.8 % w/v) weight, compared to H&S, coconut, and beer (Figure 5, Figure 6).

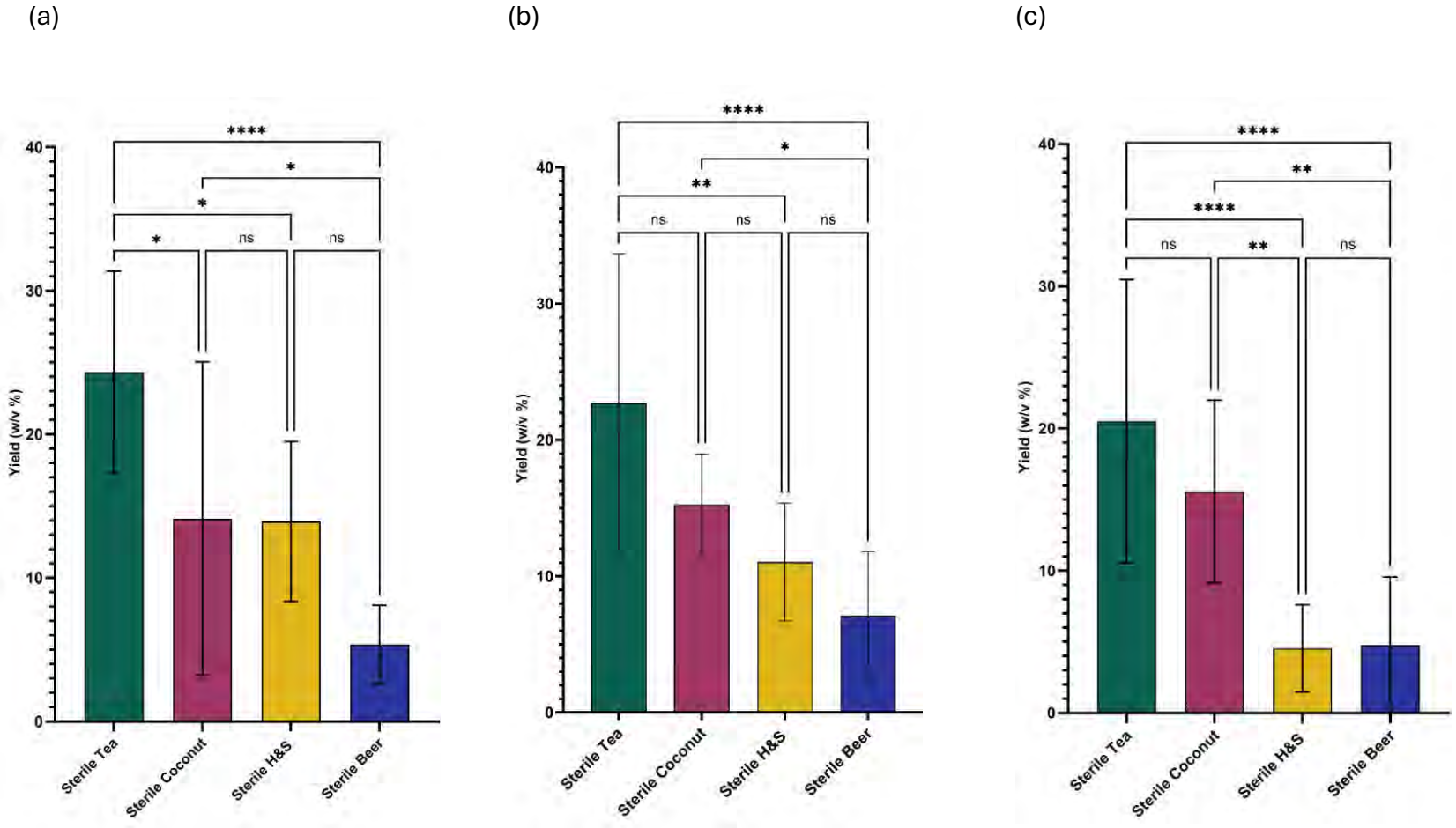


Figure 5. Mean Pellicle Wet Weight Yield in Sterile Media Incubated for (a) 27 days, (b) 34 days, (c) 42 days.

(n = 10 for all media, error bars = standard deviation, ns = not significant, * = p < 0.05, ** = p <0.01, **** = p < 0.0001)

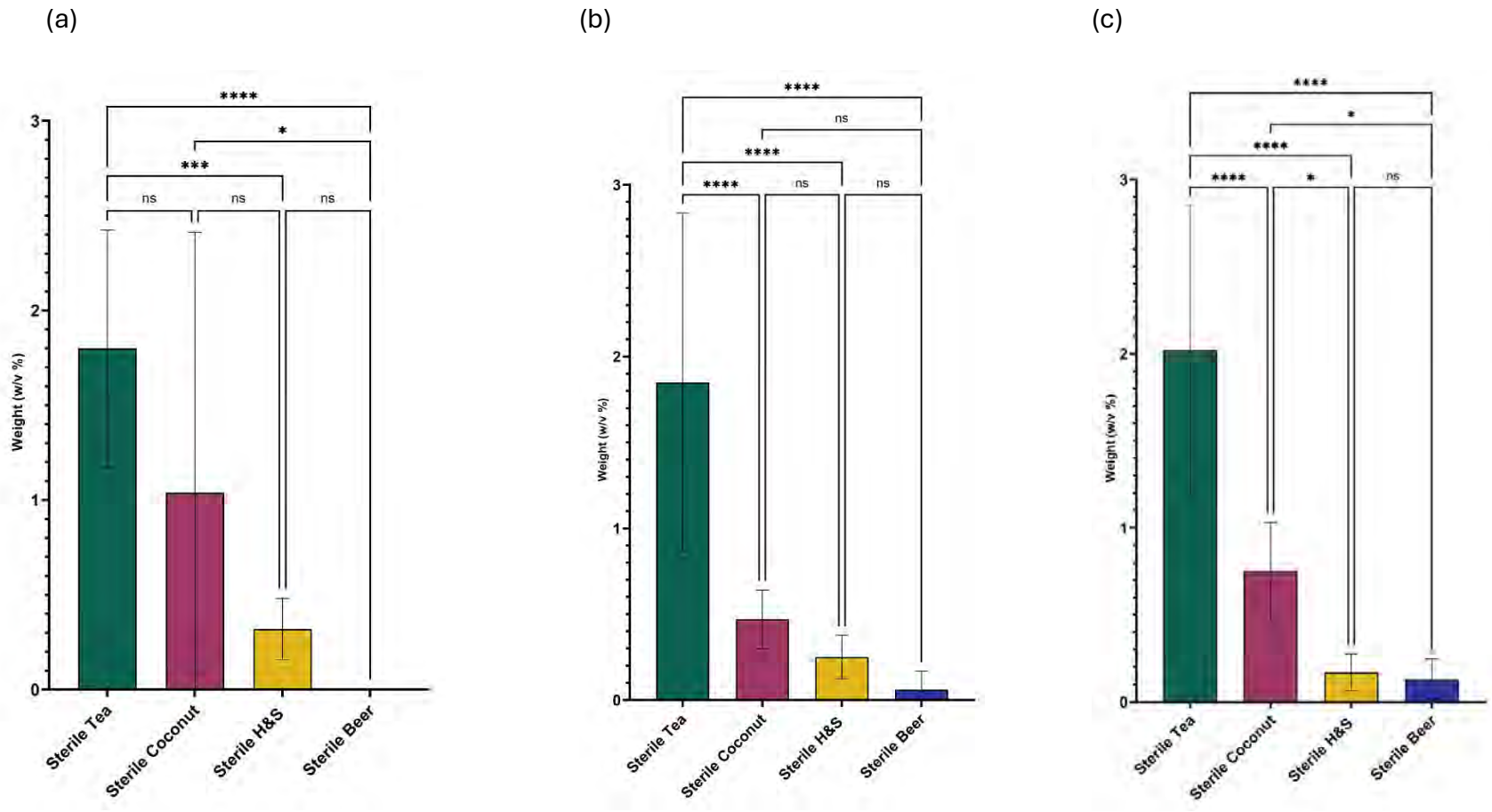


Figure 6. Mean Pellicle Dry Weight Yield in Sterile Media Incubated for (a) 27 days, (b) 34 days, (c) 42 days.

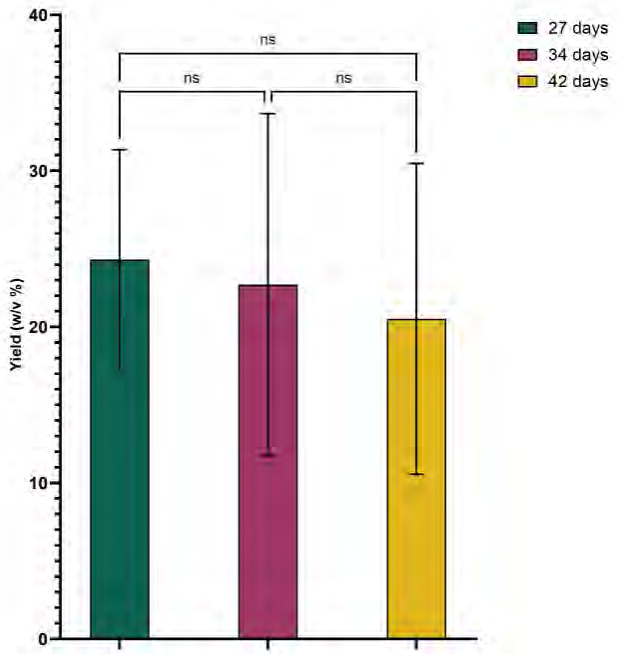
(n = 10 for all media, error bars = standard deviation, ns = not significant, * = p < 0.05, **** = p < 0.0001)

In concurrence with pellicle thickness findings, whilst initial observations suggested a trend of slight increase in sterile tea pellicle weight yield over time, statistical analysis revealed this increase was not significant (Figure 7).

It is important to note that in some of the literature examined which measured microbial activity and BC pellicle development in sweetened black tea, the tea liquid had not been autoclaved prior to inoculation; Marsh et al (2014) suggest the sterilization process causes a build-up of toxins in the tea that can negatively impact microbial community activity. Nguyen et al (2008) concur and postulate that an increase in antimicrobial polyphenols present in the tea due to sterilisation could inhibit pellicle growth. However, the experimental work discussed in this thesis contradicts this view, with results suggesting that sterile tea yields significantly higher and more reproducible pellicles. Conversely, it could be argued that an unsterile medium leads to an inadequately controlled environment for the study of community changes (due to potential contaminants and lack of control of growing conditions) and thus pellicle development.

Differences in the wet and dry pellicle yields was pronounced, irrespective of media type or sterile vs non sterile conditions (Figure 5, Figure 6). The reduction in weight yield from wet to dry was in the region of ten-fold, suggesting that the pellicles are capable of absorbing large volumes of moisture during the development process concurring with the findings of Dufresne (2012) and Rebelo (2018) who have commented on the high water retention capacity of BC pellicles.

(a)



(b)

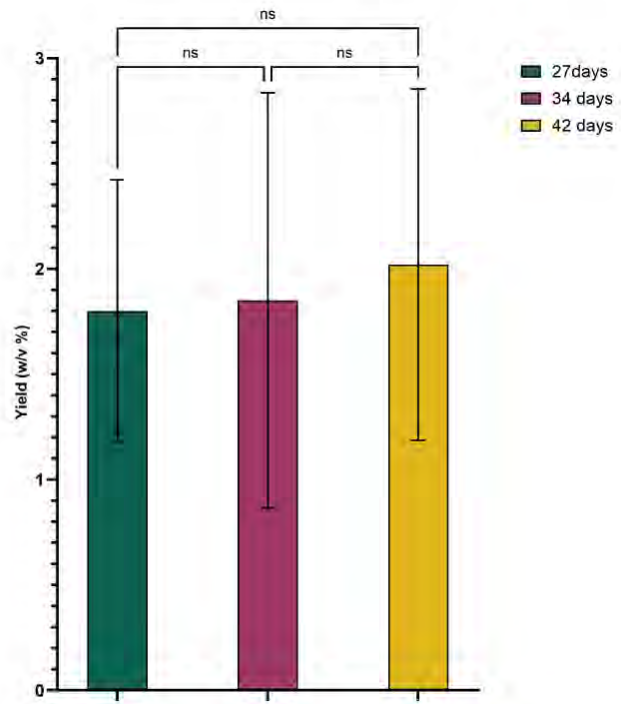


Figure 7. Mean Pellicle (a) Wet and (b) Dry Weight Yield in Sterile Tea Medium Over Incubation Time.

(n = 10 for all timepoints, error bars = standard deviation, ns = not significant)

Sterile black tea produced the highest wet and dry weight yields of pellicle ($p < 0.05 – 0.0001$) across all incubation times. Yim et al. (2017) suggested that the nitrogen content of the tea broth supports the development of pellicles. Whilst Marsh et al. (2014) and Nguyen et al (2008) acknowledge that sterilisation can produce toxins in tea that could inhibit pellicle development, they concede that aseptic conditions are preferable for BC development because the presence of contaminants is likely to affect BC yield. Sterilisation has not had a negative effect on BC pellicle development in the preliminary experiments conducted as part of this thesis.

Pellicles developed in sterile black tea medium displayed the highest variability in weight yield of the pellicles ($\sigma = 7 – 11$ w/v %). It is difficult to find evidence to explain this in terms of the growth medium; the sterile tea was prepared for this experiment in one batch and sterilisation would provide a degree of homogeneity to the medium.

Sterile H&S medium produced a significantly lower ($p < 0.00001$) pellicle yield than black tea. Whilst the yeast extract and bactopeptone added to the H&S medium supply a nitrogen source to support the pellicle development, and glucose provides a carbon nutrient source, there are no known inhibitory components in this broth. It is therefore difficult to conclude why lower weight yields were produced by this medium. H&S broth is manufactured using laboratory grade ingredients, but, due to the inclusion of yeast extract, this medium is complex (and not defined), therefore cannot be reproducibly manufactured. However, in a similar way to the tea medium, H&S medium was produced and sterilised in one batch for this experiment.

Sterile beer medium consistently produced the significantly lowest ($p < 0.0001$) yield compared to tea across all incubation times (Figure 5, Figure 6) and thus was excluded from further study. The reasons for poor pellicle production were not clear. As discussed in section 3.3.5, the presence of alcohol (specifically ethanol) has been documented as key for the support of the microbes in Kombucha responsible for producing BC. In a Kombucha SCOBY, it is normally the role of yeasts in the community to convert any sugars present into alcohol. The beer used in this part of the study contained 3 % alcohol; Lu et al. (2011) suggest that up to 1% alcohol added to the growth medium can improve BC yield when using a pure culture inocula. However, Naritomi et al. (1998) postulate alcohol concentrations more than 1.5 % inhibit BC production. It could be speculated that with alcohol already present in the beer liquid medium, the alcohol content was too high when the yeasts in the Kombucha SCOBY began to produce alcohol and microbial activity was inhibited and therefore BC growth.

Sterile coconut medium wet and dry pellicle weight yields were not significantly higher ($p > 0.05$) than H&S (except for 42 days). However, as they were significantly lower ($p < 0.0001$) than sterile tea at 34 and 42 days (Figure 5, Figure 6) and exhibited high variability in pellicle weight (Wet: σ = 3.7 – 1.9, Dry: σ = 0.2 – 1.4) they were discarded at this stage from further investigation.

3.6 Summary

- A survey of the literature to find the incubation conditions used to develop consistent, high yield pellicles was inconclusive. Whilst there was some agreement for incubation temperature and time, there were conflicting views presented regarding growth media and inoculum types. Therefore, a selection of media was chosen for further laboratory exploration, based on frequency of use in the literature (tea), range of food based (beer, coconut) and lab manufactured (H&S) liquids. Due to ease of access, Kombucha was used for the inoculum.
- Liquid media prepared under sterile conditions produced the highest, most significant yield by weight of BC pellicles, concurring with reviewed literature.
- Pellicles developed in coconut liquid displayed a large degree of variability in yield. As one of the aims of this research is to select a growth medium to support reproducible pellicle development, coconut water was excluded from further investigation.
- Beer liquid displayed a degree of pellicle formation, but to a lesser extent than the other liquid media investigated and thus was excluded from further investigation.
- Sterile black tea liquid produced the highest yields by weight of BC pellicles compared to other liquid media studied and approximately ten times greater yield than its non-sterile counterpart.
- Pellicle yield in black tea was the most variable (displayed the highest standard deviation) compared with those of the other liquid media.

- H&S broth produced the lowest BC pellicle yield across all incubation times. However, H&S could be considered the most consistent medium to support BC development because the dry pellicles developed showed the least amount of variability in weight (σ = 0.1 – 0.2 w/v %).
- No significant differences in incubation time (27 – 42 days) or conditions (room temperature vs 30 °C) were observed.

This preliminary work confirmed the production of pellicles in both laboratory-manufactured and food-based liquids using a Kombucha inoculum. Whilst it is difficult to explain the variation in yield across the different media, it should be noted that this may be simply due to the small numbers of samples (n = 10 per variant) studied. A study of a larger sample set may enable further evaluation of variability. Equally, the universal pots used in this study produced small pellicles; the use of larger vessels would produce larger pellicles (due to the increased surface area of the liquid medium in the pot) in which variability may be easier to evaluate. Since sterile black tea produced the most significantly highest yield and H&S broth produced pellicles with the least weight yield variability, with an incubation duration of 30 days at 30 °C, these parameters were chosen to support further exploratory work.

Chapter 4: The Reproducibility of the Bacterial Cellulose Pellicle Using Community Sequencing

4.1 Introduction

Chapter two investigated the conditions under which BC pellicles can be most efficiently and reproducibly developed, translating findings from the literature review into appropriate laboratory methodology.

Using Kombucha SCOBY as an inoculum, the preliminary laboratory work revealed that the highest pellicle yield by weight was produced using sterile black tea and sugar as a growth medium, albeit with the largest yield variability. Whilst a lower yield was produced by sterile H&S medium, this medium produced pellicles with less yield variability than those in black tea. Therefore, sterile black tea and H&S medium were used for further study in this section of the thesis.

As the H&S medium is more 'defined' than the black tea (it is produced using standard laboratory grade chemicals - bactopeptone and glucose – section 3.2.3) it can be controlled to a greater degree, thus limiting variability in pellicle development. As noted in previous chapters of this thesis, a degree of complexity is introduced to the medium via the use of yeast extract as a nitrogen source to support growth (Schramm et al., 1957). Additionally, the Kombucha inoculum itself is not defined, it can also be subject to a great deal of variability depending on geographical locations and conditions under which it is produced, as discussed by many researchers such as Jarrell et al. (2000), Antolak et al. (2021) and Abaci et al. (2022). This is further discussed in section 3.3.3, and is purported to affect

pellicle development and consistency. Whilst earlier work in this thesis showed H&S medium produces consistent pellicle yield, and black tea with sugar the greatest yield, the next stage of this thesis is to assess the effects of inoculum type (Kombucha vs pure culture) on pellicle production.

Section 3.3.4 discussed the diversity of the Kombucha SCOBY, highlighting the community of bacteria and yeasts found in the Kombucha BC pellicle. Many studies have explored this community in its entirety (Dutta and Gachhui, 2006; Marsh et al., 2014; Zhang et al., 2017; Mas et al., 2022), or with specific focus on either the bacterial (Yamada et al., 2012) or the yeast (Janke et al., 2004) communities and their specific attributes. There have also been specific studies to identify any differences found in the Kombucha liquid compared to that of the solid pellicle. Reva et al. (2015) found that microbial diversity was less in the solid phase than in the liquid and postulated that more investigation was needed to evaluate the core microbial species that are responsible for pellicle formation. Nevertheless, there is agreement in the literature that it is the *Komagataeibacter* genus that is the most prolific producer of BC (Dufresne and Farnworth, 2000; Iguchi et al., 2000; Yamanaka and Sugiyama, 2000; Dufresne, 2012), thus is essential in a Kombucha pellicle.

A key focus of this project is to establish the reproducibility of the BC pellicle, with a view to creating a sustainable textile material. A potential factor affecting the sustainability of the pellicles is the ability to sub-culture; that is to produce subsequent pellicles from an initial inoculum. Marsh et al. (2014) suggest that changes in microbial communities (in Kombucha) can affect pellicle development. Additionally, as mentioned in section 3.3.2 previous studies have used either growth media (liquid) or pellicles (solid) as inocula (Jarrell et al.,

2000; Marsh et al., 2014); none to date have compared the effectiveness of either inoculum in terms of BC yield, or the effectiveness of either method to reproduce subsequent pellicles. Some of the work in this chapter is also described in a peer-reviewed paper (Wood et al., 2022) (Appendix 1).

The reproducibility of BC in terms of microbial profile was evaluated using either a pure culture (*K. xylinus*) or a Kombucha SCOBY as an initial inoculum, in both solid pellicle and liquid form, and the effects on both yield and subculture over a series of three generations was observed. Changes in microbial communities over the three generations were assessed using high throughout sequencing techniques to establish the effects that any changes may have on pellicle development and yield. Additionally, the effects of subculture and inoculum on the structure of the BC nano-fibrils which form the pellicle were observed using scanning electron microscopy (SEM), to visualise notable differences which may affect the exploration of pellicle use (discussed later in this thesis).

4.1.1 Community Sequencing

Polymerase chain reaction (PCR) sequencing methods can be used to observe microbial communities without the need for culturing, thus allowing deeper insight into the community constituents (Harrison and Curtin, 2021). More specifically, 16S rRNA gene sequencing is used to observe bacterial communities. Genetic information in a cell is encoded in deoxyribonucleic acid (DNA). In a cell, DNA is usually wound into a double helix in a specific pattern of nucleic acids, which can be read by the cell's apparatus as an

instruction to build proteins using amino acids as the ‘building blocks’. The cellular apparatus that undertakes this task is known as a ribosome and is made of ribonucleic acid, termed RNA. These ribosomes are present in both microbial domains of life; prokaryotic (bacteria) and eukaryotic (fungi and yeasts) microorganisms. The rRNA in the bacterial cell is known as 16S rRNA. The rRNA present in eukaryotic microorganisms is known as 18S rRNA.

The gene that encodes rRNA in prokaryotic and eukaryotic microorganisms can be described as a ‘marker gene’. The gene is universal in all microorganisms, and almost identical (highly conserved) between different species. However, on the rRNA gene there are regions that are unique to a particular species (hyper variable regions). Knowing the sequences of these hyper variable regions allow researchers to identify microorganisms to species level (Sambo et al., 2018), and knowledge of all 16S genes in a mixed-species sample allows identification of different microorganisms at the same time (i.e. identify microbial community).

PCR, which will replicate a specific region of DNA, involves various stages. Firstly, the DNA is isolated from the individual cells (usually using an isolation kit or heat to break open cells). Once extracted, the DNA is mixed with primers and a PCR ‘mastermix’ which comprises thermostable DNA polymerase, deoxynucleotide triphosphates (dNTPs), and $MgCl_2$ in a buffer solution. Primers are short pieces of DNA that are complementary (i.e., it matches) to the gene of interest (known as the template). Primers are used in pairs, a ‘forward’ and ‘reverse’ – usually matching a short piece of DNA on either side of the template. For 16S analysis, these primers are designed to match two conserved regions within the gene, with

a hypervariable section in between. There is a wide choice of primers (Klindworth et al., 2013; Sambo et al., 2018), from which to select the most appropriate to consider the taxonomic coverage required because they will only attach to rRNA strands that are a perfect match to themselves and thus the accuracy of the process rests with primer selection (Kuczynski et al., 2012).

To allow this process to occur, the original double stranded DNA must first be split into single strands using heat (known as denaturing), breaking the existing hydrogen bonds between strands. This enables the primers to bind to the complementary sections of highly conserved template. Once the primers are attached, taq polymerase is added, allowing an extension phase to occur (Janssen, 2006). This process is repeated upwards of 20 times to allow exponential production of template DNA.

Once the PCR process is complete, there are many millions of copies of target DNA – which in the case of 16S, are copies of a hypervariable (and therefore species-unique) region. For this to be useful as a taxonomic tool, the order of nucleic acids must be known. This is achieved by a process known as 'sequencing'.

There are numerous technologies used to sequence and understand genetic information. These can be split into three broad categories: 1) Sanger Sequencing, 2) Shotgun, Short-Read or Next-Gen Sequencing, or 3) Long-Read Sequencing. Each has their own benefits and drawbacks, and there is much debate as to the most appropriate sequencing technology/platform for any sequencing task. However, for 16S sequencing of a microbial community, Short Read/Shotgun Sequencing has become most common, its value in data

collection for microbial communities being highlighted in studies such as the Earth Microbiome Project (Thompson et al., 2017). These copies of template DNA produced by PCR can be large, and first must be cut into random, shorter pieces of DNA. During shotgun sequencing, the template DNA must first be randomly cut up or shattered (Figure 8).

In order to know which DNA belongs to which sample, adapter sequences are then added and attached to a device known as a flow cell, which acts as the location for the remainder of the sequencing activity. For the sequencer to define the sequence of nucleic acid bases, the PCR process occurs once again, adding fluorescent bases one-by-one, creating clusters of the original shattered template. As each new base is added, the machine will fire a laser, emitting a fluorescent light which is recorded as its corresponding nucleic acid base. Once all the shattered DNA has been recorded, this information is entered into a programme (bioinformatics) which looks for overlapping segments of the shattered DNA sequences and assembles them back into their original length (Figure 8).

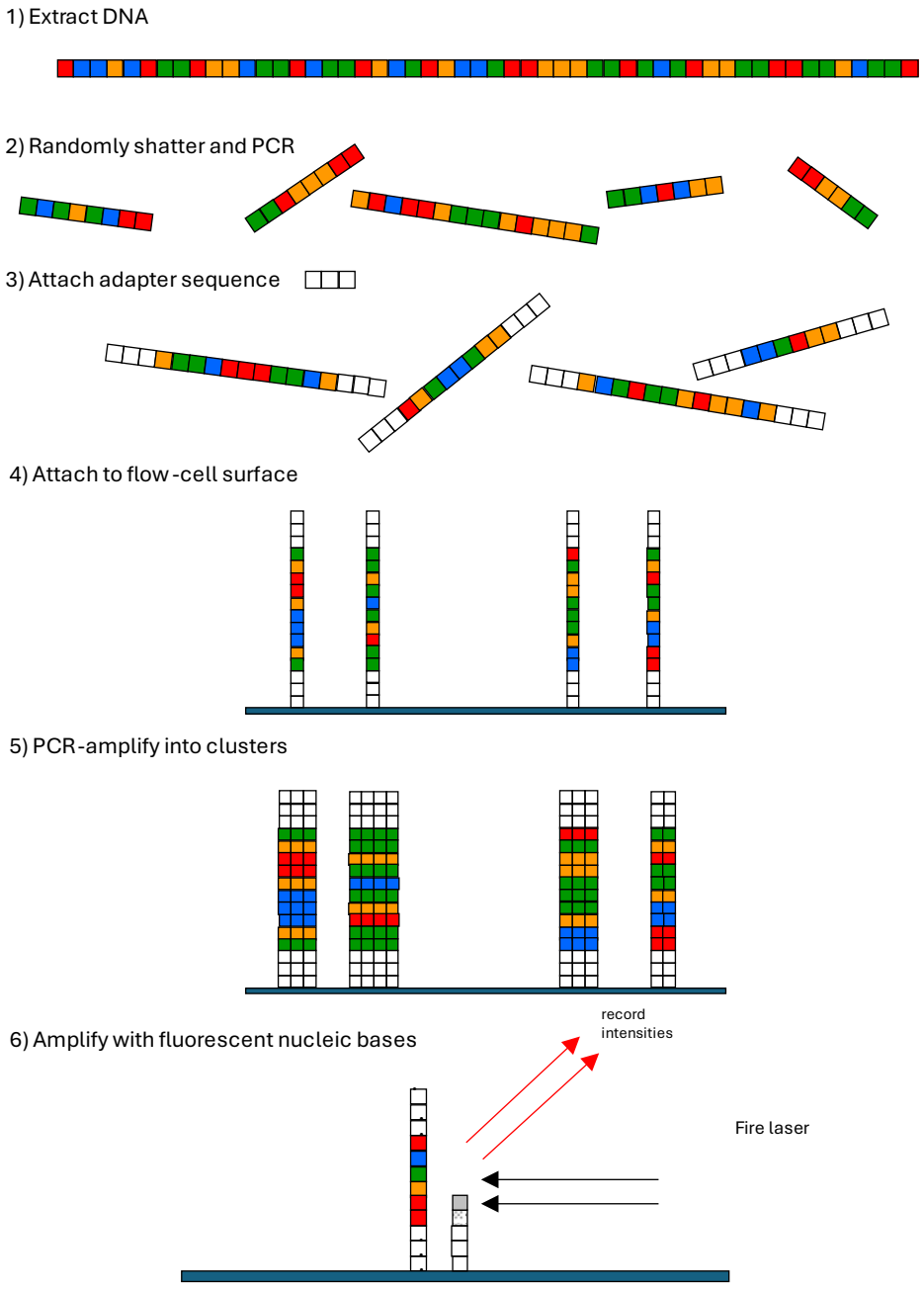


Figure 8. Step-By-Step Schematic of Next Generation / Shotgun Sequencing, Showing the Attachment of Adapters to the Template DNA, PCR Amplification and Identification of Nucleic Acid Bases.

(credit James Redfern, Manchester Metropolitan University)

Researchers suggest that the development of more recent high throughput sequencing equipment (e.g. Next-Gen and Long-Read), whilst bringing down the cost of sequencing, can also lead to an increase in inaccuracies of data (Medlar et al., 2014). It is therefore important to minimise these errors by the utilisation of efficient primers for amplification. The process of PCR 16S rRNA or marker gene analysis is faster and more cost effective than sequencing the whole genome and is often performed when many samples containing multiple species or unknown communities are to be studied (Aßhauer et al., 2015). However, other studies suggest that there are drawbacks to this type of analysis due to 'inherent differences generated in community profiles when sequencing different hypervariable regions, short read lengths and taxonomic classification difficulties due to limited resolution for closely related species' (O'Callaghan et al., 2021).

Nevertheless, the study of 16S rRNA is considered one of the most insightful when investigating microbial biodiversity, with many studies using this approach when evaluating microbial communities (Janssen, 2006; Klindworth et al., 2013; De Vrieze et al., 2018). Additionally, as the 16S rRNA marker gene has been widely studied, several large databases of reference sequences now exist, such as Greengenes (Desantis et al., 2006), SILVA (Pruesse et al., 2007) and the Ribosomal Database Project (Cole et al., 2009).

The process described above focusses on 16S rRNA, however an identical process (albeit using different primers) can be used to identify 18S rRNA and therefore gain information on the yeast communities present in a sample.

4.1.2 Kombucha Community Sequencing

In the study of the microbial species present in the Kombucha SCOBY community, 16S and 18S rRNA identification has been conducted using various extraction methods and primers (Marsh et al., 2014; Reva et al., 2015; Arıkan et al., 2020; Harrison and Curtin, 2021). Table 12 provides an overview of some of the approaches.

Table 12. Extraction Methods and Primers for 16s and 18s rRNA Extraction.

Source	16s Primers		18s Primers		Extraction Method	Dataset Used
	Forward	Reverse	Forward	Reverse		
Marsh et al. (2014)	F1	R5	ITS 1F	ITS 2R	Powerfood Microbial DNA Isolation Kit	SILVA
Reva et al. (2015)	27F	1494R	NL1	NL4	innuSPEED Bacteria / Fungi DNA Isolation Kit	Greengenes 16s
Arikan et al. (2020)	F5	R5	ITS 1F	ITS 2R	DNeasy PowerFood Microbial Kit	SILVA
Harrison &. Curtin (2021)	926F	1062R	F5	R5	DNeasy PowerFood Microbial Kit	Greengenes 16s

The Earth Microbiome Project (EMP), founded in 2010, is a collaborative program using crowd-sourced samples and DNA sequencing techniques to map patterns of microbial ecology across the planet (Thompson et al., 2017). Using shotgun sequencing techniques, thousands of samples were analysed, resulting in a large, curated dataset of 16s and 18s

rRNA amplicon data which enables the differentiation of microbial and yeast communities. The study used the primer pair 515F / 806R for 16s and 1391F / 1510R for 18s because these pairs have been reported as the best available for 16s and 18s rRNA study (Eloe-Fadrosh et al., 2016).

4.2 Experimental Details

4.2.1 Starter Culture: Single Strain Isolate

Komagataeibacter (formerly known as *Gluconacetobacter*) genus (part of the acetic acid bacteria family) is responsible for the production of BC nanofibrils (see section 3.2.1). Whilst several species of this genus (such as *K. hansenii* and *K. aceti*) have been studied in the literature, it is commonly agreed that *Komagataeibacter xylinus* is the most prolific producer of BC. Consequently, *Komagataeibacter xylinus* (ATCC 23767) (previously referred to in the literature as *Gluconacetobacter xylinus*) was purchased from the American Type Culture Collection (ATCC).

The *K. xylinus* was stored in cryopreservative solution [3.6 % KH_2PO_4, 12.6 % K_2HPO_4, 0.9 % $Na_3C_6H_5O_2$, and 1.8 % $(NH_4)_2SO_4$] at -80 °C. When required, the strain was grown on H&S agar, incubated at 30 °C for 24 hours and used to inoculate a liquid culture medium (section 4.2.3.1) to produce a working liquid *K. xylinus* inoculant. The liquid inoculant was stored in a incubator at 30 °C for 24 hours prior to use.

4.2.2 Starter Culture: Kombucha SCOBY

A Kombucha SCOBY was purchased (Happy Kombucha 2023) (KSC) and stored as per manufacturer's instructions (as described in section 3.5.1) until required. The KSC was stored in a sealed container, in tea broth, in ambient conditions as per the manufacturer's instructions.

4.2.3 Development of Pellicles

4.2.3.1 Liquid Medium

A H&S culture medium was produced by dissolving glucose (2 %), Bactopeptone (0.5 %) and yeast extract (0.5 %) in distilled water (Schramm and Hestrin, 1954b). The solution was autoclaved for 10 min at 115 °C (a lower temperature than standard autoclaving of microbiological media to prevent caramelization of the glucose) and allowed to cool to room temperature before use.

4.2.3.2 Inoculation and Generational Subculture

Sterile 25 mL universal tubes containing 10 mL H&S broth were inoculated with either 1 mL *K. xylinus* or 1 g KSC pellicle. The tubes were covered with loosely fitted lids (to allow aerobic conditions whilst minimizing particulate contamination) and placed in a 30 °C incubator for 10 days. This was labelled generation zero (G0). After 10 days, pellicles had formed on the surface of the broth; these were removed and aseptically equally split into two. One half was frozen at -80 °C for DNA extraction and analysis and the other half used as the inoculum for

generation 1 (G1P). Further G1 cultures were prepared using the broth produced in the G0 phase as the inoculum for fresh H&S broth (G1L). Each inoculation was prepared in duplicate, thus resulting in eight new cultures inoculated from either the liquid or pellicle phase of the communities started by either the *K. xylinus* or KSC (Figure 9). This process was repeated to produce subsequent generations, labelled G2 and G3.

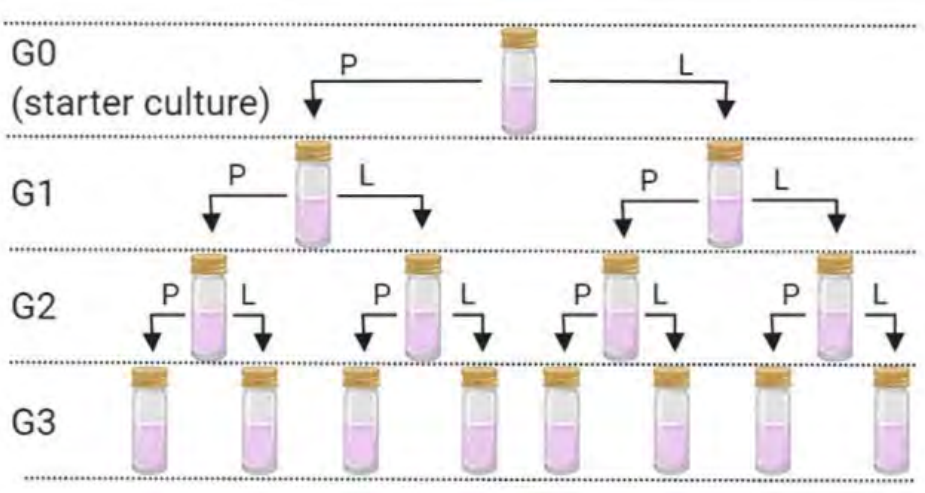


Figure 9. Generation Overview and Sampling Scheme.

P = use of the pellicle for inoculum; L = use of liquid phase for inoculum. This process was followed for each of the two starter cultures. Following G0, each inoculation was carried out in duplicate. It should be noted that liquid inoculated samples were discarded and not measured at generation 3.

4.2.4 Physical Analysis of Samples

At the end of each generation, the thickness of each pellicle (mm) was measured using a ruler before removal from the pot. Thicknesses were recorded and the pellicles photographed before and after removal from the pot, noting visual observations such as colour and opacity / transparency.

One sample of each pellicle (approx. 9 mm^2) were prepared for scanning electron microscopy (SEM) analysis by immersing each sample in 0.1 M glutaraldehyde overnight (to fix and preserve the cell structure), removing, and then dehydrating by passing through sequential ethanol baths (10 min per bath) of increasing concentrations (50 %/70 %/80 %/90 %/99.9 %). The samples were kept in a desiccator until ready to be viewed on the SEM (Carl Zeiss Ltd., model Supra 40VP). The samples were sputter coated with gold before viewing and six images per pellicle taken on the SEM, at x 20k, x 10k, x 5k, x 2.5k, x 1k, x 500 magnification.

4.2.5 Genetic Analysis of Microbial Communities

Nucleic acid extractions were carried out using the DNeasy PowerSoil kit (QIAGEN Ltd., Manchester), cleaned using the ZR-96 DNA Sequencing Clean-Up kit (Zymo Research, United States), and quantified using the Qubit dsDNA high sensitivity assay kit (Invitrogen, Paisley).

Amplicon sequencing of bacterial ribosomal rRNA genes (16S rRNA) was undertaken, as previously described (Thompson et al., 2017). Briefly, PCR reactions for initial amplifications

consisted of 5 µl NEB Q5 reaction buffer, 0.25 µl NEB Q5 High-Fidelity DNA Polymerase, 0.5 µl NEB X nM dNTPs, 15 µl DNA template (c. X ngµl−1), and 0.5 µl X pM primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806_R (5'-GGACTACNVGGGTWTCTAAT-3') or EUK1391_F (5'-GTACACACCGCCCGTC-3') and 1510_R (5'-CCTTCYGCAGGTTCACCTAC -3') for 16S and 18S rRNA genes, respectively, and made up to a final reaction volume of 25 µl with nuclease-free water. Cycling conditions comprised an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C for 60 s, and extension at 72 °C for 90 s, with a final extension at 72 °C for 10 min. Amplification was confirmed visually by 1.5 % (w/v) Tris-acetate-EDTA (TAE)-agarose gel electrophoresis. A second-stage PCR was carried out to attach barcodes for Illumina sequencing. The constituents of the second-stage PCR reaction are as follows: 10 µl NEB Q5 reaction buffer, 0.5 µl NEB Q5 High-Fidelity DNA Polymerase, 1 µl NEB (X nM) dNTPs, 0.5 µl (X pM) forward primer, 0.5 µl (X pM) reverse primer, and 20 µl cleaned amplicon template, and they were made up to a final reaction volume of 50 µl with nuclease-free water. Cycling parameters comprised an initial denaturation at 98 °C for 30 s, followed by 10 cycles of denaturation at 98 °C for 10 s, annealing at 62 °C for 20 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 2 min. Amplification and attachment were confirmed by 1.5 % (w/v) TAE-agarose gel electrophoresis.

Following second-stage PCR, all amplified DNA strands were normalized using the SequalPrep Normalization kit (Fischer Scientific, Loughborough) and pooled into libraries for sequencing on a MiSeq Illumina platform with a MiSeq Reagent Kit v2 (300-cycle) flow cell.

4.2.6 Data Analysis

Amplicon sequence variants (ASVs) were extracted from the raw sequence data using the dada2 pipeline (Callahan et al., 2016) using the default parameters. Taxa were assigned using the SILVA database and sequencing information files deposited in the NCBI BioProject database (BioProject PRJNA787576; accession numbers SAMN23827663–SAMN23827813). Relative species abundance in samples was calculated according to the number of reads assigned to the ASVs.

4.2.7 Results and Discussion

4.2.7.1 Pellicle Yield

Across all generations, the KSC pellicles displayed a consistent pale brown / cream appearance, consistent with observations previously documented by earlier studies (Domskiene et al., 2019; Jarrell et al., 2000). The *K. xylinus* pellicles, by contrast, displayed visual inconsistencies in the form of white opaque spots (diameter = 1 mm) in the gel-like structure as illustrated in Figure 10. This has not been previously documented in earlier work, but it is speculated that this could be attributed to either denser areas of formation of BC nanofibrils, or a concentration of extracellular polymeric substances (EPS). Additionally, in early generation samples (G0 and G1), from both inoculum types, the *K. xylinus* pellicles were translucent and difficult to remove from the pots due to the gel-like adherent quality of the pellicles structure. This contrasts with the KSC pellicles, which although were a similar thickness to those produced by *K. xylinus* inocula, were easy to remove from the pots across all generations and maintained structural integrity during removal.

(a) (b)

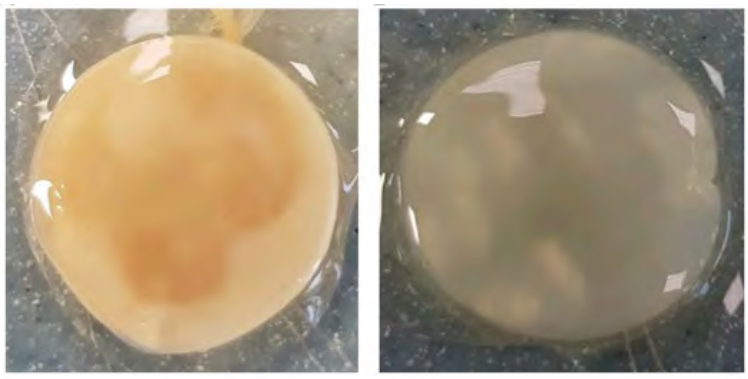
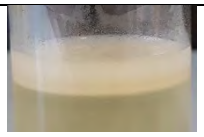
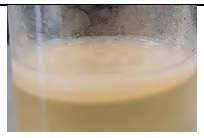


Figure 10. Examples of Pellicles Produced in Generation 2 from (a) KSC and (b) K. xylinus Isolate.

Pellicle diameters = 25mm

Across all samples, pellicle thickness increased from 0.5 mm (σ = 0) observed in generation one, to between 2.3 mm (σ = 0.4) (*K. xylinus)* and 4.1 mm (σ = 1.4) (KSC) in generation 3, despite all the pots being incubated in the same conditions (Figure 11). The trend of pellicles becoming thicker across generations was observed regardless of the starter culture used. Additionally, correlations were made of pellicle thickness in relation to starter culture. Generation one produced pellicles of the same thickness (0.5 mm), regardless of starter culture; in generation two the pellicles of KSC origin were slightly thicker (mean = 2.5 mm) compared to those from the *K. xylinus* starter (mean = 2.2 mm). However, by generation three this difference was more marked with KSC mean pellicle thicknesses of 3.8 mm compared to mean thickness of 2.4 mm in those of *K. xylinus* origin. Furthermore, in generation three, trends could be seen in line with inoculation type; the pots inoculated with

a liquid phase inoculum at any point throughout the process produced thicker pellicles than those produced by solid phase inocula only. A possible explanation for this is that the bacterial communities responsible for BC production are spread more diffusely in the liquid phase and therefore have more access to nutrients; this concurs with the findings of other studies (Dima et al., 2017; Blanco Parte et al., 2020). Additionally, a study by Goh et al. (2012) reported higher microbial counts in the liquid phase than those in solid Kombucha SCOBY; higher microbial concentrations are likely to lead to better cell distribution, higher BC production and thus greater pellicle yield.

Starter Culture	Generation One			Generation Two			Generation Three		
KSC	KP		0.5 mm ($\sigma = 0$)	KP		2.0 mm ($\sigma = 0$)	KP		2.5 mm ($\sigma = 1.5$)
							KL		3.0 mm ($\sigma = 1.8$)
				KL		4.0 mm ($\sigma = 0$)	KP		3.9 mm ($\sigma = 1.3$)
							KL		4.0 mm ($\sigma = 1.6$)
	KL		0.5 mm ($\sigma = 0$)	KP		2.0 mm ($\sigma = 0$)	KP		2.6 mm ($\sigma = 0.4$)
							KL		3.9 mm ($\sigma = 0.9$)
				KL		2.0 mm ($\sigma = 0$)	KP		3.8 mm ($\sigma = 1.8$)
							KL		4.1 mm ($\sigma = 1.4$)

Starter Culture	Generation One			Generation Two			Generation Three		
KXSC	KX P		0.5 mm ($\sigma = 0$)	KX P		3.0 mm ($\sigma = 1.0$)	KX P		2.6 mm ($\sigma = 1.5$)
							KX L		1.8 mm ($\sigma = 0.4$)
				KX L		2.0 mm ($\sigma = 0$)	KX P		2.8 mm ($\sigma = 1.0$)
							KX L		2.3 mm ($\sigma = 0.4$)
	KX L		0.5 mm ($\sigma = 0$)	KX P		2.0 mm ($\sigma = 1.0$)	KX P		2.8 mm ($\sigma = 0.8$)
							KX L		2.3 mm ($\sigma = 0.4$)
				KX L		2.0 mm ($\sigma = 0$)	KX P		2.4 mm ($\sigma = 0.4$)
							KX L		2.5 mm ($\sigma = 0.5$)

Figure 11. Observed Mean Thickness and Images of Pellicles.

KSC = Kombucha SCOBY starter culture; KXSC = *Komagataeibacter xylinus* starter culture; KP = Kombucha SCOBY pellicle; KXP = *Komagataeibacter xylinus* pellicle; KL = Kombucha SCOBY liquid; KXL = *Komagataeibacter xylinus* liquid; σ = standard deviation.

These findings suggest that a Kombucha-derived bacteria and yeast consortium as a liquid inoculum achieve pellicles of greater thickness that those produced from a *K. xylinus* starter culture. In the literature, pellicle weights (either wet or dry) are generally referred to and not pellicle thickness (which is a unique feature of this study), so it is not possible to support this hypothesis with other studies. This experiment did not weigh the pellicles produced to reduce the risk of potential contamination which could affect the sequencing profiles; however, pellicle weight measurement could assist with analysis in any future studies of pellicle yield.

4.2.7.2 Pellicle Structure.

When pellicles were observed under the scanning electron microscope, a mesh of microfibrils that appear random in distribution were observed regardless of starter culture, inoculum phase or generation. The individual microfibrils within the mesh were of comparable size and thickness to each other ranging from mean diameters of 81 mm (+/- 7.8 mm) (n = 20) in *K. xylinus* to 92.5 mm (+/- 6.6 mm) (n = 20) in KSC derived pellicles.

At magnifications less than x 20k, it was very difficult to distinguish differences in the pellicles derived from the different inocula. However, at x 20k magnification BC pellicles developed from *K. xylinus* appeared 'cleaner' than those created from KSC, i.e., they appeared to be made up of nanofibrils with very little other material visible (Figure 12). This was expected, as there was only a single strain of bacteria used as inoculum, compared to the consortium in the Kombucha SCOBY. Additional materials (aside from the nano-fibrils of BC) were observed in the pellicles developed from KSC (Figure 12). It is suggested that

these are extracellular polymeric substances (EPS), which Jefferson (2004) documented as part of the KSC microbial consortium (and therefore are less likely to occur in the single strain inoculated pellicles). Additionally, more microbial cells were visible in the KSC derived pellicles that those created from *K. xylinus*. Again, this is not surprising due to the complex nature of the KSC inoculum compared to that of the pure strain culture. Pellicles derived from *K. xylinus* pure culture appeared smoother and more consistent with nanofibrils of BC clearly visible compared to those derived from KSC; again, this is a likely effect of the diverse nature of the KSC consortium.

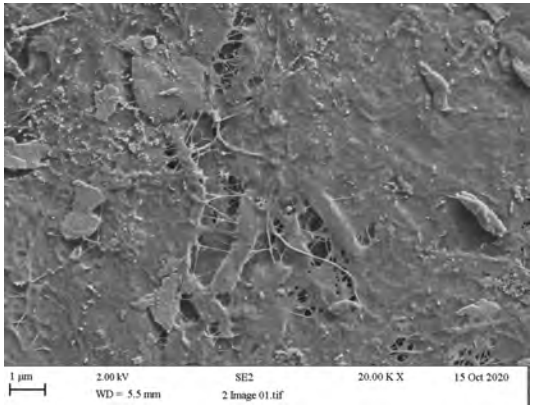
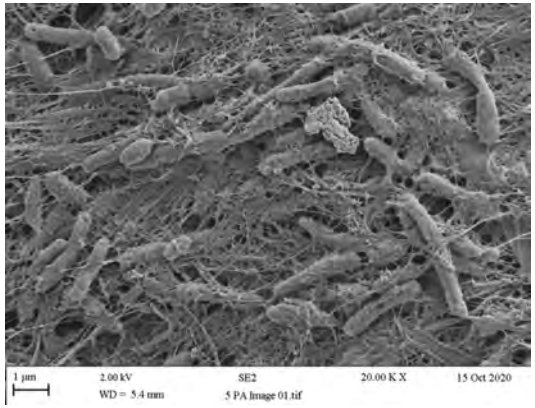
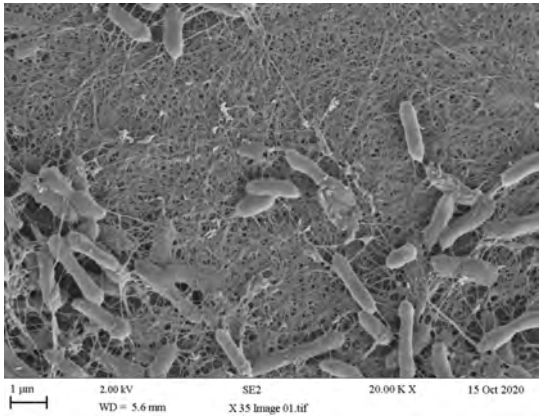
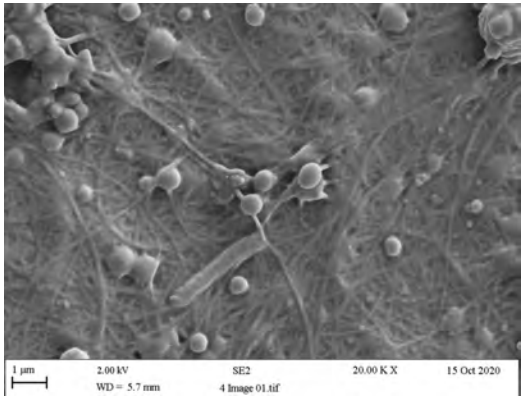
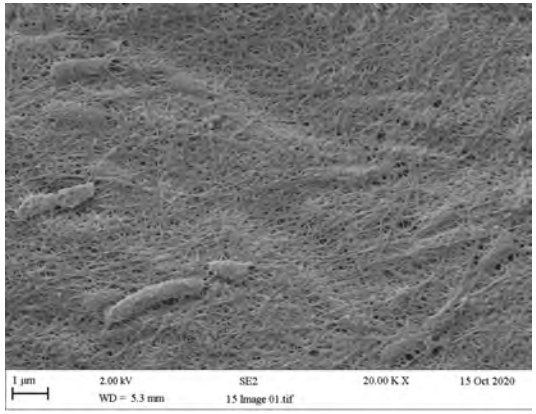
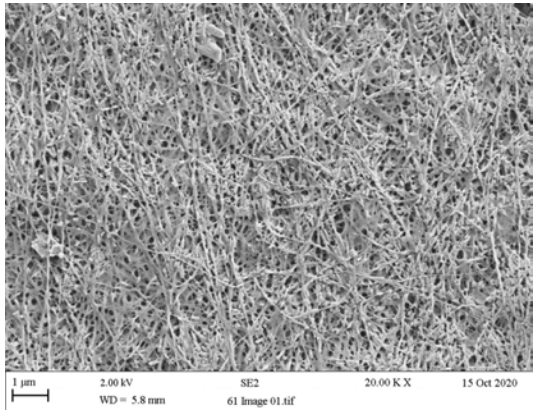
	Generation one	Generation two	Generation three
Kombucha SCOBY (KSC)			
K. xylinus **(KX)**			

Figure 12. SEM Images X 20 K Magnification of Pellicle Development Across Generations.

4.2.7.3 Community observations – bacterial species

Genetic sequencing data revealed a dominance of *Komagataeibacter* genus in all samples, (irrespective of inoculum origin i.e. KSC or KX) liquid and solid, concurring with previous studies (Jarrell et al., 2000; Marsh et al., 2014; Gaggìa et al., 2019) (Table 13, Table 14). Greater diversity was observed in the liquid samples compared to the solid, concurring with previous studies (Goh et al., 2012).

Table 13. Relative Abundancies (%) in Pellicle Samples Across Generations.

	KSC		KX	
	Komagataeibacter	**Others**	***Komagataeibacter***	**Others**
Generation 0	94.06	5.94	99.39	0.61
Generation 1	99.01	0.99	96.94	3.06
Generation 2	81.64	18.36	87.70	12.30
Generation 3	90.71	9.29	94.12	5.88

Table 14. Relative Abundancies (%) in Liquid Samples Across Generations.

	KSC		KX	
	Komagataeibacter	**Others**	***Komagataeibacter***	**Others**
Generation 0	80.56	19.44	83.11	16.89
Generation 1	93.99	6.01	67.43	32.57
Generation 2	78.20	21.8	76.39	23.61

Whilst the dominant genus observed was *Komagataeibacter*, other genera were noted in abundances higher that 1 % (genera occurring at less than 1 % were disregarded due to the low number of amplicon sequence variant (ASV) readings for these types) (Table 15).

Table 15. Relative Abundancies of Genus Types Observed in Pellicle Samples.

	Generation 0		Generation 1		Generation 2		Generation 3	
	KSC	**KX**	**KSC**	**KX**	**KSC**	**KX**	**KSC**	**KX**
Komagataeibacter	94.06	99.39	99.01	96.94	81.64	87.70	90.71	94.12
Streptococcus	-	-	-	-	4.54	4.18	-	-
Carnobacterium	-	-	-	-	2.22	-	-	-
Yersinia	-	-	-	-	2.08	-	-	-
Staphylococcus	-	-	-	-	1.02	1.20	-	-

Table 16. Relative Abundancies of Genus Types Observed in Liquid Samples.

	Generation 0		Generation 1		Generation 2	
	KSC	**KX**	**KSC**	**KX**	**KSC**	**KX**
Komagataeibacter	80.56	83.11	93.99	67.43	78.20	76.39
Streptococcus	3.53	8.97	1.31	8.02	5.46	8.18
Carnobacterium	-	-	-	-	2.42	-
Yersinia	-	-	-	-	2.30	-
Staphylococcus	-	-	-	3.21	1.22	2.01
Bacillaceae	-	-	-	4.39	-	-
Actinobacillus	-	1.32	-	-	-	-
Prevotella	-	1.32	-	-	-	-
Sphingomonas	-	1.32	-	-	-	-
Pseudomonas	-	-	-	3.06	-	-
Haemophilus	-	-	-	1.29	-	1.30
Veillonella	-	-	-	-	-	1.27

Arikan et al. (2020) discovered *Acidobacteria, Actinobacteria, Armatimonadetes, Bacteroidetes, Deinococcus-Thermus, Firmicutes, Proteobacteria,* and *Verrucomicrobia* phyla when using 16S rRNA analysis of Kombucha, as their study did not allow genus classification, but did assign 99% of the sequencing data to the *acetobacteraceae* family. As previously discussed in this thesis, this family contains the bacteria responsible for the most prolific production of BC and it is the one in which *Komagataeibacter*, (the dominant genus found in the study in this section of the thesis) is part.

Previous studies using 16S rRNA sequencing techniques on BC pellicles identified the occurrence of genera such as *Lactobacillus, Lactococcus* and *Enterococcus* (Marsh et al., 2014); Seto et al (2006) suggest that the presence of *Lactobacillus* enhances cellulose production by *Gluconacetobacter* species. However, *Lactobacillus* was not identified in the sequencing in this work. It is documented that the Kombucha SCOBY bacterial and yeast community can vary due to the geographical location and climatic conditions of the SCOBY (section 3.3.3). Studies such as those conducted by Marsh et al., (2014) and Gaggia et al., (2019) also commented on the diversity of the species identified by 16S rRNA sequencing; Table 15 and Table 16 show the relative abundancies of genus types observed per sample in this study. Whilst it is acknowledged that Kombucha SCOBY is a community of bacteria and yeasts and therefore microbial diversity is expected, the number of bacterial species present in the samples generated from the *Gluconacetobacter xylinus* starter were surprising (albeit less diverse than the Kombucha SCOBY). These could be attributed to some contamination of the samples, but also to some of the difficulties experienced with 16S rRNA analysis as described in section 4.1.1.

In the pellicle samples, the highest microbial diversity can be seen by generation two, regardless of starter inoculum (Figure 13). A large spike in microbial diversity is seen in pellicles from both starters in generation two, which then drops down by generation three. This study was conducted using H&S as a liquid growth medium, but in previous studies monitoring the microbial communities in Kombucha tea liquid, Marsh et al. (2014) and Chakravorty et al. (2016), agree that there are critical times in fermentation at which the microbial community is most stable; Chakravorty et al. (2016) suggest that this stabilization occurs after approximately 21 days. It is postulated that the drop in diversity in pellicles grown in H&S liquid medium in generation three is due to community stabilisation, concurring with the findings of Chakravorty et al. (2016) and thus a community stabilisation period in the region of 20 – 30 days is required to observe consistent BC pellicle production. Further work continuing the study to a further generation of sampling is suggested to verify this hypothesis.

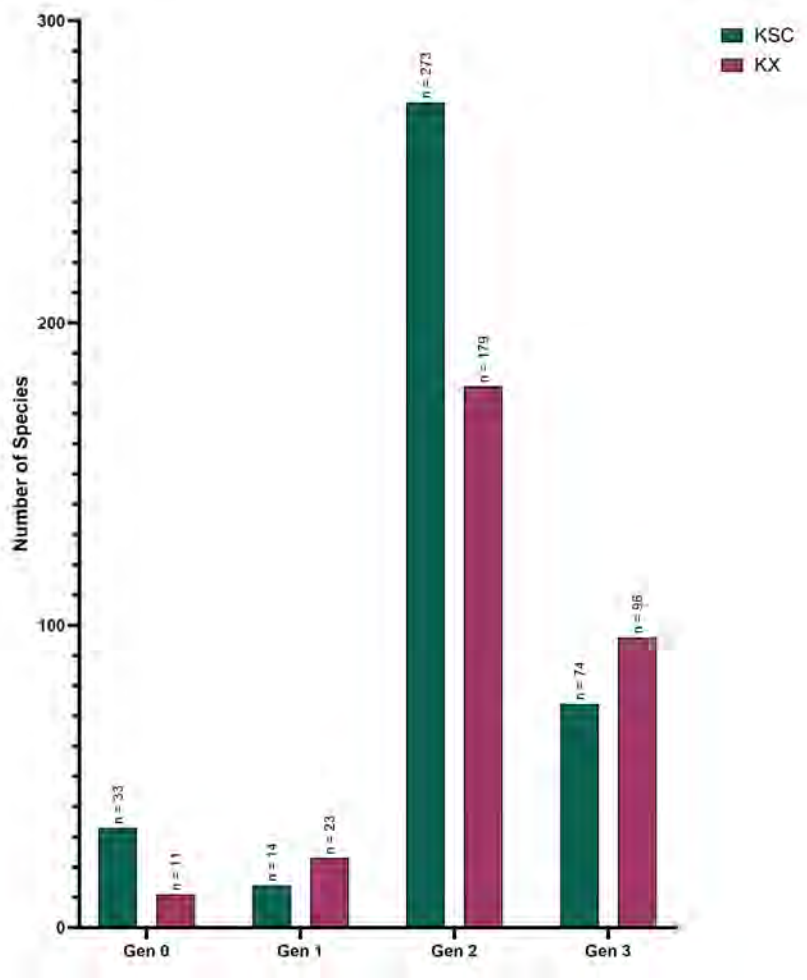


Figure 13. Number of Bacterial Species Found in Pellicle Samples.

KSC = Kombucha SCOBY starter culture; KX = *Komagataeibacter xylinus* starter culture.

In the liquid samples, similar trends to those for the solid pellicles were noted. Microbial diversity continued an upward trend until generation two. No liquid samples were measured in generation three due to the limitations in capacity of sequencing consumables; this study focussed on solid pellicles. It should be noted that more diversity was noted in the genera in the liquid samples (Figure 14) concurring with the findings of Marsh et al., (2014), however, the *Komagataeibacter* genus remained dominant.

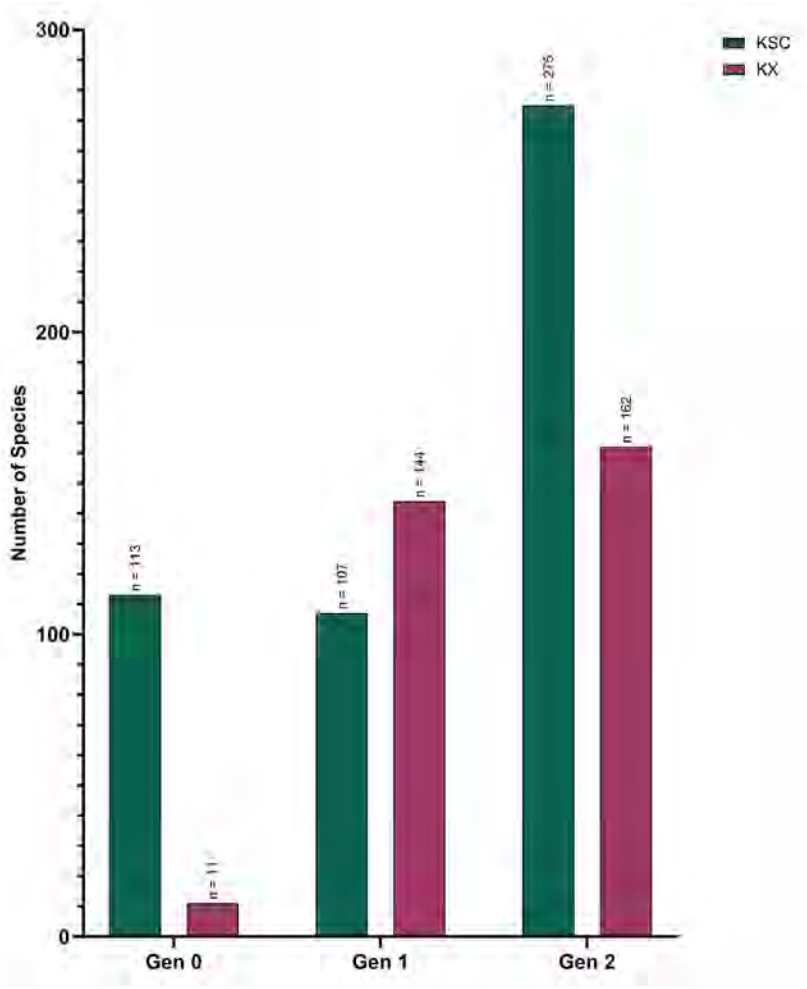


Figure 14. Number of Bacterial Species Found in Liquid Samples.

KSC = Kombucha SCOBY starter culture; KX = *Komagataeibacter xylinus* starter culture

The Bray-Curtis similarity index is used to describe the similarity in species abundance, where zero means the two samples have the same species composition and 1 means there is no similarity of the species in the samples. In this case, the Bray-Curtis index was used to explore the difference in the communities across generations, relative to the previous generation. The rate of species composition change between generations was similar, regardless of starter culture type, demonstrated by the trend lines displayed in Figure 15. The bacterial communities became more similar to the previous generation

(there was less change between generations) as more subculturing took place (as the plot is trending from one towards zero), leading to the hypothesis that the bacterial communities become more stable over time.

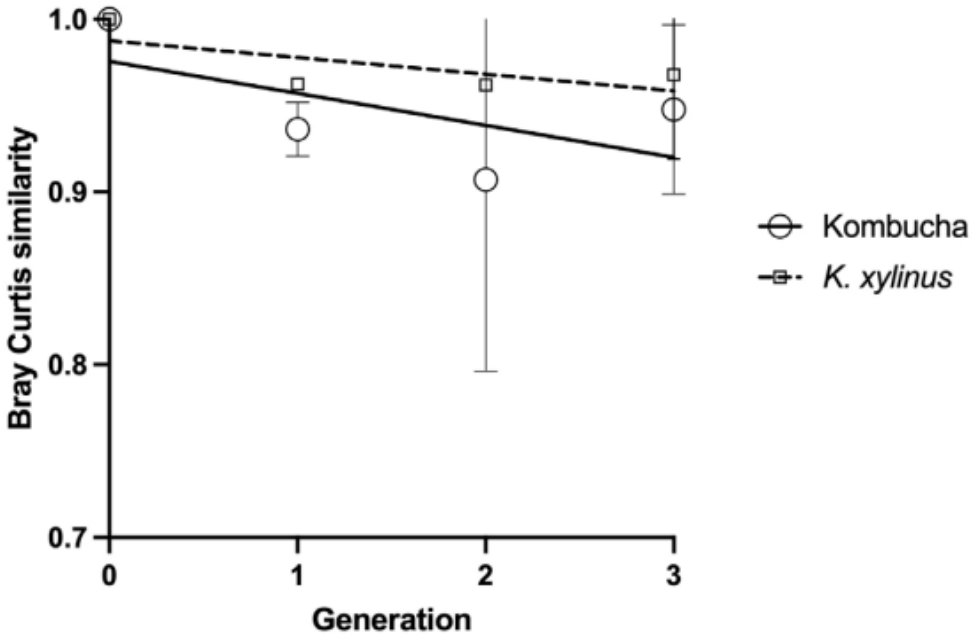


Figure 15. Trend in Changes in Pellicle Bacterial Community Relative to the Previous Generation Using the Bray-Curtis Index.

Using the Bray-Curtis similarity index to analyse the generational shift from the original inoculum (Figure 16), the gradient of the plot from one towards zero is steeper. This suggests there is a more marked drift from the original community across generations of Kombucha derived pellicles than those originating from *K. xylinus*. This leads to the hypothesis that there are species in the microbial community in the Kombucha SCOBY that are becoming more dominant over time. Since an increase in pellicle thickness was

also seen over time, it could be speculated that the dominant species are BC-forming (e.g. *K. xylinus*).

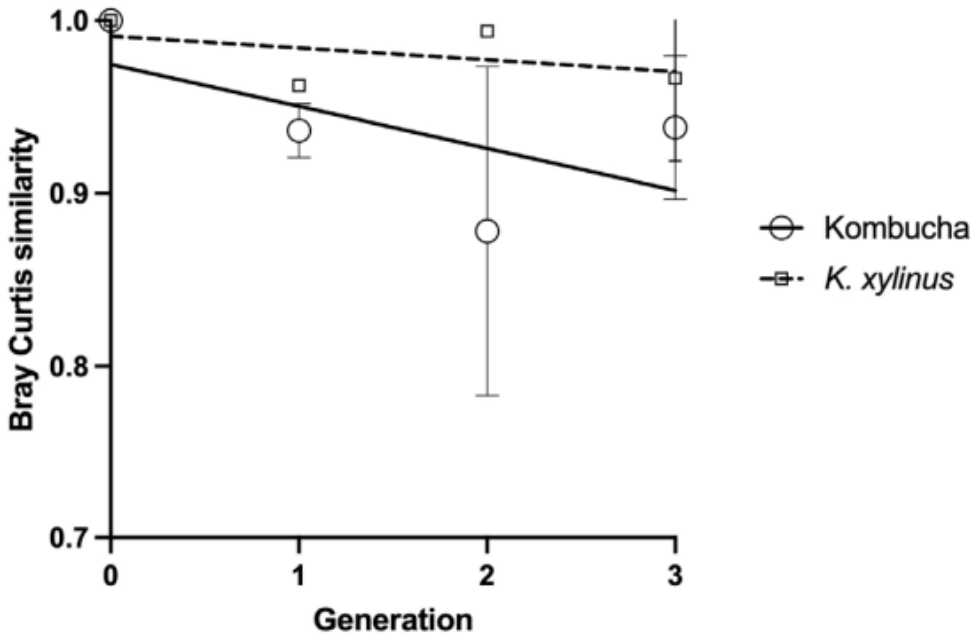


Figure 16. Trend in Changes in Pellicle Bacterial Community Relative to the Original Inoculum using the Bray-Curtis Index.

4.2.7.4 Community Observations – Yeast Species

Malassezia was the dominant yeast species observed in all samples, regardless of starter inoculum (Table 17 and Table 18). Additionally, in the liquid samples, *Candida* was present in all, with *Saccharomyces* present in all but one of the pellicle samples (it was not found in Generation 0 KX inoculated pellicle). In most cases, as expected, there

were more yeast species present in Kombucha SCOBY inoculated samples than those inoculated with pure culture.

In a similar way to the observations made of microbial communities, yeast species present in a Kombucha SCOBY are dependent on the geographic and climatic conditions of the environment. However, there should be no yeasts present in the pellicles of liquid developed from a pure KX starter culture, if handled under sterile conditions, therefore it is speculated that some environmental contamination could have occurred. It is speculated that as *Malassezia* is commonly found on human skin (Boekhout et al., 2010), and was found in both liquid and pellicle samples regardless of inoculum, human skin contamination could be the source of the yeast found in all the samples. However, the largest quantity of *Malassezia* was found in generation 0 samples, and the relative abundance of this species declines over subsequent generations; therefore, the contamination could have already been present in the pure culture sample before its use as an inoculant, rather than poor aseptic lab practice. It can also be postulated that this yeast species begins to be outcompeted by other species as the community finds stabilisation (in a similar way to the microbial communities discussed in section 4.2.7.4).

Other studies have found similar yeast species present in Kombucha SCOBY based pellicles as found in Table 18 such as *Saccharomyces* (Jarrell et al., 2000; Wang et al., 2014) and *Candida* (Teoh et al., 2004; Wang and Baoping, 2013).

Table 17. Relative Abundance (%) of Yeast Types Identified in Liquid Samples.

Genus	Generation 0		Generation 1		Generation 2	
	KSC	KX	KSC	KX	KSC	KX
Malassezia	76.60	87.61	56.02	66.38	63.33	59.20
Alternaria	4.55					
Candida	4.61	1.43	32.39	18.96	11.08	11.60
Cladosporium	2.23	2.45				
Aureobasidium	1.71					
Saccharomyces	1.10		1.82	1.55	5.10	2.47
Debaryomyces	1.10		1.13		2.86	2.55
Occultifur	1.00					
Alternaria		1.71				
Rhizopus		1.35				
Mucor			1.19			
Microglena				6.39		
Helianthus					3.14	9.01
Cladosporium					3.00	
Zymomonas					1.82	2.36
Pythium						2.84

Table 18. Relative Abundance (%) of Yeast Types Identified in Solid Pellicle Samples.

Genus	Generation 0		Generation 1		Generation 2		Generation 3	
	KSC	KX	KSC	KX	KSC	KX	KSC	KX
Malassezia	85.43	87.61	53.70	58.21	66.44	79.29	59.71	71.86
Candida	8.27		9.95	13.61		8.99		1.03
Cystofilobasidium	2.49						7.74	
Saccharomyces	1.97		2.41	1.93	1.69	2.40	7.95	16.82
Cladosporium		2.45		2.35	2.51	1.46		
Alternaria		1.71	6.33	6.47	13.11			
Candida		1.43		1.76	2.01		4.16	
Rhizopus		1.35						
Mucor			4.98					
Helianthus			4.22	6.68	1.08		3.74	
Knufia			4.22					
Candida			4.07					
Yarrowia			2.87					
Debaryomyces			2.71	2.03	1.52		1.37	1.88
Aspergillus					2.19			
Puccinia					1.80			
Hanseniaspora						1.04		
Rhodosporidiobolus						1.01	2.90	
Cryomyces							4.00	
Naganishia							2.57	2.89

4.3 Summary

- This chapter focused on the development of BC pellicles from either a single isolate *K. xylinus (KX*) or a Kombucha SCOBY (KSC) starter, studying the changes in the microbial communities over three generations, and any changes that may arise from using either a liquid or solid inoculum on BC pellicle development in H&S medium.
- Pellicle development from both starter cultures was initially consistent and comparable. Whilst pellicle yields continued to increase across subsequent generations, it was the Kombucha-based pellicles that were the thickest by generation three.
- *K. xylinus* was present in greatest relative abundance in all samples, but it is suggested that it is the consortium of microbial species in the KSC that promotes community stabilization and in turn, greater yields of pellicles in comparison to those produced by a *K. xylinus* single isolate.
- Community stabilization may have occurred by three generations in this experiment. However, further work over subsequent generations is required to assess the impact of these on pellicle production.
- Yeast species found such as *Candida* and *Saccharomyces* were found in concurrence with previous studies. More yeast species were found in Kombucha SCOBY inoculated samples than those in pure culture samples.
- These findings lead to the hypothesis that the extracellular polymeric substances and bacterial and yeast species found in the KSC, and subsequent pellicles inoculated by Kombucha could be a contributing factor to enhanced pellicle development and yield.

- Highest pellicle yield was achieved when a combination of pellicle and liquid inocula across generations were used. The microbial dispersion through a liquid allows easier access to carbon sources and better cell distribution facilitates growth (pellicle development); it is therefore suggested this enables quicker and more prolific BC pellicle production.

This thesis aims to assess the uses of bacterial cellulose as a sustainable textile material. A critical factor in this analysis is establishing the production and subsequent reproducibility of a consistent pellicle for evaluation. This section of the thesis has established that it is possible to develop reproducible pellicles using a Kombucha starter culture and inoculating further broth with a liquid inoculum over at least three generations. Thus, sub-culture does not hamper the development of a pellicle and appears to be beneficial (although further work across multiple generations would be required to prove this hypothesis). Additionally, a Kombucha SCOBY yields higher weight and more reproducible pellicles than those from a single strain isolate.

In conclusion, from the findings of chapters two and three, sterile black tea with sugar will be used as a culture medium, inoculated with Kombucha SCOBY liquid, and incubated for 30 days at 30 °C for the further stages of this study, as these conditions enable the production of the highest pellicle yield with the most consistent growth.

Chapter 5: Reproducibility of the Bacterial Cellulose Pellicle in a Public Space

5.1 Introduction

Previous studies, such as those conducted by Lee (2023), Fernandes et al. (2019), Garcia et al. (2019), and Chan et al. (2018) have alluded to the concept of 'growing your own clothes at home', exploring the potential of using bacterial cellulose pellicles in applications such as jackets and shoes. This illustrates the importance of using creativity in the wider communication of scientific principles, and how creative subjects can spark an interest in 'traditional' science outside the laboratory environment. However, the underlying principle of the research conducted in this thesis is one of sustainability, eliminating the use of synthetic chemicals to produce apparel and textiles.

Previous studies have used a Kombucha SCOBY in green or black tea as a starter to produce larger pellicle sheets to create garment shapes (Chan et al., 2018; Fernandes et al., 2019; García and Prieto, 2019; Lee, 2023). They also explored techniques common in the fashion industry such as stitching and shaping, alongside the addition of trims such as zippers, buttons, and ribs. The concept has captured the interest of some mainstream media outlets (Shaw, 2012), with 'recipes' for growing Kombucha pellicles being circulated publicly on sites such as Pinterest (Ross, 2023) and Materiom (Aberg, 2023).

This adds further gravitas is added to the lab-based findings of earlier chapters in this thesis; BC pellicle production is more vigorous when Kombucha is used as an inoculum compared to a pure culture. However, the work has either been carried out in a laboratory, or at small batch scale in a workshop. Whilst there are some researchers speculating the possibilities of Kombucha-based BC 'fabric' manufacture on a larger

scale (Lee, 2016; Chan et al., 2018), there is no work in the current literature exploring the replication and variability of BC pellicles in the public space (i.e. by non-experts).

This chapter of the thesis aims to assess the reproducibility of multiple bacterial cellulose pellicles inoculated in a public space, and the use of fashion as a context by which to drive public interest and engagement. The chapter contains published work from peer reviewed papers (Wood et al., 2022; Verran et al., 2023a; Verran et al., 2023b) (appendix 1). It evaluates the reproducibility of BC pellicles when a range of media are inoculated with Kombucha as part of 'citizen science' events, one as part of a school science club and the other as part of a city-wide public engagement science festival.

5.2 Linking Creativity, Science and Technology in Education (STEM and STEAM)

STEM is an acronym for Science, Technology, Engineering and Maths education, first introduced in the United States in the 1990s by the National Science Foundation (NSF) (Delahunty and Ríordáin, 2023, Sanders, 2008). Globally, there are varied approaches to STEM education but no internationally approved definition Decoito et al., 2024). However, the term is commonly used to define educational policy or curriculum development and is thought to have initially been driven to address shortages of such skills in the workforce (Kelley and Knowles, 2016). Although originally introduced in the US, European STEM educators also highlighted a growing skills gap in the workforce and efforts to enhance STEM education are increasingly motivated by economic considerations in both developing and emerging nations (Kennedy and Odell, 2014).

However, there is still much debate on the most effective method by which to implement STEM learning. Sanders (2008) and Abell and Lederman (2011) both suggest that whilst

STEM education is often used to imply innovation in learning approaches, the individual subjects still remain disconnected. In contrast, Kennedy and Odell (2014) argue that STEM has become a 'meta discipline' which breaks down the barriers between its component subjects and presents the opportunity to tackle complex problems using existing tools and technologies. Furthermore, STEM education '*can link scientific enquiry, by formulating questions…. Before they* [the students] *engage in the engineering design process to solve problems'* (Kennedy and Odell, 2014, p.246). This perspective introduces the importance of design thinking integration as a component of STEM education, and the application of this to real-world contexts in order to foster a deeper level of subject understanding. Stohlman (2012) agrees, further explaining that integration can provide a more engaging student experience whilst developing higher level critical thinking and problem-solving skills and improving knowledge retention.

The acknowledgment of the importance of design thinking integration in STEM learning has led to a debate regarding the separation of science- and arts-based subjects. Some educators argue that arts and science should not be taught in isolation as their integration provides a holistic approach to learning (Burrows & Borowczak, 2022). Marmon (2019) and Khine (2019) suggest that STEAM (Science, Technology, Engineering, Arts and Maths) educational approaches promote an 'imagination through innovation' view to solve STEM related problems, providing an intersection between the highly technical and highly creative.

As mentioned above, there is no internationally agreed definition of STEM and the subjects included, which had led to debate regarding social sciences (e.g. psychology, sociology and economics) as these subjects are sometimes excluded from the 'hard'

science (e.g. chemistry, physics and biology) subjects and categorised as arts based. Nevertheless, a STEAM approach would allow inclusion of social science, and thus, according to some scholars, the enrichment of the curriculum and learning experience (Lansiquot, 2016; Marmon, 2019, Khine, 2019). This, in turn has led to a variety of acronyms being developed globally to describe approaches to curriculum development (Table 19).

The approaches of STEM, STEAM, and other related frameworks are designed to address real-world challenges through the adoption of a multidisciplinary approach (Mastacusa & Snyder, 2011). While these frameworks provide valuable insights for educational practice, they also have the potential to extend to collaborative, community-based efforts, such as citizen science. More specifically, a citizen science approach presents a means to apply STEM and STEAM principles within public settings, enabling individuals to actively participate in data collection and problem-solving processes. This integration fosters broader engagement and enhances the applicability of these frameworks beyond formal educational contexts.

Table 19. An Overview of Acronyms Used for Educational and Curriculum Development Approaches.

Adapted from Abell and Lederman, 2011, Han et al., 2022, Kelley and Knowles, 2016, Marmon, 2019, Sanders, 2008.

Acronym	Subjects included	Comments
STEM	science, technology, engineering and mathematics	previously known as SMET
A-STEM	arts, science, technology, engineering, and mathematics	focus on arts and humanities–based subjects
eSTEM	environmental STEM	
GEMS	girls in engineering, mathematics, and science	used to encourage girls into scientific fields
MINT	mathematics, informatics, natural sciences, and technology	
SHTEAM	science, humanities, technology, engineering, arts, and mathematics	
STEAM	science, technology, engineering, arts, and mathematics	'A' has also been referred to as 'agriculture' or 'applied mathematics'
STEEM	science, technology, engineering, economics, and mathematics	
STEMIE	science, technology, engineering, mathematics, invention, and entrepreneurship	
STEMM	science, technology, engineering, mathematics, and medicine	
STM	scientific, technical, and mathematics or science, technology, and medicine	
STREAM	science, technology, robotics, engineering, arts, and mathematics *or* science, technology, reading, engineering, arts, and mathematics *or* science, technology, recreation, engineering, arts, and mathematics	addition of either robotics, reading or recreation to STEAM subjects

5.3 Citizen Science

Citizen science is a term that is used in relation to large scale public participation in research projects (Dickinson et al., 2012). It is a term that is distinct from 'amateur science' in that whilst the participant does not need specific scientific knowledge (Land-Zandstra et al., 2021), the project is set up by professional researchers with a clear methodology and specific aims in place, often with a view to creating a publishable set of data and findings. It is not a new concept, with the earliest examples noted as Europe-wide bird surveys in the eighteenth century or the creation of the Astronomical Society of the Pacific in 1889 (Dickinson et al., 2012). Historically, citizen science projects have been part of ecological or environmental studies, but with the increase of the reach of the internet, these types of projects have gained popularity in a diverse spectrum of disciplines. Today there are networks such as the global Citizen Science Association (CSA) (www.citizenscience.org) and the European Citizen Science Association (ECSA) (www.ecsa.ngo) which provide platforms for the sharing of ideas and development of best practice. The ECSA have developed a framework for citizen science practice, 'The Ten Principles of Citizen Science' (ECSA, 2015). These principles aim to cover the diversity of citizen science practice across a variety of disciplines as follows:

1. Citizen science projects actively involve citizens in scientific endeavour that generates new knowledge or understanding. Citizens may act as contributors, collaborators, or as project leader and have a meaningful role in the project.

2. Citizen science projects have a genuine science outcome.

3. Both the professional scientists and the citizen scientists benefit from taking part.

4. Citizen scientists may, if they wish, participate in multiple stages of the scientific process.

5. Citizen scientists receive feedback from the project.

6. Citizen science is considered a research approach like any other, with limitations and biases that should be considered and controlled for.

7. Citizen science project data and meta-data are made publicly available and, where possible, results are published in an open access format.

9. Citizen science programmes are evaluated for their scientific output, data quality, participant experience and wider societal or policy impact.

10. The leaders of citizen science projects take into consideration legal and ethical issues surrounding copyright, intellectual property, data sharing agreements, confidentiality, attribution, and the environmental impact of any activities.

(adapted from ECSA (2015))

Following on from the ten principles, the practice of citizen science can broadly fall into three models: contributory, collaborative and co-creation. The contributory model uses participants as 'data collectors', and participants are given clear guidelines in the methods by which specific information should be gathered. The other two models, collaborative and co-creation, involve the participants to a much greater degree. Whilst these types of projects are still closely supervised by professional researchers, participants are much more involved with the methods and analysis stages of the activity. However, collaborative and co-creation concepts are rapidly developing in the wake of open access platforms (e.g. www.github.com, www.instructables.com) freely

accessible on the internet, encouraging 'amateur' researchers to share knowledge and develop their own individual fields of enquiry.

There is some debate on the quality of data generated from citizen science projects (Vohland et al., 2021). There is no doubt that the extensive data sets that can be produced by such projects would be prohibitively expensive to collect by other means. However, careful planning and method consideration is required if the data produced from a citizen science project is to be of high quality i.e. accurate, complete, and relevant (Balaz et al., 2021). Reproducibility is also an important consideration, but not one that is unique to a citizen science project. A study reported by Baker (2016) claimed that over 70 % of researchers were not able to reproduce the experiments of others, and over 50 % failed to reproduce their own work.

A further consideration is the use of the data. Balaz et al. (2021) suggest that 'not one size fits all' in citizen science projects, and whilst data collection protocols may not be suitable for one discipline, they may be entirely relevant to another.

When designing a citizen science project, it is important to consider the target participants and how they will engage (De Moor et al., 2019). From a recruitment perspective, Ponciano et al. (2014) and Sauermann and Franzoni (2015) suggest that it is smaller groups of participants that make the greatest contributions, whilst larger groups the least. Some participants may be happy to interact at a superficial level, and this may still generate valuable data for the project. However, other participants (where the level of engagement is deeper) will want to become more involved and actively seek opportunities for this e.g. '*through interaction in an online forum, by becoming a moderator or trainer, or in small-scale workshops on data interpretation or policy*

involvement' (Land-Zandstra et al., 2021). It is important to consider potential further engagement of participants when designing experiments and activities, particularly when considering the reasons for further engagement requests. Several studies have attempted to examine this and suggest that it is participants feeling of contributing to 'real science' or being able to see the impact of the project outcomes that are considered the motivational drivers (West and Pateman, 2016; Frensley et al., 2017). Another consideration is when longer term engagement is a requirement of the project from a data collection perspective. As De Moor et al. (2019) explain, the voluntary nature of citizen science projects means they have no formal contracts of monetary rewards, so engagement throughout the life of the project is key from a data collection perspective.

The tenth part of the 'The Ten Principles of Citizen Science' framework (ECSA, 2015) refers to ethical considerations and the protection of participants from harm whilst maintaining standards of integrity in the collection of quality data. Each citizen science project and event should be considered from this perspective as part of the methodological approach to the experiment.

The next part of this thesis will describe a citizen science event and a schools science club in which the public participants and school students respectively were engaged as 'contributors' to data collection. The data collected was used to compare and validate findings of literature and lab work discussed in previous chapters and further explore objective four: the reproducibility of multiple bacterial cellulose pellicles inoculated in a public space.

5.3.1 Manchester Science Festival

Manchester Science festival has taken place annually since 2007 and hosts a city-wide schedule of events in a range of venues. It is based on the concept of bring practical science activities to a wide range of public audiences across the city. Whilst many of the activities are promoted as giving people the 'chance to get up close and hands-on with all things loud, lively, explosive and experimental' (Gill, 2015) it has also been used as a platform to host large scale citizen science projects such as Turing's Sunflowers (Ochu, 2012) and Hooked On Music (Morgan, 2015).

5.3.2 HandsOnBiofilm! Event

This event was created across various disciplines: microbiology, fashion, and food, and was funded by a grant received from the National Biofilms Innovations Centre (BBSRC grant number BB/R012415/1 PE014). The aim was to raise public awareness of the role of biofilms in these disciplines via a series of engaging activities, whilst encouraging participation in a wider citizen science project; producing data on the conditions required to develop pellicles with the highest and most reproducible yield by weight. The event was held at the Manchester Science and Industry Museum in October 2022.

The event attracted approximately 1200 visitors, predominantly in family groups and comprised of a series of 'stalls' with activities relevant to each theme as follows:

- The microbiology section allowed participants to first create a model of a biofilm using 'bunchems' (plastic balls that clip together to create 3D shapes, (Spinmaster, 2022).

- Participants were then asked to choose a liquid broth for inoculation (beer, coconut water, black tea with sugar, H&S), choose an inoculum (Kombucha pellicle or liquid) and decide at which temperature they would like to incubate their selection (room temperature or 30 °C). These variables were chosen because they were reflective of previous studies in literature and the evaluation work undertaken previously detailed in this thesis. Thus, the findings of this citizen science activity could be compared to those found in the initial lab work as part of this thesis.
- The fashion section encouraged participants to draw a piece of apparel and use these to instruct researchers to build a 3D model of their design on a quarter scale mannequin using larger pieces of BC pellicle. This activity was designed to encourage participants to engage with sheets of BC 'fabric' and imagine their use in clothing in a creative context.
- The food section provided participants with information on the role of bacteria and fermentation in nutrition, offering participants a bottle of Kombucha drink on successfully completing all activities in the event. Whilst this part of the event engaged and educated visitors, findings from this activity were not evaluated as part of this thesis.

5.4 Experimental Details

5.4.1 Event preparation

Five weeks prior to the event, two five litre Kilner jars containing black tea and sugar (as described in section 3.5.1) were inoculated with a Kombucha pellicle and the tea in

which it was delivered (Happy Kombucha 2022) (pellicle = 16 cm diameter / 1.5 cm thick, the broth = 50 mL). The jars were and stored at room temperature (22 +/- 2 °C) to produce both a solid pellicle and liquid inoculum for the event. After five weeks, aliquots of the liquid medium and portions of the pellicle developed on the surface of this were removed for use as inocula. The newly formed pellicle was cut into 1 g portions prior to the event and stored in a petri dish until required.

Three hundred 25 mL universal pots were prepared containing sterile culture medium as follows:

- 75 x black tea with sugar
- 75 x beer
- 75 x coconut water
- 75 x H&S

5.4.2 Inoculation and Incubation Conditions

An A1 poster board was created to inform participants of their choices for growth medium, inoculum and incubation conditions (Figure 17). Participants were advised to select a culture medium, inoculate this with their chosen inoculum, and indicate which incubation temperature they would like to be used. Each inoculated universal tube was given a unique reference number, which was marked on the side of the bottle, a record given to the participants, and the variables independently noted.

The lids of the universal pots were screwed on tightly to avoid spillage during transportation and taken back to the laboratory for incubation. However, the tightly fixed

lids limited the oxygen supply in the pot and therefore after five weeks incubation, very little pellicle development had occurred. This supports previous work reported in literature that describe the importance of aerobic conditions in BC pellicle development due to the nature of the microbes responsible for this (section 3.4.5) (Schramm and Hestrin, 1954a; Chao et al., 2000; Dufresne, 2012; Gullo et al., 2014; Zheng et al., 2018; Aswini et al., 2020). The lids were loosened to allow the ingress of oxygen from the atmosphere and thus provide aerobic conditions. Incubation continued for a further three weeks after which pellicle development was observed.

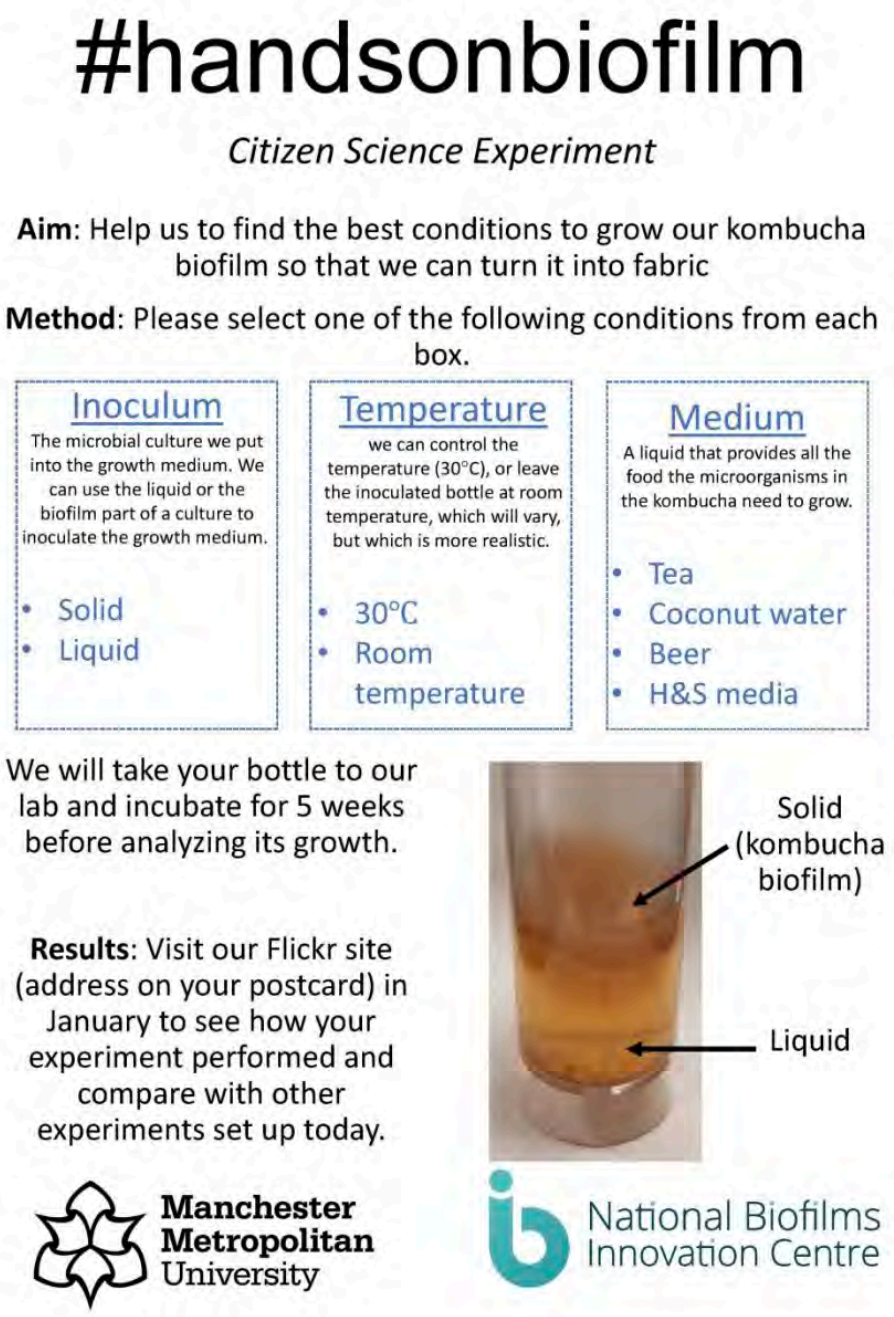


Figure 17. Poster Board from the Citizen Science Project Detailing Incubation and Inoculation Choices.

5.4.3 Pellicle Assessment

After a total of eight weeks photographs were taken of all the universal pots. Any pots that had not successfully developed pellicles were discarded (n = 140). The thickness of any developed pellicles (n = 81) was recorded using a ruler on the outside of the pot. Pellicles were then extracted from the pots and placed on filter paper to remove excess surface water. The pellicles were then weighed (wet), dried in a fan oven at 60 °C for 48 hours (to constant weight) and their dry weight recorded.

Evaluation of the data collected revealed the most popular inoculum, growth medium and incubation conditions alongside the most successful combination of these for pellicle development in terms of dry weight yield.

5.4.4 Ethical considerations

This project was assessed and approved by the Manchester Metropolitan University Ethics Committee (application number 41586), which included the retention of personal data for purposes of contacting visitors after the event. Health and safety risk assessments were conducted as required by Manchester Science and Industry Museum.

5.4.5 Results and discussion

221 bottles were inoculated, with coconut water the most popular medium, liquid the most popular inoculum and 30 °C the most popular incubation temperature (Figure 18). Whilst there is no conclusive evidence as to why these choices proved most popular, it can be speculated that coconut water could have been most chosen as participants

would associate this as ‘natural’ and therefore link this to a more environmentally friendly product.

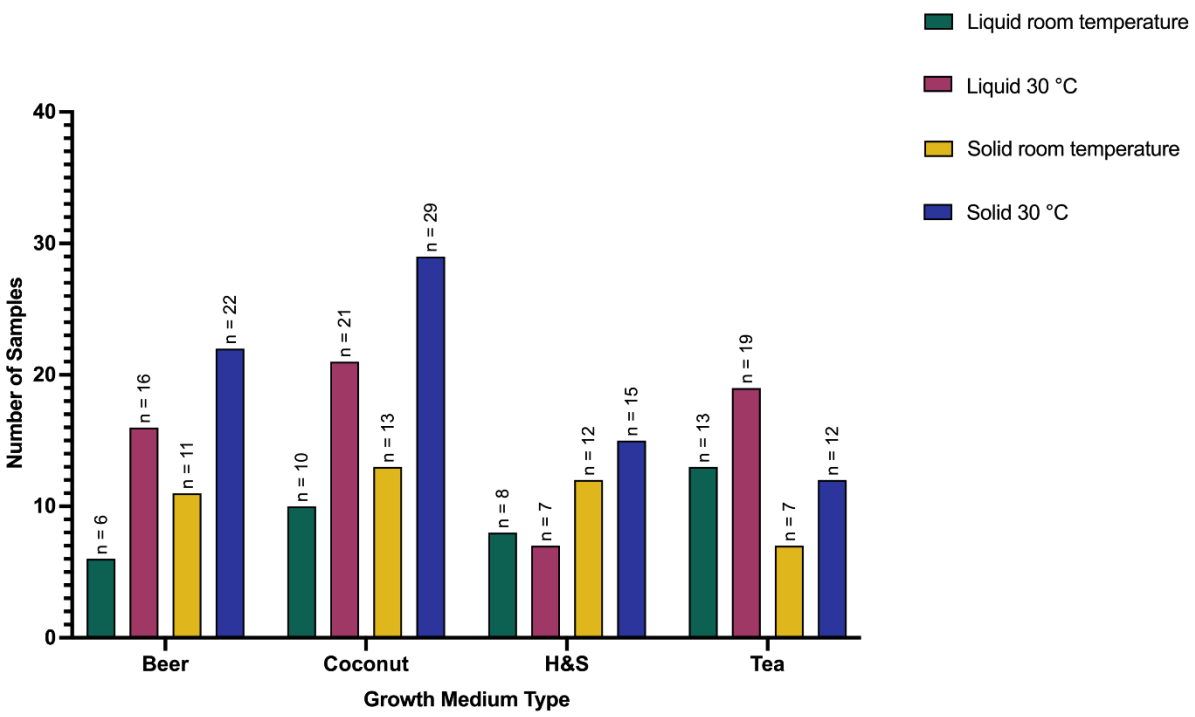


Figure 18. Number of Samples, Media and Incubation Conditions Selected by Citizen Science Participants.

12.7 % of coconut water (n = 28) and 12.2 % of tea broth (n = 27) pots inoculated produced pellicles compared to only 4.5 % beer (n = 10) and 7.2 % H&S (n = 16) (Figure 19). It is well documented that tea is the most widely used liquid medium to support the fermentation of Kombucha and thus the most used to support Kombucha pellicle production (Jayabalan et al., 2014; Coelho et al., 2020), so this result was not surprising. As coconut water is likely to provide a highly nutritious environment to support pellicle growth, the

high number of successful pots in this case was again not surprising, concurring with the initial work as part of this thesis where coconut water was identified as a viable supporting liquid broth for pellicle development. However, the possibility of the failure of pellicle growth in any medium due to anoxia cannot be conclusively discounted.

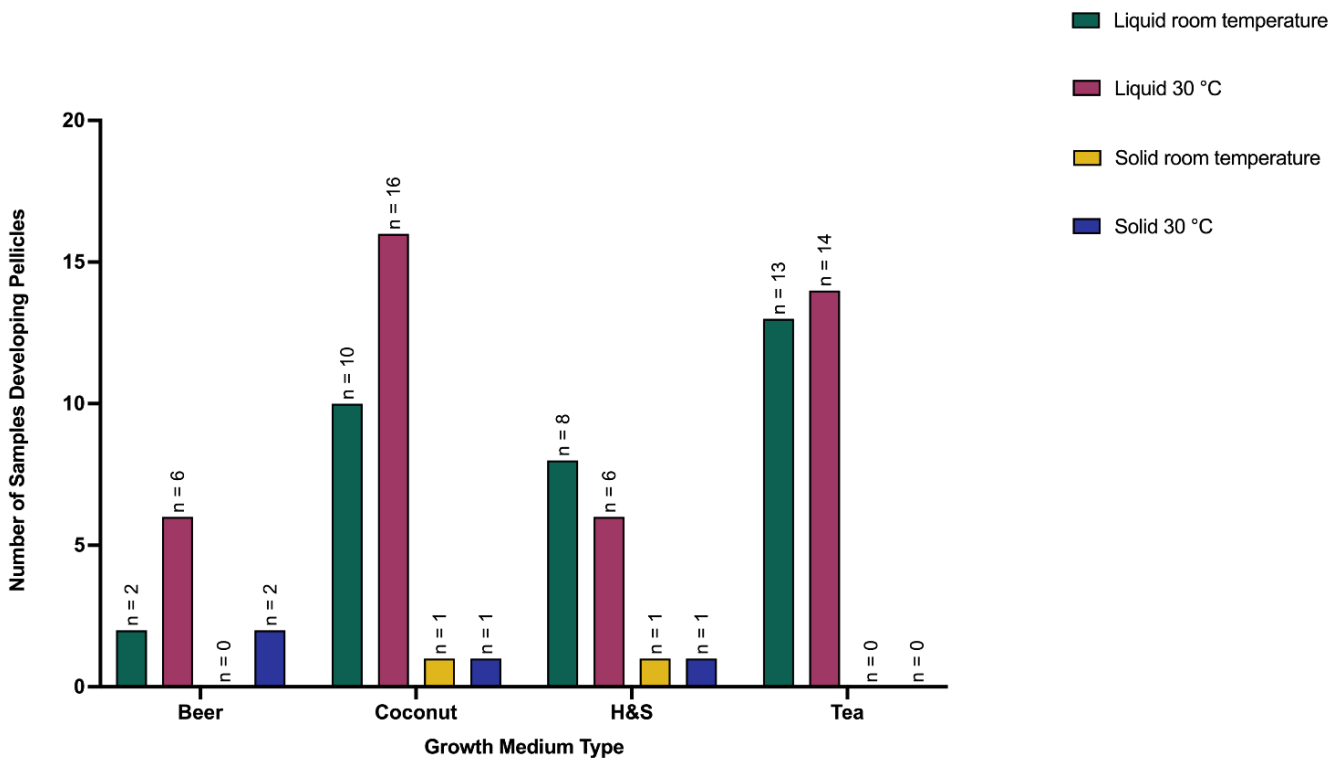


Figure 19. Successful Pellicles by Medium Type and Incubation Conditions Grown in Citizen Science Experiment.

The lower number of pellicles developed in beer broth agreed with initial observations in this thesis. It is not clear why beer 'broth' does not support pellicle growth in the same way as other liquid media explored. An observation (also discussed in section 3.5.3) is that there is alcohol present the beer broth, which is not present in other media explored.

Several studies have reported the beneficial effect of additional ethanol in the growth medium in the production of BC (Lu et al., 2011; Agustin and Padmawijaya, 2018) stating that yield is improved because the alcohol is considered a nutrient for the active bacterial species. However, Naritomi et al. (1998) suggest that increased concentrations of alcohol beyond a threshold of 15 g/l (1.5 %) cause BC production to be inhibited. A speculation is that the level of alcohol already present in the beer (27 g/L, 2.7 %) used in this study is beyond the threshold level observed by Naritomi et al., (1998) thus inhibiting the production of BC, impacting pellicle development and concurring with findings discussed in section 2.5.3.2.

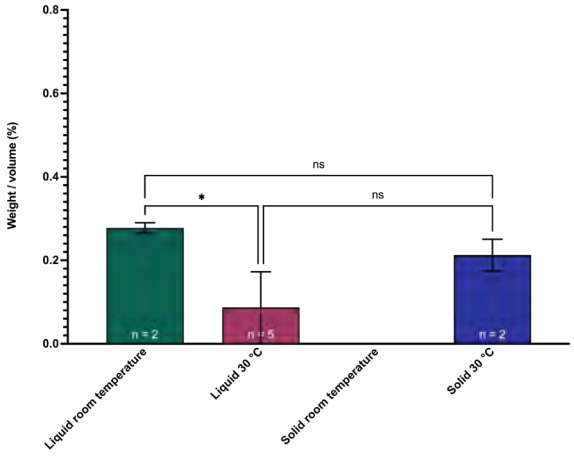
However, as H&S broth has been developed specifically to support the growth of BC pellicles, it is difficult to explain the poor success rate in comparison to the other growth media used in this experiment (and that observed in section 4.2.3). A possible explanation is that H&S was developed for aseptic laboratory use. Whilst the H&S broth was sterilised prior to the event, the inoculation was not conducted under aseptic conditions and therefore contamination could have entered the pots. However, this was the case for all broths inoculated and it is therefore postulated that H&S broth has a lower resistance to contaminants than the other broths explored. This theory is supported by the findings of the preliminary work in Chapter Two of this thesis. Preliminary work inoculated H&S, beer, tea, and coconut broths under aseptic conditions; there was no failure of pellicle production in any of the H&S pots.

Findings in chapter two suggested that liquid inoculum produces higher yield pellicles than solid inoculum. When considered as a proportion of the total number of successful pellicles, liquid inocula produced 92.9 % (n = 26) in coconut, 87.5 % (n = 14) in H&S, 80

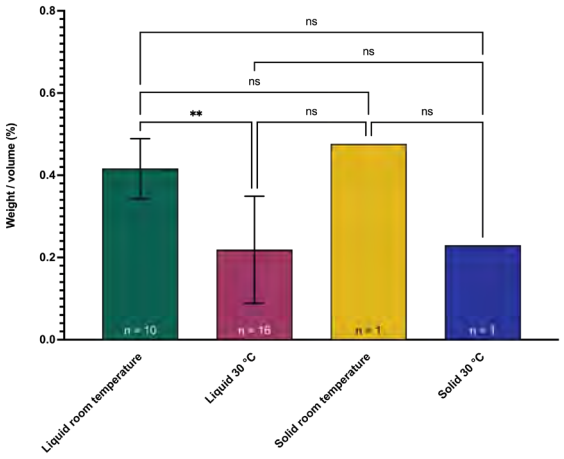
% (n = 8) in beer and 100 % (n = 27) in tea, although it should be noted that no inoculations were selected for a solid inoculum in tea medium (Figure 19). However, statistical analysis of these measurements shows in most cases the variations between liquid and solid inocula (and incubation conditions) are not significant ($p > 0.01$) (except for coconut and beer media inoculated with liquid and incubated at room temperature vs 30 °C, and H&S media inoculated at room temperature). Tea could not be analysed as there were no solid inoculations (Figure 20).

Comparison of the pellicles harvested from the different growth media revealed that those grown in tea produced the thickest and heaviest pellicles ($p < 0.0001$) (Figure 21), agreeing with work described earlier in this thesis (section 2.5.3.2).

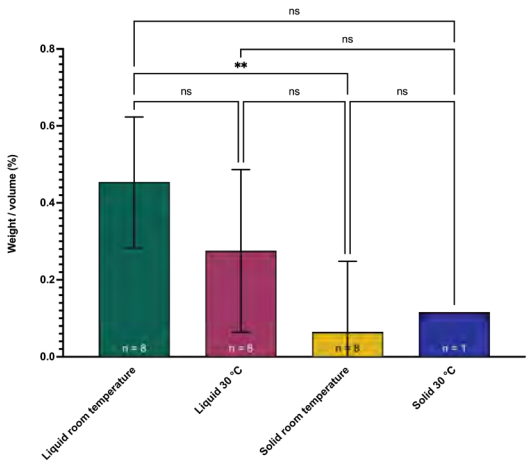
Variability in pellicle yield by weight also concurred with earlier findings in this thesis; whilst tea gave the highest weight /volume % dry yields (1.433 % at room temperature and 1.873 % at 30 °C with a liquid inoculant), this medium also displayed the highest variability (σ = 0.327 and 0.487 respectively), whilst pellicles developed in H&S medium were the lightest and least variable (Figure 21).



(a)



(b)



(c)

Figure 20. Mean Dry Weight Yield of Pellicles Grown in (a) Beer, (b) Coconut, (c) H&S by Inoculation Type and Incubation Conditions.

(error bars = standard deviation, ns = not significant, * = $p < 0.05$, ** = $p < 0.01$)

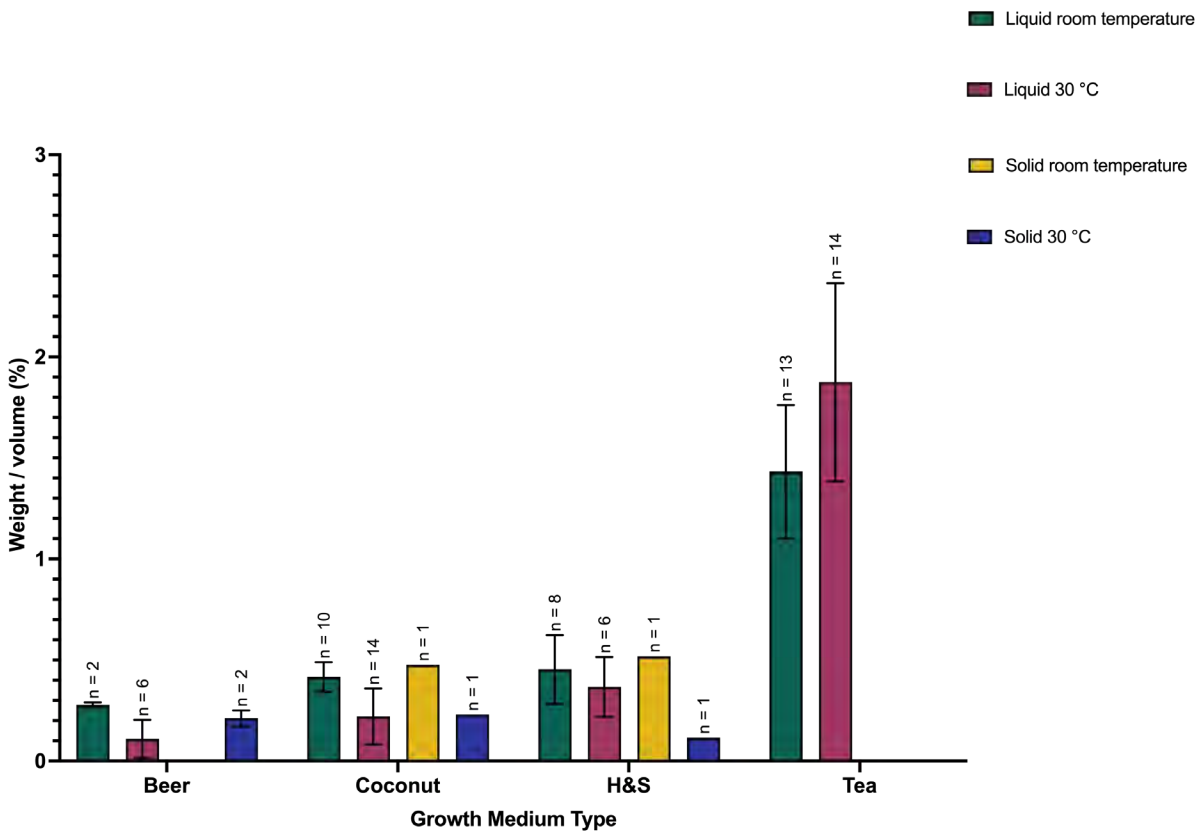


Figure 21. Mean Dry Weight Yield of Pellicles by Medium Type and Incubation Conditions.

(error bars = standard deviation)

5.5 STEM in Secondary Education

Science, technology, engineering, and mathematics (STEM) are core parts of the secondary curriculum. Several studies (section 5.2) have investigated the importance of the types of activities involved in ensuring an inclusive environment in the classroom for the communication of important STEM concepts (Chan et al., 2020; Banks and Barlex, 2021). The role of 'real world' experiences for pupils studying STEM subjects is also well documented, with UK initiatives, such as STEM ambassadors (2024) encouraging industry professionals from outside the educational system to engage with school pupils to offer 'real life' experiences of STEM professions. Alongside traditional careers fairs,

initiatives such as STEM ambassadors encourage industry professionals to go into schools to engage with pupils in their own environment and deliver activities relevant to the specific profession.

Textiles and fashion technology fall into the category of STEM subjects in the UK national curriculum, and the development of BC using the techniques described earlier in this thesis straddles learning outcomes for key stage three in both biology and textiles. An activity was therefore devised to be delivered in a secondary school to meet the needs of the national curriculum and to gather further data regarding the inoculation of media in a public space to develop BC pellicles.

5.5.1 School Science Club

A local secondary school was chosen as it was known to the author and therefore provided ease of access. The school runs a science club for year seven pupils covering topics in chemistry, biology, and physics. A project was devised to run over six consecutive weeks whereby the participants would be introduced to the concepts of environmental pollution by the fashion and textiles industry and then invited to explore BC as a novel material that could be grown in the school laboratory.

The participants were 15 year seven students (a mix of genders) who regularly attended a lunchtime science club on a voluntary basis. The student group had been provided with basic details of the activities to ascertain their interest in the project and asked to commit to attending all 3 sessions across a 6-week period.

5.5.2 Event Preparation

As described previously (Table 11) five litres of sterile black tea with sugar and five litres of H&S liquid medium were prepared to support pellicle growth. Forty sterile 500 mL Kilner jars (www.kilnerjar.co.uk) were filled with either 250 mL black tea or 250 mL H&S medium and the lids securely sealed (thus creating 20 jars per medium).

The liquid inoculum for each of the tea or H&S culture media was taken from a Kombucha pellicle culture which had been stored for 30 days in a quantity of tea and sugar mixture (as advised by the pellicle manufacturer). A total of 40 × 25 mL sterile 'universal' tubes (25 mL total possible volume) were filled with 10 mL of the inoculum and stored at room temperature.

5.5.3 Ethical Considerations

As this activity took part on a school premises, ethical protocols were put into place by the school; the observer was DBS checked (DBS certificate number 001800326153) and was always chaperoned by a DBS checked member of school staff. Pupils' personal data was not recorded.

5.5.4 Inoculation and Incubation Conditions

In the first session, the students engaged in a brief discussion of the impacts of the textile and fashion industries on the environment and were asked to participate in an activity of exploring a different way of producing textiles (the development of BC pellicles). Each student was then provided with two pre-prepared Kilner jars, one containing tea culture

medium and one containing H&S culture medium, alongside two universal sample pots containing the inoculum mixture. Each student inoculated each Kilner jar by pouring in the contents of one of the universal sample pots, closing (but not sealing) the Kilner jar after inoculation. Each jar was labelled with the student's name and placed on a shelf in the biology classroom at room temperature (approx. 22 °C).

5.5.5 Pellicle Assessment

Two weeks later, the students returned to the science club to evaluate the activity in the inoculated Kilner jars. The students used free text to note their comments and discussed their observations with their fellow participants. The jars were then closed again for further incubation at room temperature. After another two weeks had passed the activity in the jar was noted via free text. The thickness of any developed pellicles was measured before the pellicles were removed for weighing and photographing. The pellicles were then dried (to constant weight) at room temperature on a bench top for 48 hours and photographed before weighing again. The weight measurements were converted to percentage yield weight / volume to allow comparisons with previous work in the literature and earlier in this thesis (section 2.2.2).

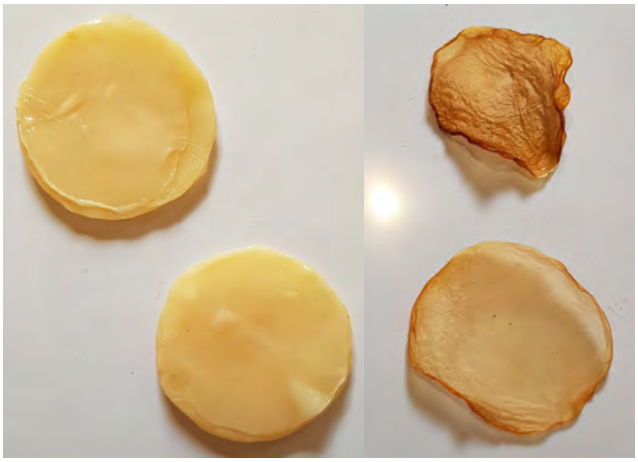


Figure 22. H&S Pellicles (a) Wet and (b) Dry.

Pellicle Diameter = approx. 4 cm.

Figure 23. Black Tea Pellicles (a) Wet and (b) Dry.

Pellicle Diameter = approx. 4 cm.

5.5.6 Results and Discussion

In the first session the students were keen to discuss the environmental impact of their clothing and were knowledgeable about the need to take action to reduce textile pollution. Linking the real-world knowledge of the students to scientific principles connected the learning and promoted the enquiry conversations in the classroom,

illustrating the importance of linking creative thinking with scientific practice and further illustrating the importance of the STEAM principles of learning discussed in section 5.2.

All students inoculated one jar each of H&S and black tea growth medium with the pre prepared Kombucha SCOBY liquid inoculum.

The second session was designed for the students to monitor the progress of the pellicle development in the jars. In the jars containing the inoculated tea culture medium, all students could see the development of a transparent film on the surface of the culture medium, measuring between 1 and 5 mm in thickness. The students commented on the jelly-like consistency of the pellicle, and many mentioned the vinegar-like smell of the culture medium when the pots were opened.

In the second session, the students observed that half (n = 10) of the jars containing the H&S culture medium had not developed a pellicle and there were instances of contamination with the development of obvious fungal growth in these jars. In these cases, the students were advised not to open the jars (to prevent the release of spores). These jars were removed, and the contents safely disposed. This engaged the students in conversation with their biology teacher regarding the development of fungal spores into mould, linking to previously covered year seven curriculum topics. These observations also correlated with the results obtained from the citizen science project (section 5.4.5) where a reduced number of H&S inoculated medium were seen to have any pellicle development. In a similar manner to the citizen science event, the H&S broth was sterile, but was not inoculated under aseptic conditions, further supporting the speculation that sterile H&S broth has a lower resistance to contaminants than sterile tea broth.

Figure 24. Contaminated H&S Medium in Kilner Jar.

In the last session with the students, they observed that some of the remaining H&S jars not disposed of in session two had developed fungal contamination and these jars (n = 6) were removed and disposed of appropriately. However, four of the H&S jars had developed pellicles and the students noticed vigorous development of these; the pellicles were opaque, white in colour and 10 mm thick.

Most tea culture medium pellicles had developed further (n = 15), with students noting bubbles in the liquid underneath the pellicle, and pellicles measuring approximately 10 mm in thickness. It was also noted that some jars contained multiple thinner pellicles, rather than one thicker sheet.

In terms of yield mean dry pellicle weights by volume measured 0.344 % in H&S and 0.592 % in tea growth media (Figure 25). Variability was greater in tea medium (n = 15, σ = 0.377) compared to H&S (n = 4, σ = 0.1). This concurs with earlier findings in this thesis, tea growth medium supports greater yield by weight pellicle development (Chapter 2).

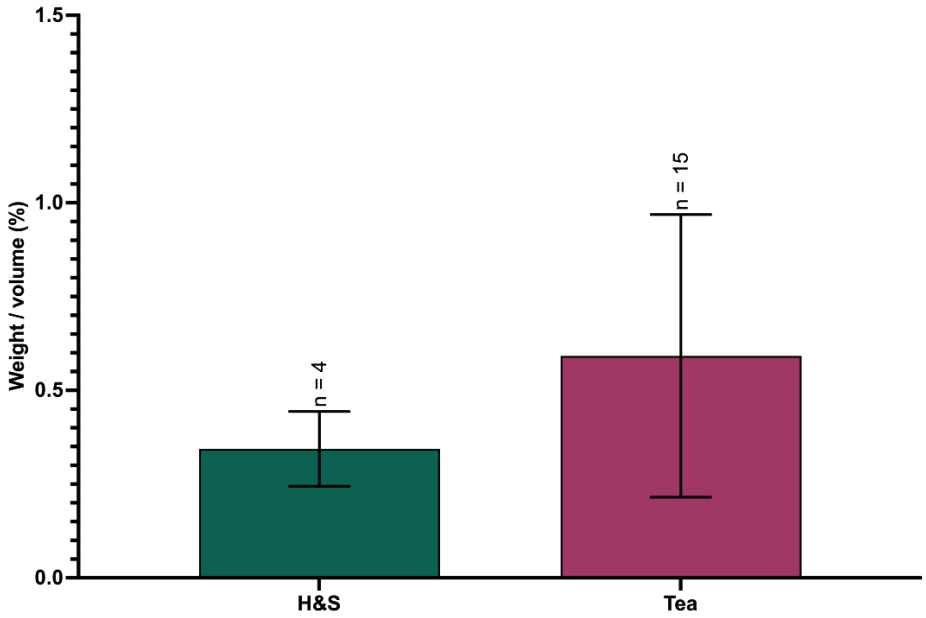


Figure 25. School Science Club: Dry Pellicle Weight by Volume Measurements. (error bars = standard deviation)

5.6 Participant Engagement

Whilst there was a large degree of engagement in the HandsOnBiofilm! event (over 1200 interactions) and the school science club was at maximum capacity (15 students), it was noted that it was difficult to maintain long term participant interest. For the HandsOnBiofilm! event, whilst over 200 inoculations took place which yielded valuable data in terms of optimum growth conditions for pellicle development, there was relatively little engagement post event. Participants had the opportunity to view the photographs of their pots after the incubation period (Figure 17), but there was low engagement with the Flickr site on which the images had been uploaded. It was only after

reminders were sent out that hits on the site improved, resulting in a total of 5340 views from 122 individuals (Verran et al., 2023a).

Equally, whilst engagement in the activity during the lunchtime science club was good, with all students inoculating pots in week one, subsequent attendance started to fall in the following weeks. Due to half term holidays, the students were not able to harvest the pellicles themselves, although the dried pellicles were returned to the students for them to further explore in their own time (and potentially via their timetabled textiles classes).

Whilst the difficulties in long term participant engagement concur with findings in literature (West and Pateman, 2016; Frensley et al., 2017), group size in this case did not have bearing on data quality, disagreeing with the findings of Ponciano et al. (2014) and Sauermann and Franzoni (2015). Each set of participants provided valuable data on optimal incubations conditions and yield. The citizen science experiments also provided valuable insights into the behaviour of the inoculated media in a public space which was a key purpose of the experimental design.

Engagement with both sets of activities was excellent at the point of delivery and assisted data collection for the thesis. However, when considering future citizen science projects (Citizen Science, 2023), strategies for longer term engagement will need to be considered more carefully. One approach for consideration could be that of using future participants as co-creators in future activities; an observation from the school science club was one of enhanced interest once the biofilms (or pellicles) had started to develop in their pots and there was a tangible output to the inoculation experiment. Future citizen science activities could involve participants growing BC pellicles for a specific end use e.g. making an item of apparel, thus potentially deepening both engagement and lines of

enquiry. Additionally, the influence of creative practice in conjunction with physical science cannot be overlooked. Participant engagement in all events was at its highest when the growing of the pellicles (the 'science') was contextualised in fashion sustainability and creativity. This further supports the thinking discussed in section 5.2; creative and science subjects can serve to enhance outputs when considered as subjects together, rather than isolation.

5.7 Summary

- The findings of the lab-based work to establish the conditions that reproducibly developed the greatest yield by weight of BC pellicle were explored in the public arena via a citizen science event. The event, which attracted in the region of 1200 visitors, invited participants to choose a broth to inoculate, choose an inoculum and then select incubation conditions to support pellicle development. This resulted in the inoculation of 221 bottles of pre prepared sterile broth (black tea with sugar, H&S, coconut water and beer) with either a Kombucha solid pellicle or liquid broth inoculum. Inoculated pots were incubated for six weeks at either 30 °C or at room temperature (22 °C + / - 2 °C).
- Whilst coconut water was the most popular broth, the least successful was beer and it is postulated that the alcohol content in the beer has an inhibitory effect on BC production.
- H&S is more susceptible to the effects of contamination when not inoculated under aseptic conditions. This was supported by the results of both events, where

H&S medium showed a higher susceptibility to contamination (and therefore less development of pellicle) than the pots containing black tea.

- No significant differences were found between liquid and solid inocula or 30 °C and room temperature incubation conditions.
- Black tea with sugar, inoculated with liquid Kombucha produced the greatest yield by dry weight of pellicles, albeit also displaying the highest variability in weight.

The findings of part one of the thesis conclude that sterile black tea with sugar, inoculated with a Kombucha SCOBY (liquid or solid) inoculum and incubated for 30 days at a temperature of 30 °C provide the conditions to reproducibly develop the greatest yield by weight of BC pellicles. These conditions will be used to develop pellicles for further exploration in the next part of this thesis.

The engagement of the participants of the citizen science projects were mixed and a future approach may be that of engaging the participants as co-creators of or collaborators, using the approach of STEAM subject delivery in the experiments. This could lead to better engagement with the growing of the biofilm (or pellicle), but importantly, could also lead to deeper understanding by the public of the advantages and disadvantages of BC pellicles as sustainable textiles for clothing.

Part Two

Chapter 6: Uses and application of Bacterial Cellulose

6.1 Introduction

Textiles produced from 'non-traditional' routes (i.e., those not created using plant or animal fibres / skins or those created from fossil fuel origins) are often referred to as 'next generation' textiles. They are seen as an environmentally friendly or sustainable alternative to current production routes. However, many do not live up to these claims and use harmful synthetic chemicals to render them fit for purpose.

BC as a next generation material has great potential, with several studies reviewing current and speculated applications (Laavanya et al., 2021; Choi et al., 2022; Mishra et al., 2022). The explored uses of BC are diverse, ranging from biomedical applications to uses in food, electronics, and textiles. The interest in BC stems from its ability to be manufactured without contaminants associated with plant cellulose, its enhanced strength (Dufresne, 2012) and moisture absorbency properties (Rajwade et al., 2015), and the purported ease with which it is able to biodegrade in the natural environment (Gallegos et al., 2016). The latter renders BC a material which could impact many industries seeking to reduce their environmental footprint in terms of the effects of waste products and end of life disposal. Another attractive feature of BC is its potentially relatively low cost in manufacture (Yim et al., 2017); lab-based formulations to support growth can be costly and difficult to procure, however many studies (including part one of this thesis) have illustrated how BC can be produced relatively cheaply using food-based liquids.

This chapter provides an overview of some of the current uses of BC, alongside those being explored, presenting BC as a novel alternative to current practice, thus addressing objective five: review current applications of bacterial cellulose.

6.2 Biomedical

BC has been used widely and in a variety of ways in the biomedical industry. According to Davis et al. (2003) and Rajwade et al. (2015) ideal biomaterials should display properties such as biocompatibility (not toxic to the biological system, thus minimising adverse tissue reaction); promotion of cellular interaction and tissue development; biodegradability; have inherent porous structure and exhibit good wear resistance and mechanical properties. Many studies have shown that BC displays all the above properties and therefore is suitable for biomedical applications (Mohite and Patil, 2014a). Whilst examples of these applications will now be discussed, it is worthy of note that the field of biomedical applications for BC is already well developed and therefore will not be a focus of further exploration in this thesis, beyond this brief overview.

6.2.1 Artificial skin / Wound dressing

The main goal of a wound healing structure is to create a skin like protective barrier against infection (Huang et al., 2014), whilst allowing the regeneration of the epidermis and repair of the dermis (Mohite and Patil, 2014a). There are many different wound healing dressings on the market but the most effective are those that most closely resemble skin in terms of structure and function (Mohite and Patil, 2014a; Torres et al., 2019). BC has been considered as a '*semi-permanent artificial skin'* (Huang et al., 2014:

8) and has been explored as a treatment for damage to epithelial skin layers by enhancing the BC with additives such as collagen (Lin et al., 2013b). The ability of the BC dressing to retain moisture provides a favourable moist environment for wound healing (Hoenich, 2006; Chawla et al., 2009) and has been trialled as a treatment for severe second degree burns and diabetic foot ulcers (Picheth et al., 2017). Improved performance over conventional dressings was observed in terms of adherence to the contours of the wound site, moist environment maintenance, pain reduction, increase in epidermal regeneration rate, reduction in scar tissue formation and assistance in the removal of necrotic residues (Czaja et al., 2007; Mohite and Patil, 2014a; Picheth et al., 2017). BC sheets, depending on preparation techniques, can also be transparent, allowing wound healing to be observed without the removal of the BC dressing. Additionally, when BC is in contact with blood, it reduces the tendency to clot (Fink et al., 2010), which is often a point of failure in biomedical materials (Braune et al., 2018).

However, mass market development of the products has been limited, due to prohibitive problems with the development of efficient bulk manufacture techniques (Hoenich, 2006; Czaja et al., 2006; Czaja et al., 2007). Johnson & Johnson developed BC based wound care products in the early 1980s and a US company, Xylos licensed the technology to manufacture the XCell® range of dressings (Czaja et al., 2006; xcellbiologix, 2019). Rival brands such as Gengiflex®, Biofill®, Bionext® and Membracell® have also been brought to market and are claimed to be more effective than traditional gauze rivals, but, the market reach and development of these products still appears stifled (Picheth et al., 2017).

Additionally, the BC dressing can be enhanced with additives to further promote healing or administer drugs to the wound site (Mohite and Patil, 2014a). Whilst BC is biocompatible and non-toxic (Dufresne, 2012), it does not possess any inherent anti-microbial activity (Huang et al., 2014). The ideal anti-microbial should 'be inexpensive, non-toxic, non-carcinogenic, fast acting and not interfere with the wound site' (Sulaeva et al., 2015: 1560).

Amalgamation of silver nanoparticles (which have inherent anti-microbial activity) into the BC structure using precipitation (Yang et al., 2011) and ultrasonic methods (Cai and Yang, 2011) have attempted to address this issue with encouraging results achieved by Yang et al. (2011) who found a 99.2 % reduction in *Escherichia coli* activity using silver particles. Chitosan also shows inherent anti-microbial activity, and BC – Chitosan composites have displayed some success in inhibiting the growth of *Staphylococcus aureus* and *E. coli* (Lin et al., 2013b; Sulaeva et al., 2015; Wahid et al., 2019), whilst BC – Chitosan – Nanodiamond composites have also showed promise when used as a wound dressing (Ostadhossein et al., 2015).

Immersion of the BC membrane in antimicrobial agents (such as benzalkonium chloride) also displayed promising results with effects of up to 24 hours when exposed to the bacterium *S. aureus* and *Bacillus subtilis* after exposure to the agent (Wei et al., 2011). The broad-spectrum antibiotic, chloramphenicol was embedded into a BC membrane and showed prolonged effectiveness against *E. coli, Staphylococcus. aurae* and *Staphylococcus pneumoniae* (Sulaeva et al., 2015) and work by Juncu et al. (2016) successfully embedded ibuprofen into a composite carboxymethyl cellulose / bacterial cellulose structure.

Wu et al (2014) and Moritz (2014) both claim that the microporous structure enables slow release of drugs at the wound site to counteract unwanted microbial activity. However, other studies have suggested that the release of antimicrobial agents and other drugs embedded into the BC membrane is too rapid and is therefore restricting the wider scale use of BC in wound dressings (Picheth et al., 2017).

6.2.2 Tissue engineering

Treatment of coronary heart diseases using replacement blood vessels often relies on the creation of these replacements using synthetics such as polyethylene terephthalate (PET) or expanded polytetrafluoroethylene (ePTFE) (Rosamond et al., 2008; Fink et al., 2010). Whilst these materials are useful for larger blood vessels, they have limited success in vessels < 5mm in diameter (Fink et al., 2010; Huang et al., 2014). BC has potential in this field; it can be shaped into tubes and diameters of almost any dimension (Bodin et al., 2007). Trials of BC tubes have shown good blood compatibility (Esguerra et al., 2010; Fink et al., 2010) and the mechanical properties of BC are similar to those of arteries (Bäckdahl et al., 2008). Recent developments using BC as a composite structure with Polyvinyl alcohol (PVA) have been promising in the replacement of cardiovascular tissues where they have shown very similar characteristics (Torres et al., 2019).

In addition to the already mentioned characteristics of BC (high water retention, good thermal stability, and high strength), a BC / PVA composite also displays high visible light transmittance and good UV absorbance. This placed the composite as a promising candidate for cornea replacement in work by Wang et al. (2010), although the study does recognise the need for further development to ensure the material is entirely suitable.

However, it is suggested that the BC composite could go some way to 'reducing rejection rates of transplanted corneas' (Picheth et al., 2017: 101). Other studies in the field of ophthalmology have used different BC composites to treat a variety of eye conditions. The versatility of BC in the manufacture of a variety of 3D shapes has found application in contact lenses (Patchan et al., 2016) with US patents being filed in this field (Li et al., 2011), whilst post-operative wounds have been dressed with BC composites and a reduction in recovery times have been noted (Picheth et al., 2017)

This work has led to explorations of BC in wider tissue engineering applications such as tissue scaffolds and nerve surgery (Petersen and Gatenholm, 2011). The microfibrillar network structure of BC lends itself well to tissue scaffolding applications as it has a similar microfibrillar structure to that of collagen, but the pore size of BC is measured on the nanoscale. This means that the pores are often too small to allow efficient penetration of human cells, thus restricting the growth of new tissue and thus developments in this field were somewhat restricted (Ludwicka et al., 2016). Bäckdahl et al. (2008) suggested a novel method by which paraffin wax and starch were introduced during the growth period of BC from *Acetobacter xylinum*, thus altering the porosity of the structure to promote the cellular in-growth of smooth muscle tissue, whilst Rambo et al (2008) used a physical template of pins (60 – 300 μm in diameter) to impart additional porosity. Chemical modification of porosity has also been developed, with acetylation and alkaline pre-treatment being two of the areas successfully explored (Petersen and Gatenholm, 2011).

Whilst porosity has limited some development, BC has been used as cartilage reconstruction material. When used as a meniscus replacement, the small pore size and

improved mechanical properties of BC have been advantageous; in clinical trials the BC has been able to withstand wear and tear at the joint site, allowing cells to penetrate the scaffold, albeit at a reduced rate. This means that the cartilage renewal takes place more slowly, but the growth is protected by the sturdy BC structure (Ludwicka et al., 2016).

6.3 Cosmetics and Beauty Care

In a similar way to textiles, the cosmetics industry is seeking to reduce its environmental impact by investigating alternatives to traditional approaches in beauty care (Bianchet et al., 2020). As BC can biodegrade in the natural environment, it is attractive to industries wishing to use products that cause less environmental harm at the end of their useful lives.

As a material with a potential to impact the sustainability of the beauty industry, it is interesting to note that food waste has been explored as a medium via which BC can be produced for facemasks, in a similar way to pellicle production described in chapter two. Guo et al. (2019) filed a patent for a method to produce BC masks using fermented banana skin waste, thus addressing some of the principles of environmentally harmful practices being used in the manufacturing of beauty products.

As BC is reported to have an enhanced ability to retain moisture (Dufresne, 2012; Rajwade et al., 2015; Wood et al., 2023) it has been trialled in cosmetic applications such as beauty treatment face masks where this ability ‘enhances moisture uptake by facial skin’ (Amnuaikit et al., 2011; Mohite and Patil, 2014a: 105). However, trials report that whilst the BC mask did improve skin moisture uptake, in comparison to the application of a moist towel it did not improve other qualities such as skin texture and elasticity and

therefore needs more development to be an innovative product in this field (Amnuaikit et al., 2011). A study by Almeida et al. (2014) added glycerine to the BC mask and found this to have a significant effect in improving moisture retention by the skin, whilst Chi et al. (2016) reported the addition of ginseng extract improved skin elasticity. Pacheco et al. (2018) reported positive feedback from users of BC facemasks, with trial volunteers responding positively to the comfort aspects of using these types of products.

There are some BC beauty face masks commercially available, with the suggestions that the presence of nanofibers in the structure are of benefit, as their size (<100nm) means they can penetrate the pores of human skin. It is thought that the nanofibers also help with the addition of supplementary ingredients which are claimed to impart additional benefits (such as vitamins B and C to alleviate uneven skin tone appearance or liquorice root extract acting as an anti-inflammatory and skin brightener) (Ludwicka et al., 2016).

The above studies have focussed on the beauty industry; however, other studies suggest such face masks may be useful in the treatment of medical conditions such as dermatitis, psoriasis, eczema and even scar reduction (Ludwicka et al., 2016; Ullah et al., 2016). In these cases, the approaches have been twofold; those which aim to treat the condition using BC facemasks to administer medication directly to the skin, and applications which aim to treat skin discolouration because of these conditions. This is an emerging market for the use of BC in facemasks for medical applications, with patents being filed for masks which facilitate the lightening of the skin (Jiong et al., 2018).

However, whilst BC does offer enhanced benefits (such as moisture retention) over current materials used in the cosmetic industry, there have been no studies to date analysing its impact on this industry as a biodegradable material.

6.4 Food and Packaging

When not used in sheet or pellicle form, the gel like texture of BC enables its use as a thickener, stabiliser, gelling and suspending agent in foods (Chawla et al., 2009; Shi et al., 2014; Ullah et al., 2016). Its increase in volume on contact with moisture, high fibre content and its indigestibility in the human intestinal tract means it is also useful as a bulking agent in dietary foods such as lower calorie ice creams and desserts, as a fat replacement in food products such as meatballs and as the basis for the development of vegetarian meats (Ng and Shyu, 2004; Dufresne, 2012; Shi et al., 2014; Choi et al., 2022). Its high fibre content is also reported to have beneficial health properties such as decreasing the risk of obesity, diabetes, cholesterol, and cardiovascular disease (Torres et al., 2019; Choi et al., 2022). Traditionally, it is consumed in Southeast Asia as 'nata de coco' in the form of a gelatinous cube, synthesised as a static culture from coconut water and served in a sugar syrup (Chawla et al., 2009; Esa et al., 2014). The BC takes on the flavour of its growth medium and in recent times new desserts, such as 'nata de pina' (grown in pineapple juice) have become popular (Shi et al., 2014).

As previously mentioned, BC is also used directly in drinks, commonly known as Kombucha or Manchurian tea. It is thought to have originated in Japan and uses a symbiotic culture of yeast and bacteria (SCOBY) to ferment the tea and sugar liquid. The fermented liquid is consumed (not the cellulose) (Dufresne and Farnworth, 2000; Chawla et al., 2009). It is purported that the fermented tea has a variety of health benefits.

BC has also been explored as a potential alternative to plastic food packaging. There are rising concerns in the food industry regarding the waste generated from oil-based

packaging and biodegradable options are a viable alternative (Esa et al., 2014). BC is biodegradable and exhibits the required strength properties for such an end use. However, in its pure form it does not exhibit inherent anti-microbial or antioxidant properties, which are crucial for food preservation and restriction of contamination. BC composites have been explored to alleviate this issue (Gao et al., 2014), with BC / nisin composites addressing the anti-microbial (Nguyen et al., 2008) and BC / polylysine composites exploring biodegradability issues (Zhu et al., 2011). Composites of polylactic acid (PLA) and BC have also been tested showing good mechanical properties, whilst 'retaining transparency and biocompatibility' (Xiao et al., 2012; Shi et al., 2014: 543) whilst composites of BC / Chitosan have also been explored with limited success (Ashrafi et al., 2017). A study by Abral et al. (2020) compressed paper made from BC and impregnated this with ZnO, suggesting that the resultant improved properties of higher moisture resistance and tensile modulus made the composite an ideal candidate for exploration as food packaging.

Whilst active food packaging aims to increase the shelf life of food products via antimicrobial activity, it can also be used to indicate food safety (Shi et al., 2014). Kuswandi et al. (2014) added methyl red to BC film during manufacture, creating a sensor which changed colour as changes in pH due to microbial activity were detected. This, when used in the packaging of chicken, alerted consumers to the freshness of the meat. Kuswandi et al. (2012) also developed a BC / curcumin membrane that reacted with similar colour change on contact with volatile amines produced by out-of-date fish. In the same field, a BC / bromophenol blue film indicated the freshness of guava fruit (Kuswandi et al., 2013). A more recent study by Pirsa et al. (2018) suggests that using composites such as BC / polypyrrole / zinc oxide could lead to the development of active

food packaging, with preliminary studies showing links between the conductivity of the composite film and indicators of food spoilage such as volatile compound presence and pressure.

6.5 Paper

BC has found use as an enhancement to the performance of functional paper when added during the pulp phase as a binder (Huang et al., 2014; Torres et al., 2019). The microfibrillar structure can lead to a paper product with enhanced strength (Surma-Ślusarska et al., 2008; Campano et al., 2018) and durability (Dufresne, 2012; Hervy et al., 2018; Urbina et al., 2019). However, this is dependent on the effective dispersion of the BC microfibrils throughout the paper structure, an issue addressed in a study by Xiang et al. (2019) via the addition of additives such as glucomannan and polyethylene oxide. Additionally, by modification of the BC polymeric chain, other properties can be introduced. Basta and El-Saied (2009) used glucose phosphate as the carbon source during BC production, resulting in the addition of phosphorus to the BC polymeric chain. When this was added to the paper pulp, it significantly reduced smoke production and enhanced flame retardancy properties, without the production of toxic effluent (which is common in traditional processes). Studies have highlighted the potential of these developments in currency and high strength speciality paper (Iguchi et al., 2000), however, the costs of BC production are still a prohibitive factor to mainstream application (Lee, 2018).

In a study by Sriplai et al. (2018), layers of BC sheets with $CoFe_2O_4$ incorporated within the BC structure were used to create a white magnetic paper. This paper showed

advantages over magnetic papers currently on the market as it visually appears very similar to ‘regular’ paper, was magnetic on both sides and could be produced at a reduced cost. Additionally, the BC paper held its white appearance, which has not been achieved by other methods to date. Sriplai et al. (2018) suggest that this development could make a real contribution in the field of counterfeit detection.

Fluorescent papers are traditionally manufactured by coating a paper base with a fluorescent material. However, this is a costly process resulting in a finish with poor durability (Zhang et al., 2019). Using a composite of Eutropium (an earth element with good photoluminescent properties) and bacterial cellulose in combination with plant cellulose, Zhang et al. (2019) produced a paper displaying high fluorescent intensity whilst maintaining good stability and durability. Additionally, the combination with plant cellulose gave the material the stiffness properties associated with traditional paper products.

Luo et al. (2019) used graphene dispersed in BC sheets to create a composite paper, in addition to the enhanced strength properties already reported, this work developed a conductive yet flexible BC paper product. The researchers claim that this could have impacts in fields such as electromagnetic interference shielding and energy storage. Developments in this specific field are discussed in the next section.

6.6 Electrical

BC composites can be widely found in developments for flexible electronic substrates. Shim et al. (2019:1) suggest that ‘from the variety of compounds that might be incorporated into BC, …. conductive substrates represent an important market niche…

still poorly explored'. As electrical applications cover a variety of end uses, the following section aims to give a brief overview of the major findings in these fields.

6.6.1 Energy Storage

BC is non-conducting in its pure state, but conductive properties can be imparted via coatings such as polypyrrole (PPy) and polyaniline (PANI) (Huang et al., 2014), whilst Yoon et al. (2006) developed a BC conductive film via the incorporation of carbon nanotubes.

Super capacitors have been developed by Bai et al. (2019) by incorporating both graphene nanotubes and PPy to develop a flexible BC film and by Hu et al., (2016) using carbon nanowires derived from BC, whilst Hosseini et al. (2019) used a BC aerogel containing silver nanoparticles and polyaniline (PANI) to explore this field. The high degree of flexibility and good levels of conductivity combined with the enhanced mechanical properties of BC make this type of development suitable for exploration in wearable electronic applications (Bai et al., 2019). Studies by Shim et al., (2019) support this and suggest that combining polyaniline and BC provide a viable conductive alternative for 'technical textiles, wearables and other applications' (Shim et al., 2019:1)

Additionally, Celik et al. (2018) made use of the non-conductivity of BC in the development of lithium sulphur batteries, which are currently being explored for mobile technologies requiring high energy storage, such as electric car batteries.

BC membranes have also been used as flexible substrates in the manufacture of Organic Light Emitting Diodes (OLEDs). Legnani et al. (2019) used a BC composite as a substrate

coated with silicon dioxide and indium tin oxide, achieving results within 14% efficiency of those measured using traditional glass substrates. This suggests that a BC composite could be a viable proposition for flexible, biocompatible OLEDs.

6.6.2 Sensors

The large number of hydroxyl groups in the BC structure contribute to its high moisture absorbency and retention and it is this sensitivity to moisture that suggests BC could be used as a humidity sensor. Hu et al. (2011) and Jia et al. (2016) found success using this principle and created simple formaldehyde and ammonia sensors respectively by coating a quartz crystal microbalance with BC nanofibers. Both studies suggest that the BC sensors have potential to provide a real alternative to expensive monitoring equipment currently employed and suggested methods by which the sensors could be mass produced (Hu et al., 2011; Jia et al., 2016).

6.6.3 Acoustics

BC maintains 'high sonic velocity over a wide frequency range' (Ashjaran et al., 2013: 127) and has therefore found applications in audio speaker diaphragms. Acoustic membranes need a material with high Youngs modulus and high sound propagation velocity and paper was traditionally used because it can be processed into a lightweight membrane. In addition to the enhanced sonic velocity values, BC has good dimensional stability and better Youngs modulus than paper. The Sony Corporation of Japan found BC in this application to have superior performance (Torres et al., 2019), but the elevated costs of manufacture did not justify mainstream production (Iguchi et al., 2000).

6.6.4 Conductivity

In addition to the development of conductive BC paper by Luo et al., (2019) discussed above, other researchers have investigated the potential of BC composites as electrically conductive materials. Yoon et al., (2006) created electrically conductive BC membranes (grown from *Gluconacetobacter xylinus*) by incorporating carbon nanotubes. However, Marins et al., (2006) developed an electrically conductive BC membrane by coating the BC with polyaniline. Both studies suggested that whilst the membrane was found to be conductive, it could also have applications as an electromagnetic shield (Yoon et al., 2006; Marins et al., 2014)

6.7 Wastewater and Toxic Spill Treatments

The characteristics of high porosity, high tensile strength and high moisture absorbency place BC perfectly as a bio adsorbent (Zhu et al., 2011; Mohite and Patil, 2014a), with particular use as a heavy metal remover from wastewater (Torres et al., 2019). Bio adsorbency studies with heavy metals such as chromium (Kumar et al., 2009; Stoica-Guzun et al., 2016), arsenic (Ramezani et al., 2016), cadmium (Mohite and Patil, 2014a) and lead (Zhu et al., 2011) all showed some degree of success, leading to suggestions that this approach could be further widened to that of toxic spill clean-up and mineral recovery from oil mining. A study by He et al., (2018) used a three-dimensional skeleton of BC to support a silica aerogel. This structure displayed large compressive strain resistance, allowing shape recovery whilst exhibiting super-hydrophobicity and super-oleophilicity. The impact of this is excellent oil and organic solvent absorption rates with the ability to recover absorbed materials by mechanically squeezing the filter, leading to

the possibility of oil recovery and oil – water separation (He et al., 2018). A different approach was adopted by Luo et al. (2018), who combined a three-dimensional BC structure with two-dimensional graphene oxide to create an aerogel. The researchers reported a structure with 'multi-scaled pores and a large specific area' (Luo et al., 2018:824), which can absorb various oils and organic solvents and describe the potential of the aerogel as a 'superior all-carbon absorbent for environmental protection' (Luo et al., 2018:824)

6.8 Fabrics and Dyes

6.8.1 Garment Applications

As the textile and fashion industry increasingly focus on the idea of 'sustainable fashion' (Henninger et al., 2016), BC has been investigated as a candidate in this arena with items such as shoes, jackets and blouses being developed to a conceptual stage (Launch, 2019). Exploratory projects with groups such as fashion designers and professional milliners (Wood et al., 2022; Appendix 1) have illustrated the diversity of the potential applications in fashion and apparel through conceptual design led projects.

BC has often been referred to as 'vegetable leather' (as a potential replacement for animal leather) but research conducted as part of this thesis has indicated BC is considerably weaker in terms of tensile and tear strength when subjected to apparel standard tests, compared to animal leather counterparts of a similar mass and thickness (Chapter Six). Whilst early testing work suggests BC displays superior abrasion resistance properties compared to animal leather (Wood et al., 2023; Appendix 1), well documented hydrophilic properties of BC inhibit its use as an apparel textile (Rajwade et

al., 2015). Textiles with a high propensity to retain moisture cause high levels of wear discomfort in clothing (Fourt and Hollies, 2002; Almeida, 2015; Watkins and Dunne, 2015).

Nevertheless, there have been some studies exploring the use of BC in garments (da Silva et al., 2021). A common approach to minimising environmental impact in garment design is the 'zero waste' principle which employs pattern cutting and design techniques to ensure none of the materials used in construction are wasted (Ramkalaon and Sayem, 2021). To this end, a study by Chan et al. (2018) explored the development of BC pellicles on the surface of a tea liquid medium inoculated with a Kombucha SCOBY in a method similar to that discussed in section 2.5. The growth medium was incubated in a vessel prepared by an injection moulding technique to create a pot in the shape of a garment panel. Several vessels were made to reflect all the panel shapes required to create a shirt. In tandem to this, garment panel shapes were also developed in square vessels, this time the specific shapes created by 'blocking off' areas of the surface of the growth medium. This restricted the oxygen supply to the BC forming bacteria and thus only BC pellicle formed at the exposed areas of the surface. Both methods produced the panels of BC in the shapes required, thus achieving the goal of zero waste, and the authors claimed the panels could be manipulated and stitched in the same way as a traditional textile. However, the resultant BC garment needed to be lined to provide wearer comfort, and the issues of hydrophilicity remained problematic. To address the issues resulting from the hydrophilic nature of BC, Kaminski et al. (2020) treated BC sheets with glycerol and stearic acid. Whilst there were some term effects on the durability of the BC in terms of flexibility and water repellency, these effects were tested on wristbands and not full

garments; further work is required to fully assess the suitability of BC sheets in whole garment applications.

BC has also been investigated as a potential alternative for animal leather used in shoes. Lee (2023) first explored this concept, creating a shoe to explore the aesthetic capabilities of the material. However, there have only been further explorations in shoe applications using BC as a composite with synthetic chemicals such as perfluorocarbons, which reduce the hydrophilic properties of the BC (Fernandes et al., 2019). Whilst Fernandes et al. (2019) suggest the application of these chemicals renders BC a viable material for shoe manufacture, the composite nature of BC with synthetics negates its 'environmentally friendly' credentials and thus its impact in reducing the polluting nature of the textile and leather industries.

6.8.2 Textile Colouration and Processing

Whilst the enhanced hydrophilic nature of BC has limited the development of mainstream apparel (Wood, 2019), it has not inhibited the exploration of colouration techniques normally attributed to standard textile manufacture. Costa et al. (2019) dyed BC pellicles (before drying) with natural dyes such as those extracted from the *Hibiscus rosa-sinensis* flowers, reporting that natural pigments can be used successfully to colour BC sheets, eliminating the need for harsh polluting chemicals normally associated with textile dyeing. Similarly, Song et al (2018) explored the potential of flavonoids in the colouration of BC sheets, discovering strong depths of colour and high resistance to fading by washing. Traditional preparation for dyeing techniques has also been explored on BC sheets, with Han et al. (2018) reporting effective removal of residual impurities

from the growing process (e.g. bacterial cells) using treatments of NaOH and H_2O_2. However, this study also reported an increase in crystallinity of the BC sheets after treatment which could affect dye absorbency, although this has not yet been explored. Shim and Kim (2019) explored industrial dyestuffs – direct, acid and reactive – in the colouration of BC sheets, suggesting that more effective dye take up is achieved by adding the dyestuff to the growing medium (during pellicle development) than attempting to dye the pellicle after it has been formed. Reactive dyes (those which form a covalent bond with a fibre) produced the greatest dye exhaustion and deepest hues in this study.

Traditional colouration techniques of textiles often result in dyestuffs being discarded in effluent and there is increasing pressure across the global textile industry to address this practice and its harmful environmental effects (Muthu, 2018). Mohite and Patel (2014b: 106) state that BC 'is one of the new biosorbents ... showing renewable, biodegradable and biocompatible qualities.' This suggests that as well as the previously mentioned application of BC as a heavy metal adsorbent, BC could be used as a potential effluent filter in the textile industry, removing excess dyestuff before release into the water system. Experiments with aniline Blue (Mohite and Patil, 2014a) and Dimarene Red (Vyjayanthi and Suresh, 2010) both showed 90% effluent decolourisation in under 30 minutes.

6.9 Summary

BC has been explored by a diverse range of industries and product manufacturers as an innovative material with the potential to relieve some of the pressures of waste reduction and end of life product disposal due to the ease with which BC biodegrades. As

illustrated in part one of this thesis, BC can be manufactured in a sustainable manner and replicated over multiple generations with little impact on the yield or the properties of the resulting pellicle.

- Biomedical applications appear to be the most researched, advanced, and successful areas of use, due to the observed biocompatibility of BC with human tissue. However, whilst the uses in this area are diverse and widespread, none of the literature refers to the sustainable manufacturing or the end-of-life disposal credentials of products developed in this field.
- Cosmetic and beauty applications have found use for BC in products such as facemasks due to its superior moisture retention properties. However, whilst this industry is also scrutinized for its single use products and therefore huge amounts of waste generated, there is no evidence in the literature to suggest these credentials for BC are being utilized.
- Paper, electronics, sensors, and acoustics have all explored BC from the 'novel materials' perspective, rather than as a material with any specific environmental impact credentials. In these fields, many of the applications identified required BC to be a composite material to ensure fitness for purpose.
- Food applications have identified the potential of BC to impact on their environmental footprint. Whilst already used widely in foodstuffs, BC shows promise in biodegradable food packaging.
- It should be noted that in all the applications discussed above, BC has been developed using pure culture types in laboratory manufactured media (except for beauty face masks, where food wastes were explored).

Fashion and textiles have been highlighted as one of the most polluting industries on the planet (Ellen MacArthur, 2017). Alongside its novelty as a textile, BC has also been evaluated from a sustainable angle, with its relatively low natural resource requirements, ability to be developed with zero waste, and ease of end-of-life disposal very attractive to both product developers and manufacturers. Additionally, the production of BC for clothing has been approached from a sustainable angle, using food waste, or naturally occurring liquids (such as fruit juices) to support pellicle development, and often using a reproducible Kombucha SCOBY as an inoculum in an uncontrolled environment, rather than a pure culture which requires costly carefully controlled conditions for pellicle growth. Nevertheless, BC still encounters problems in apparel applications due to its high hydrophilicity. Whilst the hydrophilic issue can be addressed by using synthetic finishes well established in textile and clothing production (usually based on hydrocarbons) this does negatively affect the environmental impact of the finished material. There are some 'natural' solutions, such as beeswax, which would not impair the environmental credentials of BC, but these are generally less durable than their synthetic counterparts.

Nonetheless, there has been very limited research in terms of BC performance as an apparel textile. Most research to date has focused on the design and creation aspects of apparel, but there has been no consideration of the performance characteristics of BC in this application. The next chapter of this thesis will address this issue and explore the performance characteristics of BC when compared to textiles used in apparel applications.

Chapter 7: Part 1 - Evaluation of the Properties of Bacterial Cellulose Pellicles Using Textile Testing Methods

7.1 Introduction

The previous chapter reviewed the applications of BC revealing that current and predicted uses are varied, ranging from biomedical (Mohite and Patil, 2014a), food and packaging (Chawla et al., 2009; Pirsa et al., 2018), paper (Torres et al., 2019), electronics (Shim et al., 2019) through to textile applications (da Silva et al., 2021). In biomedicine, BC has been explored in depth and is already widely used in wound dressings and tissue engineering. However, in terms of textile applications, although BC has been explored by artisans as a fashion material (and a suggested animal leather alternative) there is very little research on its performance properties as an apparel textile.

Part one of this thesis focused on the most efficient methods to reproducibly manufacture BC pellicles. A review of literature and small batch laboratory-based techniques revealed that a liquid medium comprising sterile black tea and sugar inoculated with Kombucha SCOBY and incubated at either 30 °C or room temperature for 30 days reproducibly produced the greatest yields by pellicle weight. Observations of 16s rRNA sequencing data showed that the microbial community in the Kombucha SCOBY reached a stabilisation point after approximately 30 days incubation and that the most effective sub - culturing to reproducibly manufacture further BC pellicles was achieved using Kombucha SCOBY liquid inoculum.

This chapter contains peer reviewed published work (Wood et al., 2022) (appendix one) and is split into two parts. In the first part of this chapter, BC pellicles are produced using the methods described in part one of the thesis and the resultant material is explored under the lens of textile testing for apparel. The properties of the pellicle are evaluated using textile testing methods to enable identification of potential uses of bacterial cellulose pellicle as a textile. In the second part of this chapter, the hydrophilic nature of the BC pellicle is explored as a potential material for the reduction of colour in dyehouse effluent.

7.2 Experimental Details

7.2.1 Bacterial Cellulose Sheet Production

A Kombucha SCOBY pellicle was purchased from Happy Kombucha (2022) as the starter culture for the experiments. 100 g of SCOBY was placed in a 500 mL sterile pot containing 250 mL sterile H&S medium and stored for 15 days in a 30 °C incubator to produce a standardised inoculum. After 15 days any solids were removed from the pot by filtration (section 3.5.1) and the remaining liquid broth used as an inoculum.

As previously discussed in part one of this thesis, BC growth is supported in a variety of media, and sterile black tea with sugar has been shown to produce the greatest yield by weight. Therefore, for the textile testing evaluation, 5 L sterile tea broth was inoculated with the standardised liquid inoculum in a ratio of 1: 10 (inoculum: tea) in sterile plastic containers (170 (l) x 50 (d) x 110 (w) mm). Lids were loosely placed on the containers, and the containers incubated for 30 days at 30 °C to create larger sheets of BC than those produced previously in this thesis. After 30 days the sheets were removed from the pots,

rinsed with water, and left to dry on a bench top in ambient conditions for 1 week, to constant weight. In total, five sheets were produced.

7.2.2 Selection of Samples for Comparison

When manufacturing apparel, textiles are chosen in line with both physical performance and comfort properties to meet the wearer's expectations. The tests to evaluate these properties are selected with consideration of the physical structure of the textile, alongside the desired properties for analysis.

As BC has been introduced to apparel as a 'vegetable leather' (Wood, 2019; Lee, 2023) it was deemed pertinent to compare its properties to traditional animal leathers used in clothing, thus two types of cow hide (a 'natural grain' skin and a napped surface suede) were selected for comparison. Additionally, under an electron microscope, BC structure is revealed as that of a dense mat of microfibrils, and therefore structurally not comparable to either knitted or woven fabrics commonly found in apparel. However, the microfibrillar structure is similar to that found in non-woven products (such as felted wool or fusible linings used in apparel) and therefore its performance was compared to non-woven fabrics in this evaluation. Three non-woven fabrics were selected for comparison, a plant-derived 100 % cellulose fibre sheet (mechanically manufactured), a 100 % wool felt (mechanically manufactured) and a polyamide (PA) sheet (thermally and chemically manufactured). The comparison fabrics were all sourced from university stores.

When selecting the 'traditional' fabrics for comparison to BC, care was taken in selection to ensure that fabrics of a mass per unit area and thickness were as close to that of the

BC samples used in the test to reduce any variability in performance that could be attributed to these factors.

Figure 26 presents the fabrics used in the analysis of performance properties and their mass per unit area and thicknesses. Mass per unit area was measured in accordance with BS EN 12127 (1998) using a Ohaus precision balance (Scales and Balances, UK). Fabric sample thickness was measured in accordance with BS 2544 (1954) using an Oxford thickness gauge (Cromwell, UK). Visual appearance of the surface of each sample was recorded in daylight at 2 x magnification using a Samsung SM-N950F camera.

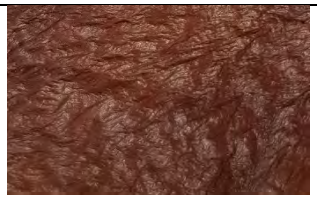
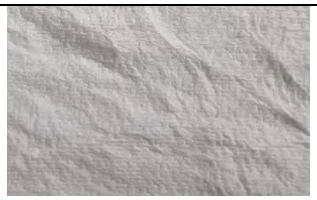
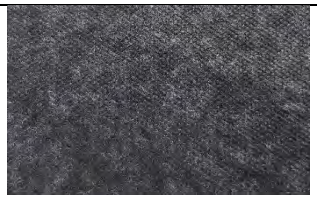
Sample (image x 2 magnification)	Mean (n = 3) mass per unit area (g/m^{-2})	Mean (n = 3) thickness (mm)
Bacterial cellulose 	323.67 ($\sigma = 2.87$)	0.62 ($\sigma = 0.14$)
Animal skin (suede) 	234.64 ($\sigma = 0.47$)	0.60 ($\sigma = 0.02$)
Animal skin (leather) 	685.00 ($\sigma = 0.82$)	1.31 ($\sigma = 0.06$)
Cellulose nonwoven 	178.67 ($\sigma = 2.36$)	1.88 ($\sigma = 0.14$)
Polyamide nonwoven 	208.33 ($\sigma = 0.47$)	0.95 ($\sigma = 0.03$)
Wool felt 	169.00 ($\sigma = 2.83$)	1.09 ($\sigma = 0.04$)

Figure 26. Mass per Unit Area and Thickness of Samples.

(σ = standard deviation).

7.2.3 Selection of Tests for Evaluation

The claim that BC could be used as a replacement for animal leather (Lee, 2023) suggests that it could be used for specific purposes in apparel, such as outerwear (e.g., jackets and coats). Apparel performance testing for outerwear consists of assessing characteristics such as strength (tensile), resistance to abrasion and, in some cases, resistance to water. However, assessment of physical performance in apparel is only part of the picture. The comfort of the wearer is an essential part of garment development and therefore fabric selection. Critical considerations pertaining to wearer comfort are moisture vapour transfer (breathability) and moisture retention (Watkins and Dunne, 2015). Therefore, to assess BC as a potential textile for apparel, tests were selected to evaluate reaction to moisture, abrasion durability and tensile strength and elongation. All test methods were selected and performed in line with commonly used industry standards described in the following sections.

7.2.3.1 Tensile Strength and Elongation (BS EN ISO 13934)

In accordance with the specified method, three specimens were taken at randomized points from each sample fabric. Samples were marked on the fabric using a rectangular template (300 mm x 50 mm) and cut using scissors. The samples were mounted on the tensile tester (Instron, UK; model 33R4465), with a jaw separation width of 200 mm. The machine was set to an extension rate of 100 mm/min and all specimens were extended to breaking point with maximum force at break (N) and breaking elongation (mm) recorded.

7.2.3.2 Martindale Abrasion (BS EN ISO 12947-1)

In accordance with the specified method, three specimens were taken at randomized points from each sample fabric. Samples were marked on the fabric using a circular template (40 mm diameter) and cut using scissors. The samples were mounted on the Martindale Abrasion tester (James Heal, UK: model NU 864) in accordance with test standard BS EN ISO 13934 and the machine set to rub at 5000 revolution intervals against an abrasive cloth (James Heal, UK: original SM25), with a 9 kPa weight per specimen. The specimens were assessed after each 5000 rubs and removed at the point of destruction with the number of rubs recorded.

The test was repeated using a 12 kPa weight for the animal skin and BC specimens only and specimens were assessed after each 5000 rubs and removed at the point of destruction with the number of rubs recorded.

7.2.3.3 Taber Abraser (BS EN ISO 17076-1:2020)

In accordance with the specified method, three specimens were taken at randomized points from the animal leather, animal suede and BC sample fabrics. Samples were marked on the fabric using a circular template (100mm diameter) and cut using scissors. The samples were mounted on a Taber Abraser (Taber Industries, USA; model 5135), using Calibrase CS-10 (Taber Industries) abrasive wheels, 1000 g weights and a textile specimen clamp with double-sided tape to ensure no movement of the sample on the mount during the test. The appearance and weight of each sample was assessed and noted before the test and at 1,000 revolution intervals until the sample broke down or a total of 10,000 revolutions was reached.

7.2.3.4 Breathability: Water Vapour Transmission (BS 7209: 1990)

In accordance with the specified method, three 90 mm diameter circular specimens were taken at randomized points from each sample fabric and individually weighed. Each sample was mounted on an individual pot containing 40 mL distilled water and a gauze cover affixed covering the fabric sample and the pot. Each pot was weighed, mounted on the rotating disc for 24 hours, removed and reweighed, in accordance with test standard BS 7209:1990. The fabric samples were removed from the pots and reweighed immediately.

The water vapor transmission (WVT) of each fabric was calculated by comparing the mass of the pot at the start and end of the test and using the following calculation:

$$\mathrm{WVT}=M/A$$

where M is the mass lost (g), d is the diameter *(mm)* A is the area (m^2) ($A = (\pi d^2/4) * 10^{-6}$) of the dish (mm).

As an additional measure in this test (additional to the standard test method), each fabric sample was weighed before and after the test was completed, and these weights compared to establish the moisture retention of the material. Water vapor absorbed by the fabric and still present at the end of the test was calculated as a percentage mass by weight as follows:

$$\frac{MET-MST}{MST} * 100$$

where *MST* is the mass of the sample at the start of the test (g), *MET* is the mass of the sample at the end of the test (g).

7.2.4 Results and Discussion

7.2.4.1 Tensile Strength and Elongation

The most common tests for establishing the tensile strength and elongation of textiles for clothing use one of two techniques, a strip or a grab test. A strip of fabric of a standard measurement is fixed between the jaws of the machine (set at a predetermined distance apart) and the jaws moved apart, subjecting the fabric to a force until the sample ruptures. In the grab test, the rate at which the jaws move apart is set so that the sample ruptures in 20 (+/- 3) seconds. The strip test moves the jaws apart at a constant rate until the point of fabric rupture. There are slight nuances in sample preparation for both tests, depending on the fabric structure.

As there was no information in previous literature to indicate that BC had been tested as a textile for clothing, there was no pre-existing information to indicate the most suitable test method. However, the strip test (cut strip method) is commonly used for both non-woven and leather fabrics, so this method was selected for evaluation of BC. As this method uses a constant rate of extension (CRE) (500 mm / min), this allowed the mode of breakdown of the samples to be observed.

The animal leathers and BC sheet all performed in a similar fashion in terms of rupture under tensile force. 'Clean' breaks at failure were observed in all samples (Figure 27). However, the force required for rupture was considerably lower for the BC sheet (0.196kN) than either of the tested animal leathers (0.844 kN for leather, 0.515 kN for

suede) or the force to rupture animal leathers found in existing literature (Von Hoven, 2002). Additionally, elongation at break was lower for the BC sheet than the animal leather or suede (17.85 mm, 29.77 mm, 59.32 mm respectively) (Figure 28).

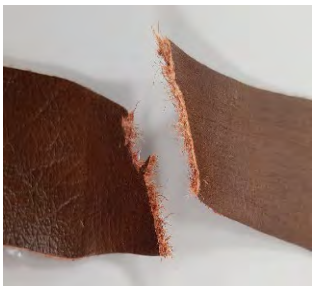
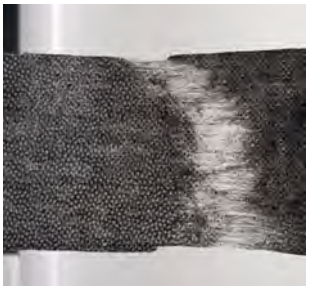
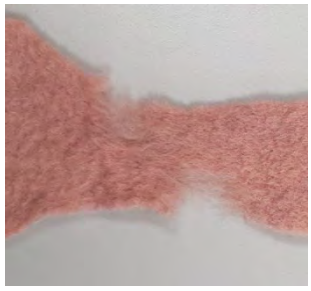
Bacterial cellulose	Animal skin (leather)	Animal skin (suede)
		
PES non-woven	**Wool felt**	**Cellulose non-woven**
		

Figure 27. Physical Sample Breakdown Appearance After Tensile Test.

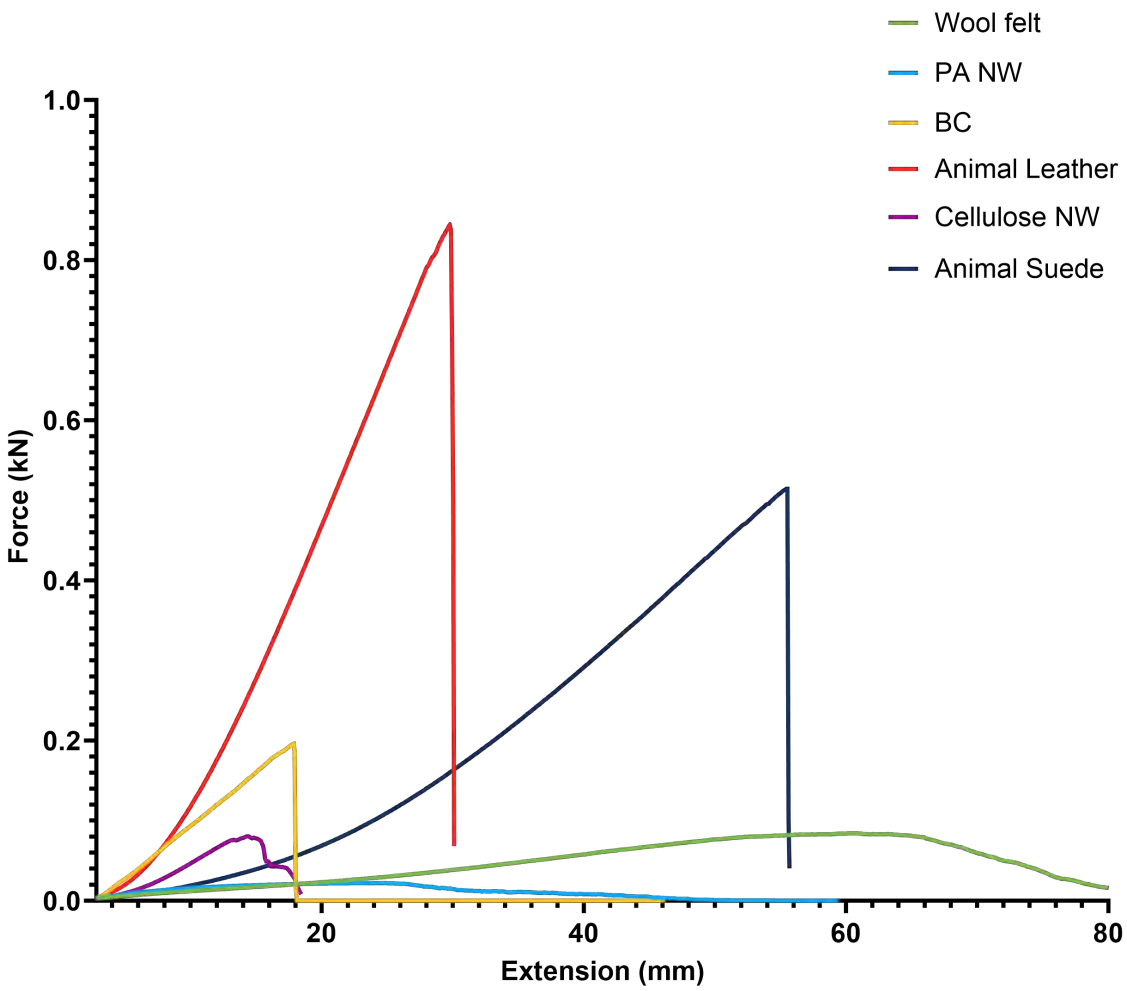


Figure 28. Tensile Strength and Elongation Performance at Break of Samples.

The wool, PA and cellulose non-woven fabrics all displayed gradual breakdown at low force (rather than the abrupt rupture of the leather and BC samples, indicated by the sudden drops in the plots displayed in Figure 28). This is unsurprising as non-woven structures do not usually display high tensile strength characteristics. However, it should be noted that the mode of breakdown is different from that of the BC, suggesting that in terms of tensile strength, it is not appropriate to consider BC as a non-woven textile, regardless of its nanofibrillar structure.

However, whilst the results of the tensile and elongation tests suggest that it would not be appropriate to use BC as a replacement for animal leather in applications such as outerwear or close-fitting garments, it could be used in looser clothing, where resistance to tensile stress is less critical.

7.2.4.2 Abrasion Resistance

Textile testing for apparel uses the Martindale abrasion test to establish the potential of the fabric to 'wear out' over a lifetime of use, for example under the arms where sleeves rub against a garment body, or between the legs where thighs rub together in trousers, thus assessing the quality and consistency of the fabric. The test consists of the fabric sample mounted in a holder and rubbed against a standard abradant cloth under a standard pressure (specified in the standard test method, either 9 kPa or 12 kPa), for a predetermined number of cycles. The rubbing action follows a Lissajous figure to maintain consistency of abrasion across the fabric and abradant cloth. Industrial standards for fashion apparel set Martindale abrasion standards (at 9 kPa pressure) at 20,000 rubs before failure (fabric breakdown); higher performance garments (such as workwear or protective garments) can approach pass standards of 50,000 rubs. However, for textiles in which a higher abrasion resistance is required (for example upholstery applications), Martindale testing can be performed at a higher pressure (12 kPa), or a harsher test method can be adopted, such as the Taber Abrader test.

The Taber Abrader test is usually reserved for applications where exceptionally high abrasion resistance is required (such as plastics, ceramics, carpets, and leather), as it is a more destructive test using a series of abrasive papers. In contrast to the Martindale

method, the Taber Abrader test sample is cut into a disc shape and mounted on a turntable. Two abrading wheels (covered with standard abrasive paper) are lowered onto the disc. The turntable rotates, moving the abrasive wheels in opposite directions thus creating a motion that allows abrasion at all angles of the test specimen. The device is also fitted with a vacuum hose placed close to the abrasion site, removing any debris that may alter the abrasive action. Additionally, the abrasive wheels are balanced to ensure consistency of load across the test specimen. Each wheel applies a 250 g load to the specimen; this load can be increased by the application of additional auxiliary weights to the wheels. Common practice to assess the deterioration of specimens due to the abrasive action of the test can be performed visually, or the specimen can be removed and weighed at predetermined points in the test to assess the loss of mass of the material.

Non-woven fabrics are well known for their poor abrasion properties in comparison to 'traditional' textile structures (such as knit or woven). When testing using the Martindale system, the non-woven fabric broke down rapidly; cellulose at 5000, PA at 10000 and wool felt at 15000 rubs (9 kPa). This performance behaviour concurs with the literature (Textor et al., 2019). No further abrasion testing was performed on the non-woven samples.

However, animal leather is noted for its resistance to high level of abrasion (BNP Media, 2011). The BC, animal leather and animal suede all resisted abrasion to a high number of rubs in the Martindale test at 9 kPa; animal leather and suede showed signs of breakdown at 90,000 rubs at 9 kPa, whilst at 150,000 rubs BC still maintained structural integrity and thus the test was abandoned. When the Martindale test was performed at

12 kPa pressure, failure points for BC, animal leather and animal suede were observed at 105,000, 92,000 and 15,000 rubs respectively. Apart from the suede, this still showed a high degree of abrasion resistance. However, it should be noted that 15,000 rubs at 12 kPa is still in line with acceptable industry standards for some types of home furnishings (such as bedding or curtains) (Figure 29).

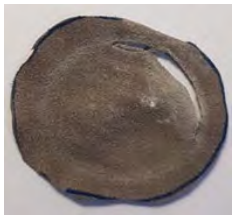
	BC	Animal skin (leather)	Animal skin (suede)	Cellulose nonwoven	Polyamide nonwoven	Wool felt
Rubs to destruction @ 9 kPa	150,000* 	90,000 	90,000 	5,000 		15,000 
Rubs to destruction @ 12 kPa	105,000 	92,000 	15,000 	n/a	n/a	n/a

Figure 29. Physical Appearance of Samples at Points of Breakdown after Martindale Abrasion Test.

(* = test abandoned)

The BC, animal suede and animal leather were then subjected to the Taber Abraser test. As the suede broke down earlier than the other specimens, it was unsurprising that it reached a point of failure at 2000 revs at 100 g load, much earlier than the BC or animal leather samples. Whilst the animal leather showed signs of surface disruption at 1000 revs, breakdown of the textile did not occur until 10,000 revs, when all the surface of the leather had been removed. At this point, the BC material was still intact, albeit showing some degree of abrasion. The BC and animal leather showed a steady and comparable degree of mass loss throughout the test (Figure 30). The test was abandoned at 10,000 revs as the animal leather had failed, thus BC had been shown to be superior in terms of abrasion resistance (Figure 31). As an indication of performance level, European standards (EN388-2016) dictate that a textile should withstand over 8000 revs at 1000 g if it is to be considered suitable for protective gloves against mechanical risks (such as protective motorcycle gloves). The BC exceeded the performance expectations of this standard.

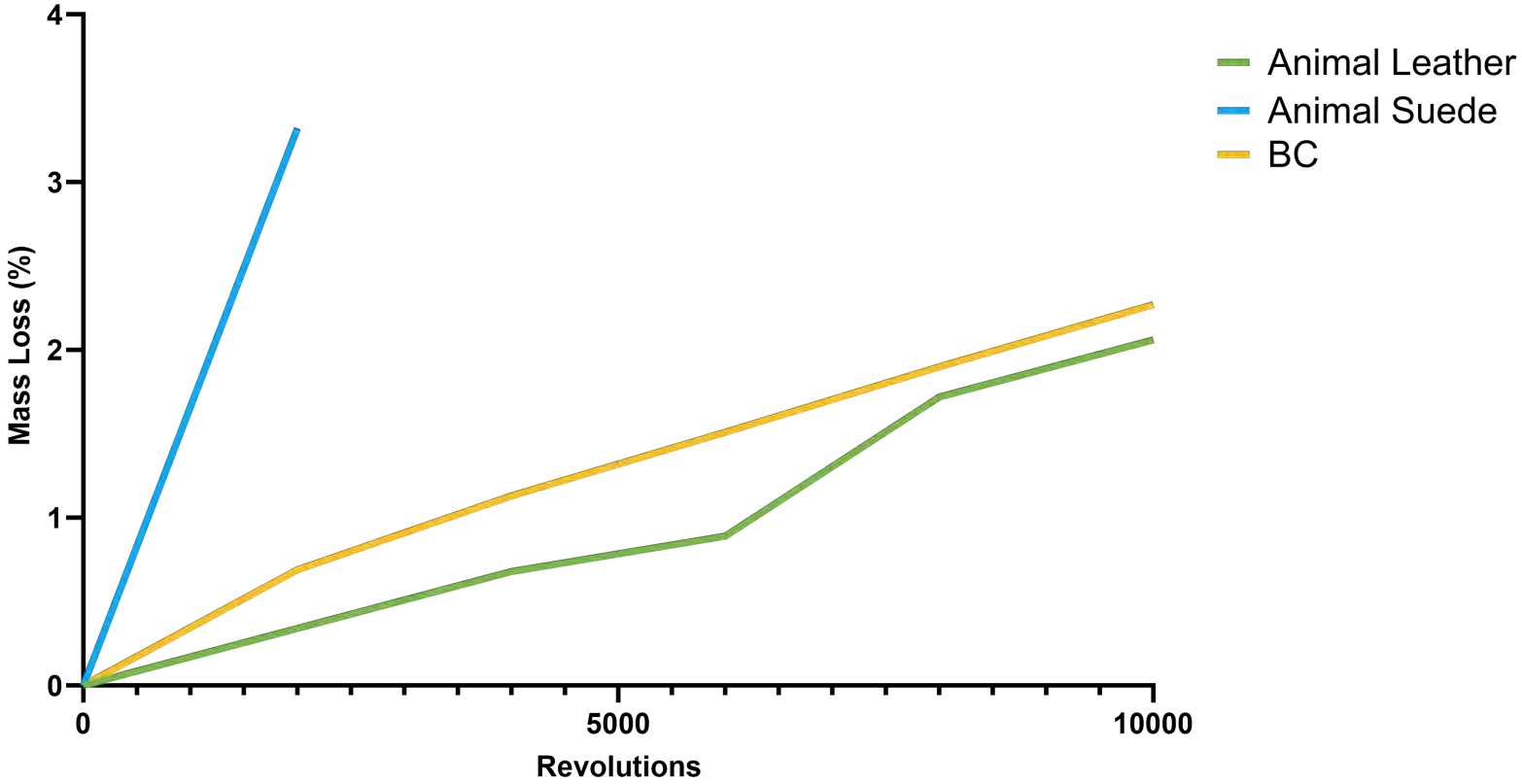


Figure 30. Taber Abraser Sample (n = 3) Mass Loss Throughout Duration of the Test.

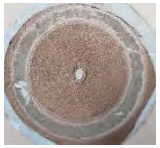
	Before test	1000 revs	2000 revs	3000 revs	4000 revs	6000 revs	8000 revs	10000 revs
Brown leather								
Bacterial Cellulose								
Suede				n/a	n/a	n/a	n/a	n/a

Figure 31. Physical Appearance of Samples at Points of Breakdown after Taber Abrader Test.

7.2.4.3 Moisture Performance

When discussing the reaction of textiles to moisture, it is important to first define key terms used in this field.

Waterproofness is the impermeability of the textiles to any penetration of water in either liquid or gaseous form. Waterproofing in textiles is commonly addressed by the application of a coating such as polyurethane or polyvinyl chloride, creating a solid barrier to oils, aqueous liquids, and gases (Williams, 2018). As the coating is nonporous, it prohibits any breathability characteristics and is therefore seldom used in apparel contexts due to the resultant comfort issues (section 7.2.3) (Özek, 2018). Waterproof textiles are commonly found in technical applications such as awnings or inflatable boats, and only in apparel for extreme weather protective clothing such as rainsuits for commercial fishermen (Özek, 2018). Textile waterproofness is measured by resistance to hydrostatic pressure.

In apparel, the term *waterproof* is often used when referring to clothing, when the garment is water *resistant*. The difference is that the garment is not completely impermeable to water in liquid or vapour form but can repel liquid water for the working period of the garment. For example, a fashion raincoat may be marketed as 'waterproof' as it offers protection from a light rain shower but will not offer protection from extreme weather conditions (such as those experienced during winter hillwalking). Conversely, a textile used in a hill walking jacket will have a much higher resistance to water but will also have specific elements of garment construction engineering to further enhance its protection for the wearer against the elements (such as taped seams or waterproof zips).

Inherent water repellency is achieved by considering the textile structure and composition. Whilst many synthetic fibres (such as polyester) are hydrophobic, this will not impart water repellency alone; the structure of the textile must be dense enough to not allow liquid water through. For example, cotton, whilst an absorbent fibre, can still offer a degree of water repellency when woven into a dense structure, such as Ventile® (McLoughlin and Sabir, 2017). Water repellency, if not inherent, can be imparted onto a textile by a finishing process. The finishing process usually involves the application of a synthetic chemical, often fluorocarbon or silicone based, both of which are documented for their poor environmental impacts (Williams, 2018). Natural alternatives to these chemicals do exist, such as beeswax but this is less durable than its synthetic counterparts.

Water vapour transmission (WVT) (also referred to as *moisture vapour permeability* (MVP)) measures the rate of water vapour transmission through a material; in textiles, this is referred to as the breathability of the fabric. In apparel applications, breathability of textiles is important as it contributes to the comfort of the clothing. To ensure wearer comfort, moisture vapour emitted by the skin (perspiration) needs to escape through the garment to assist the body in cooling; if the moisture cannot escape, it builds up in the area between the garment and the skin (referred to as the microclimate). As the relative humidity of the microclimate increases, the thermal conductivity also increases and makes the clothing uncomfortable to wear (Qu and Ruckman, 2006; Watkins and Dunne, 2015).

In modern performance wear, the sweating guarded hotplate is commonly used to measure the breathability of textiles used in apparel. This is also referred to as the ‘skin

model' and is capable of measuring both thermal and moisture vapour properties of a textile worn against the body (SDL Atlas, 2023). Thermal and moisture vapour characteristics are considered in tandem in this test and are considered co-dependent when evaluating the comfort properties of a textile used in clothing (Huang, 2006).

However, although this test is considered the most accurate in terms of evaluating clothing comfort from a moisture transmission perspective, it requires a relatively large fabric sample size (300 x 300 mm^2) and complex equipment to be performed. In contrast, the upright cup or turl dish method requires smaller fabric samples (90 mm diameter) and is comparatively straightforward and inexpensive to carry out (McCullough et al., 2003). Additionally, the turl dish method is commonly used in fabric samples that are not considered for use in performance wear i.e. it is assumed the wearer is not excessively perspiring and the skin is in a state of equilibrium in terms of moisture loss (McCullough et al., 2003; Huang and Qian, 2008). The test is therefore deemed appropriate for 'fashion' apparel.

The turl dish test consists of filling small pots with water and sealing the fabric to be tested over the surface of the pot. The pot is then mounted on a turntable that rotates for 24 hours. The pots are weighed before and after the test and the change in weight determines the amount of water lost over the 24-hour period, which is used to calculate the breathability of the fabric.

In clothing the test is used to assess the rate at which perspiration moisture can move away from the body, through the clothing and evaporate out into the atmosphere (Hu, 2008).

However, as an additional measure in this work, the fabric samples were weighed individually before and after the test to establish how much moisture was physically retained in the fabric structure.

In this test, all the traditional non-woven structures tested as the most breathable, with mean results of 647.10, 586.32 and 690.06 g/m^{-2}.24h obtained for the cellulosic, PA and wool fabrics respectively (Figure 32).

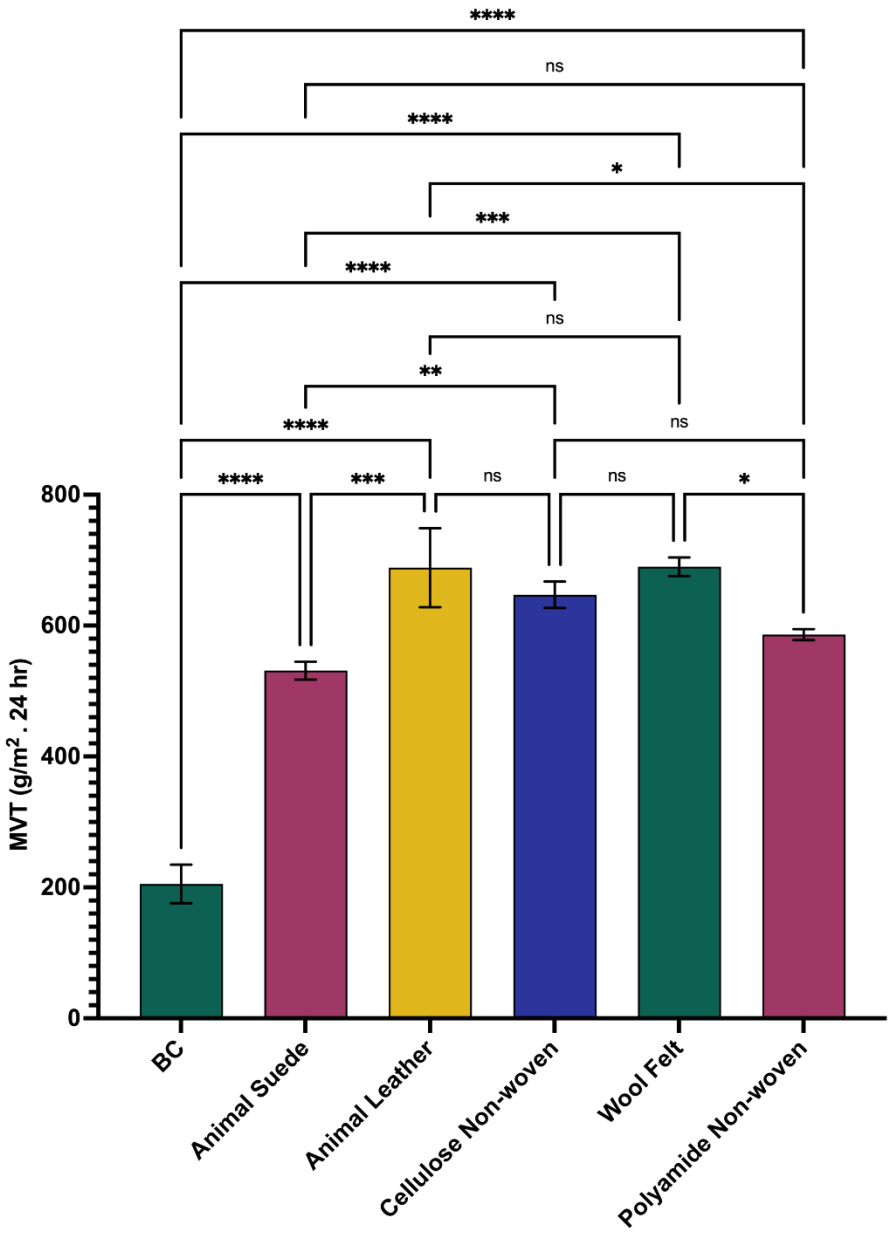


Figure 32. Moisture Vapour Transmission (MVT) of Fabric Samples.

(n = 3 for each fabric, error bars = standard deviation, ns = not significant, * = p < 0.05, *** = p <0.001, **** = p < 0.0001)

This was unsurprising as these types of non-woven structures have large gaps between the fibres, allowing the moisture vapour to pass through easily. Whilst animal leather and suede displayed similar breathability characteristics (animal leather 688.49 $g/m^{-2}.24h$ and animal suede 531.30 $g/m^{-2}.24h$) to the non-woven samples, it was the BC material

that gave the significantly lowest readings ($p < 0.0001$) in the test, 205.40 $g/m^{-2}.24h$. indicating the lowest level of moisture vapour transmission of all samples tested. This can be explained when looking at the residual moisture levels in the samples at the end of the test (Figure 33).

Animal fibres are susceptible to atmospheric moisture absorption with the wool felt and both animal skins retaining between 3.18% and 5.31%. However, at the end of the test, BC material retained 14.67%, a significantly ($p < 0.0001$) higher amount of moisture than the other samples tested. Whilst the ability of the BC to absorb moisture is not surprising (this feature is well documented in the literature, section 3.1.3), it does present an issue when considering the BC for clothing because if a fabric does not allow moisture to readily move from the body and out into the atmosphere, wearer discomfort is caused. However, this characteristic could be advantageous in other applications where a high levels of moisture absorbency is required, such as beauty, sanitary or wound dressing applications.

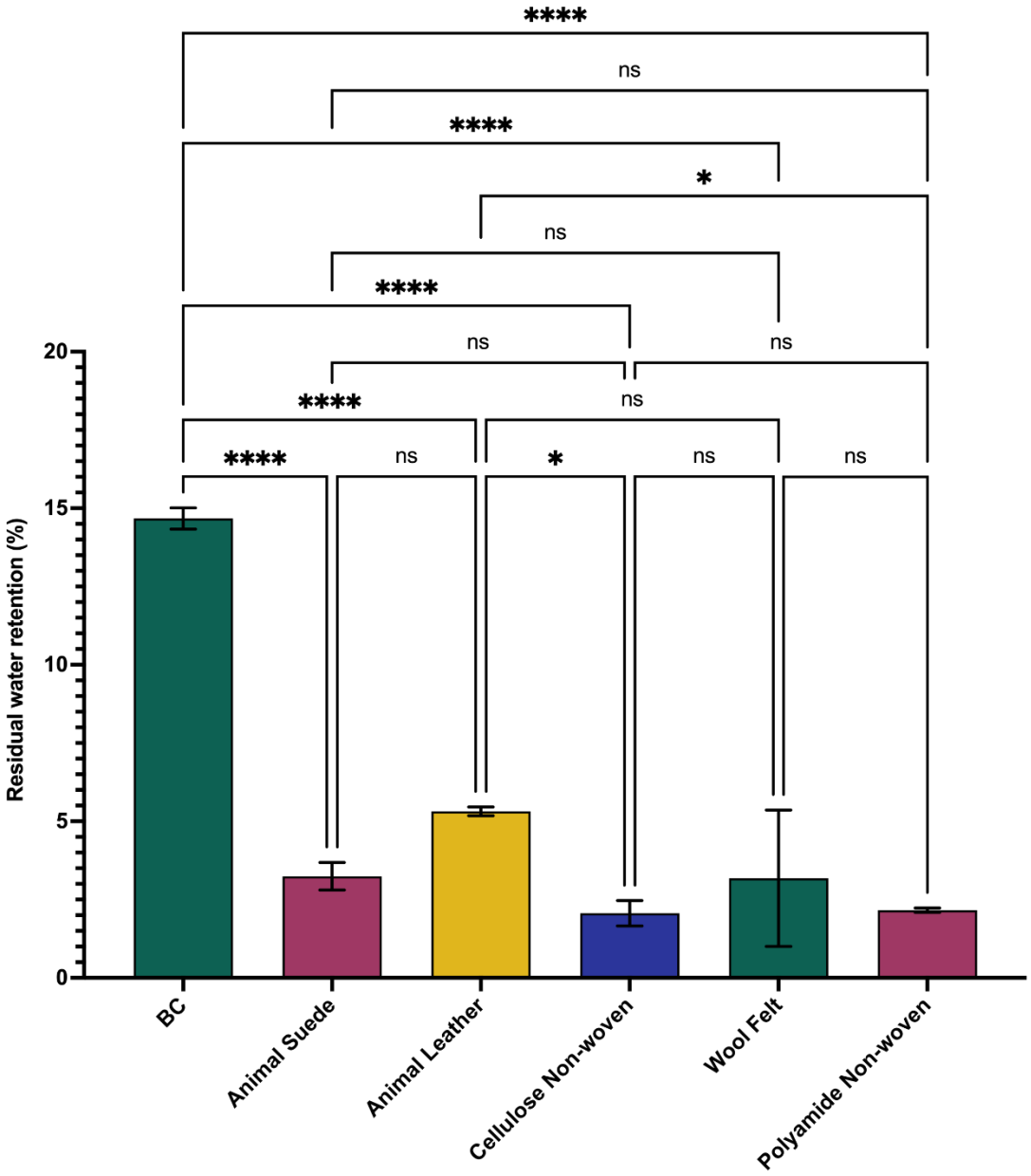


Figure 33. Residual Water Retention of Samples After Moisture Vapour Transmission Test.

(n = 3 for each fabric, error bars = standard deviation, ns = not significant, * = p < 0.05, **** = p < 0.0001)

7.3 Summary

- This section focussed on the use of BC in apparel, using standard textile testing methods performed in industry to assess the suitability of the textile for a specific application. As BC has been purported as a replacement for animal leather in fashion apparel it was tested as such; durability was assessed using tensile and abrasion testing whilst moisture transfer properties were evaluated as one of the key indicators of wearer comfort. Under a microscope, BC is a mat of random microfibrils, similar to the formation of fibres found in non-woven fabrics, albeit its physical appearance is leather-like. Thus, the performance of the BC was compared to both leather and non-woven structures.
- Tensile strength and elongation testing revealed that BC behaved in a similar way to leather (and not non-woven structures) in that it broke down abruptly, albeit under considerably less force than the animal skins used in the test. This suggests that BC would not be suitable for close fitting garments, but may be suitable in looser fitting designs, where tensile strength is not a prohibitive factor.
- The moisture vapour transfer properties of the BC were much poorer than any of the other samples tested, indicating that the BC would impart a level of wearer discomfort if used in clothing, particularly clothing worn directly against the skin. However, there could be a potential application in looser fitting outerwear, where moisture transfer or repellency is not a key consideration (such as fashion clothing without wet weather protection).
- BC showed exceptionally high abrasion resistance, considerably outperforming all the other samples tested and exceeding one of the most rigorous European standards for abrasion resistance in clothing textiles. This finding suggests that

there could be an application for the BC in protective clothing. However, the issues of tensile strength and moisture absorbency would need to be addressed if this type of clothing were close fitting; textiles for garments must have a balance of the properties required by the end user / application (Williams, 2018). Nevertheless, this issue could be remedied by lamination of the BC with another textile, or the creation of a composite as discussed in chapter five, for end uses such as energy storage and sensors. Whilst this has potential to impart greater strength to the BC pellicle, the high hydrophilicity could still cause problems in apparel due to a reduction in wearer comfort (section 7.2.4.3). One suggestion could be to encapsulate the BC to prevent moisture ingress, whilst still retaining the high abrasion characteristics thus rendering the BC useful in garments such as motorcycle wear in which there are areas of padding design for impact protection and abrasion resistance.

As has been noted previously, fashion and apparel are just one area in which BC could be used. As BC pellicles have not previously been tested using standard methods to assess performance in apparel, this work provides useful indications of the characteristics of BC pertinent to the apparel context.

The relatively poor strength performance and moisture vapour transfer renders the BC unsuitable for apparel applications without (potentially synthetic) intervention which could affect the sustainable aspect of the BC in terms of production and environmental impact. The aim of this thesis was to explore the use of BC in textile applications whilst

making use of its sustainable credentials. Therefore, synthetic treatments and modifications will not be considered in the context of this study.

However, hydrophilicity is an outstanding property of the BC pellicle and whilst this is a disadvantage in apparel, it could be capitalised in other applications. Chapter five of this thesis reviewed the current uses of BC and reflected on applications, such as beauty and medical textiles, which take advantage of its hydrophilic nature. Nevertheless, the hydrophilic feature suggests BC could also have applications which could impact the global textile industry without it being considered as a textile itself. As discussed in the introduction to this thesis, environmental pollution (for example the coloured effluent discharge from dyehouses), is a problem for the global textile industry. This leads to the suggestion that the hydrophilicity of BC could be used in the treatment of such effluent; this hypothesis will be explored in the next part of this chapter.

Chapter 6: Part 2 – A Preliminary Investigation of Bacterial Cellulose Pellicles as Colour Removers from Dyehouse Liquid Effluent

7.4 Introduction

Previous chapters in this thesis have discussed the devastating impact of the textile industry in terms of waste fabrics and garments. However, another area of the industry which causes severe environmental damage is that of the disposal of the by-products of textile manufacture (Al-Tohamy et al., 2022). Environmental pollution in the textile industry due to the discharge of liquid effluent is a global issue, one which varied approaches have attempted to resolve. It is possible that BC could offer a solution by utilising its excellent moisture absorption properties to store effluent for future safe extraction and disposal or reuse. Studies such as those conducted by Costa et al. (2019), Shim and Kim (2019) and Song et al. (2018) suggest that BC pellicles are receptive to traditional dyestuffs in terms of dyeing the pellicles for apparel applications. The next part of this chapter will address objective seven: analyse a selected properties of bacterial cellulose in a practical application using lab-based methods, by exploring the use of the BC pellicle from the angle of removal of colour from dye solutions and thus its suitability as a potential effluent treatment.

7.5 Effluent Treatment in the Textile Industry

Fibres used in the textile industry can be categorized into three main types: natural cellulosic (e.g., cotton, linen), natural protein (e.g. wool, silk) and synthetic (including

modified fibres - e.g. polyester, nylon, acrylic, acetate, viscose) (Yaseen and Scholz, 2019). Most textiles require colour to be imparted on the fibres and the techniques and chemicals used to undertake this process are varied (Holkar et al., 2016; dos Santos et al., 2018). Many end-uses require fabrics consisting of a mixture of fibre types, thus there are a mixture of dyestuffs and chemicals used in any one process to achieve the desired output.

The process of dyeing and finishing textiles require large volumes of water, with estimates suggesting 120 – 220 L required per kilo of fabric (Öner and Sahinbaskan, 2011). Traditional methods generate vast quantities of highly polluted liquid waste or effluent (Holkar et al., 2016; Yaseen and Scholz, 2019; Al-Tohamy et al., 2022). This waste contains a diverse spectrum of organic and inorganic chemicals in both dissolved and suspended solid form, such as sizing agents, finishing agents, inhibitors, salts, chlorine compounds and phosphates alongside the actual colour (Khandegar and Saroha, 2013; Shehzadi et al., 2014; Holkar et al., 2016; Yaseen and Scholz, 2019; Kishor et al., 2021). The composition of the effluent is highly dependent on the specific manufacturing process, and it is not uncommon to see large fluctuations in pH, colour, salinity, temperature, biochemical oxygen demand (BOD) and chemical oxygen demand (COD), which are all strictly measured before the liquid is deemed safe for discharge (dos Santos et al., 2018; Yaseen and Scholz, 2019). Discharge of effluent into natural waterways, untreated to a sufficient standard, can cause irreversible damage to aquatic life and potable water (Masmoudi et al., 2014; Kishor et al., 2021). Figure 34 illustrates the dramatic effects of dyehouse effluent discharge on the river Trent (Staffordshire, UK) in 2023; the UK Environment Agency claimed there had been no damage to aquatic life on this occasion but advised the public to avoid the area.

Figure 34. The River Trent (Staffordshire, UK) Contaminated with Dyehouse Effluent.

(credit BPM Media)

Whilst traditional dyestuffs from natural sources are still used, most modern dyehouses use synthetic dyestuffs (Khehra et al., 2005). Cao et al. (2019) estimate that globally, in the region of 280,000 tonnes of these dyes are annually discharged as waste. They are categorized according to their chemical structure (e.g., azo, anthraquinone, sulphur) and mode of application (e.g., reactive, basic, disperse, acid) (Best, 2012; Popli and Patel, 2015). For example, reactive dyes react with fibres to form covalent links and thus are suitable for cellulosics, whilst acid dyes are applied in a dyebath with a low pH to promote adherence to the fibre and are generally used to dye fibres of a protein base. Dyes are developed to improve ease of application (Kishor et al., 2021) and to resist breakdown by exposure to sunlight, water, soap, bleaching and perspiration to improve the colour's longevity, performance and customer satisfaction of the product (McKay et al., 1999; Cristea and Vilarem, 2006) Dyestuffs displaying these properties are complex

structures with a high molecular weight and therefore a high biodegradability index (Seshadri et al., 1994; Holkar et al., 2016). For example, anthraquinone – based dyes have an aromatic molecular structure and are more resistant to breakdown in nature, whilst some metal dyes are based on chromium and release carcinogenic chromium into the environment upon breakdown (Banat et al., 1996). Christie (2001) suggests azo compounds make up approximately 65% of commercially used textile dyes as they provide a wide range of hues with high colour intensity. Azo compounds, characterized by a -N=N- linkage, often contain two or more aromatic ring structures and therefore can be difficult to breakdown by natural processes. However, if they are broken down, they can release potentially toxic by-products, underlining the importance of the removal of these compounds from effluent. Whilst there is a general trend in the industry to seek alternatives to some of the more toxic dyes and chemicals (with some already banned from use), another issue is the amount of dyestuff that is not fixed to the textile during the dyeing process and therefore is discharged in effluent.

The textile industry is constantly evolving in line with developing technologies to address the issue of effluent, but it is still the case that colour is often the most visual and obvious contaminant (Chatzisymeon et al., 2006). There is some uncertainty of how much colour is discharged in effluent, with studies suggesting that anything between 15 % - 50 % of dyestuffs used do not fix to the fibre and are therefore discharged as waste (Kalyani et al., 2008; Harrelkas et al., 2009). Dong et al. (2019) suggest that in the case of reactive dyes used for cotton fibre, a fixation (or exhaustion) rate of 50 – 85% is achieved, thus 15 – 50 % of dyebath colour could be discharged as effluent, which if not treated or captured, could cause contamination of the natural environment. Other studies suggest that this could be in the region of 280,000 tons of textile dyes worldwide every year (Maas

and Chaudhari, 2005). However, the presence of less than 1 ppm of dyestuff can still be highly visible, affecting the aesthetic merit of the water (Banat et al., 1996; O'Neill et al., 1999). Whilst this is not always the most toxic element of the effluent, the presence of colour can provide an indication of water pollution (Figure 34) and affect gas solubility in natural waterways (Kishor et al., 2021) Additionally, some dyestuffs can degrade into toxic by-products when exposed to sunlight and water (Ahlström et al., 2005). Therefore, the removal of colour is often the primary concern in textile effluent treatment, and is a serious environmental challenge, although other colourless soluble organic chemicals present may pose a much greater risk to the environment (Banat et al., 1996; Baldrian and Gabriel, 2003). As global standards become more restrictive, the adoption of effective colour removal technologies becomes more important (O'Neill et al., 1999).

7.6 Global Standards

Legislation around the globe regarding the discharge of effluent is managed by local government bodies (Kishor et al., 2021). For example, in the UK, the Environment Agency (EA) has responsibility for adherence to effluent legislation and issues compliance permits for the levels of various contaminants in water discharges from dyehouses. As an example of the limits, effluent colour content is assessed by light absorbance and should read < 0.1 when measured in the wavelength range of 400 - 800 nm (Environment Agency, 2009).

As mentioned previously, standards for effluent discharge around the globe vary according to geographical location, but are also dependent on different processes, dyes and chemicals used, depending on the nature of the textile being manufactured (Holkar

et al., 2016). In some countries, there are either few or no standards, or the practice is not strictly monitored, leading to non-adherence. For example, in Nigeria, the practice of discharging untreated dyehouse effluent is widely accepted, causing public and environmental concerns (Okareh et al., 2017). This practice is emulated in countries such as India, Bangladesh, Pakistan, and China, which are major contributors to global textile production and are undergoing rapid economic development (Wu et al., 2012; Kishor et al., 2021; Al-Tohamy et al., 2022). It is suggested that the standard physio-chemical treatments available for water treatment are expensive to maintain and therefore not widely adopted (Okareh et al., 2017; Hussein and Scholz, 2018).

The impact of continued water pollution across the globe is causing not only environmental damage but is detrimental to human health; Table 20 illustrates some of the detrimental effects of chemicals associated with imparting colour onto textiles. Additionally, environmental impacts include increased mortality rates and changes in shape and size in aquatic life, decreased growth rate and metabolic process alterations in aquatic microorganisms, and necrosis, chlorosis and damaging of root systems in plants (Al-Tohamy et al., 2022). However, it should be reiterated that whilst colour in effluent is responsible for some of the environmental damage there are many other (colourless) chemicals involved in the finishing of textiles (e.g., to impart properties such as stain- and water-resistance), that also cause severe environmental impacts. It is therefore important that reliable and cost-effective techniques are developed to allow the efficient treatment of dyehouse effluent (Wang, 2019).

Table 20. Toxic Effects of Textile Chemicals.

Adapted from Kishor et al. (2021).

Chemical	Use	Toxic Effects (Human)
Chlorine based solvents	scouring	central nervous system liver kidneys
Carbon disulphide	viscose yarn manufacture	neurological, psychiatric, gastrointestinal, reproduction, birth defects, leukaemia, skin, kidney
Alkylphenols	wool scouring, dye dispersing agent	endocrine, breast cancer, neuro-developmental delays
Chlorobenzenes	dye carrier, dyestuff, azo pigments.	carcinogenic, liver, thyroid, endocrine
Phthalates	printing / dyeing / coating to soften textile	carcinogenic, endocrine disruptor, reproductive system
Heavy metals	preparation for pigments & dyes	carcinogenic, respiratory, reproductive system, dna damage, kidney, liver
Azo dyes	dyes (wool, silk, viscose, synthetics)	carcinogenic, respiratory
Volatile organic compunds	solvent based inks	*skin / eye irritation, depression, dizziness, headaches, drowsiness, numbness in fingers and toes
Organophosphorous compounds	preparation for pigments	eye, nose, skin irritation

**Common effects from gas, but can be found in water*

7.7 Current Effluent Treatment Techniques

Traditional wastewater treatments can be inefficient in the removal of many dyestuffs from effluent (Banat et al., 1996; Zheng et al., 2013). Methods such as flotation, flocculation, ion-exchange, precipitation, and adsorption using activated carbon have been explored with varying degrees of success (McKay et al., 1999; Malik et al., 2007; Harrelkas et al., 2009; Santos and Boaventura, 2015; Oluwakemi Kehinde and Abdul Aziz, 2016). However, no one technique alone has been completely successful, leading to the conclusion that perhaps a combination of treatments is appropriate for colour removal. Additionally, the cost and complexity of the processes are generally considered to be prohibitive (Mohite and Patil, 2014b; Nawaz and Ahsan, 2014). A coagulation-flocculation technique was found to be effective in the removal of disperse dyes from wastewater, but less effective with reactive and vat dyes (Yeap et al., 2014). Adsorption techniques using activated carbon have found varying degrees of success with effluent containing a mixture of dyestuffs, but activated carbon is deemed expensive for commercial applications (Mohite and Patil, 2014b; Holkar et al., 2016). Other materials such as food wastes (e.g., ground nut shells, date stones, potato waste) have been explored as adsorbents with varying degrees of success (Holkar et al., 2016). However, all adsorption techniques produce significant amounts of sludge (a viscous mixture of solid and liquid waste), which needs to be disposed of at the end of the process (Holkar et al., 2016). Additionally, this sludge can contain many soluble dyes, which are not extracted and recycled, contributing to the unnecessary waste and unsustainable practice of the textile industry (Hussein and Scholz, 2018).

7.7.1 Physical Techniques

Physical effluent removal techniques do not use chemicals. An example of a physical technique is that of membrane nano-filtration (Masmoudi et al., 2014). The reduced pore size of the filter is a successful solution for catching small particles of colour but fouling and therefore blocking of the filter membrane is high, thus reducing its useable life (Masmoudi et al., 2014). Nevertheless, when this technique is used in combination with an activated sludge method (sludge containing aerobic microorganisms), around 90 % of colour can be removed (Fersi and Dhahbi, 2008). In other studies, a coagulation / flocculation technique was used in combination with a membrane nano-filter and again, approximately 90 % of colour was removed whilst reducing the amount of fouling of the filter (Riera-Torres et al., 2010; Zahrim et al., 2011). A colour reduction of 99 % was achieved by Aouni (2009), when using the membrane nano-filter alongside an electro coagulation technique. Masmoudi (2014) demonstrated a near 100 % colour removal rate when combining membrane nano- and micro- filters. Whilst these studies have all shown high degrees of success, each of the methods proposed are expensive and, in some cases, complicated. It is acknowledged that simpler and cheaper methods need to be further investigated and researched (Masmoudi et al., 2014).

7.7.2 Chemical Treatments

Chemical techniques used in the treatment of textile effluent can alter the molecular structure of the waste compound to render it harmless to the natural environment. Oxidation techniques using reagents such as Fenton's reagent have proved effective in

treating dyebath additives, but sludge is still generated due to residual dye molecules (Babuponnusami and Muthukumar, 2014).

7.7.3 Biological Techniques

Biological techniques for decolouration of dyes include biosorption, enzymatic degradation, or a combination of both (Wu et al., 2012; Imran et al., 2015). The techniques can be classified as aerobic, anaerobic, or facultative (Holkar et al., 2016; Zabłocka-Godlewska et al., 2018) depending on the physiology of bacterial strain being utilised (Masi et al., 2019).

Biological techniques are usually most effective in dealing with dissolved matter (Holkar et al., 2016) and are more suitable for acid and reactive dye classes (Willmott et al., 1998). Biological techniques are found to be more eco-friendly, cheaper and produce less sludge when compared to other chemical or physical techniques (Wang et al., 2009a; Hayat et al., 2015), particularly when used in conjunction with other methods such as adsorption (Masi et al., 2019). Studies have shown that biological techniques such as treatment wetlands have proved successful in the removal of water-soluble Acid Orange (Ong et al., 2010), Basic Red and Acid Blue azo dyes (Hussein and Scholz, 2018). Further studies have been conducted to find the bacterial species responsible for the decolourisation of dyestuffs in waste, with bacterial species being isolated from in wetlands, polluted rivers and sludges resulting from other treatment processes (Zabłocka-Godlewska et al., 2018). Zablocka-Godlewska et al., (2018) found the most effective bacteria to decolourise triphenylmethane, fluorone, and azo dyestuffs were Gram-negative, rod shaped facultative anaerobic species.

Imran (2015), states the selection of the bacteria, either as a single species or as a consortium, is critical to the successful decolourisation and degradation of the dyestuffs. This is further supported by studies evaluating the treatment of azo dyes. Synthetic azo dyes are widely used in the textile industry due to their versatility and high degree of durability and degradation resistance to UV light, and microbiological attack (Patel and Vashi, 2015). Whilst biological processes have difficulty breaking down synthetic dyes (Cao et al., 2019), Mishra (2018) suggests the decolourisation of the dye can be achieved either by adsorption or biological degradation of the molecular structure, with careful selection of bacterial strains. Table 21 presents an overview of some of the studies in this field. However, these approaches should be taken with caution; anaerobic digestion causes decolourisation, but the by - products are aromatic amines which are carcinogenic, toxic, and prone to further mutation (Cao et al., 2019). Nevertheless, aerobic degradation has proven successful in breaking down such structures rendering them inert (Solís et al., 2012).

Additionally, studies using strains of strains of algae and fungi (Table 22) have indicated that it is the production of extracellular ligninolytic enzymes which are critical in the degradation of the complex dye molecular structures (Sen et al., 2016). However, Mishra and Maiti (2018) argue that the long growth times of algae and fungi (in comparison to bacterial species) reduce their efficacy at decolourisation, as they remove dye predominantly by adsorption, rather than degradation.

Table 21. Treatment of Dyestuffs with Bacterial Strains.

Bacterial Species	Experimental Conditions	Initial Dye Concentration	Efficacy of Colour Removal	Dyestuff	Source
Escherichia coli	Static incubation 20 – 45 °C, 10 hours	2000 ppm	100 %	Reactive Red 22	Chang and Kuo (2000)
Pseudomonas luteola	Static incubation 37 °C, 20 hours	200 ppm	90 %	Reactive Red 22 azo	Chang et al. (2001)
Pseudomonas sp	Static anaerobic 30 °C, 1 hour	50 ppm	99.28 %	Reactive Red BL1	Kalyani et al. (2008)
	Static 30 °C, 114 hours	5 ppm	80 %	Reactive Red 2	Kalyani et al. (2009)
Pseudomonas aeruginosa	Static 30-40 °C, 5.5 hours	300 ppm	91 %	Reactive Red BS Ci 111	Sheth and Dave (2009)
Citrobacter	Anaerobic 33 °C, 25 hours	1000 ppm	95 %	Reactive Red 180	Wang et al. (2009a)
Brevibacillus laterosporus	Static 30 °C, 48 hours	50 ppm	87 %	Reactive Golden Yellow HER	Gomare et al. (2009)

Bacterial Species	Experimental Conditions	Initial Dye Concentration	Efficacy of Colour Removal	Dyestuff	Source
Bacillus lentus	Static anaerobic 35 °C, 12 hours	1500 ppm	92 %	Reactive Red 120	Oturkar et al. (2011)
Enterobacter sp	Anaerobic 35 °C, 30 hours	50 ppm	91.4 %	Reactive Black 5 diazo	Chen et al. (2011)
Alcaligenes faecalis	Anaerobic 37 °C, 16 hours	400 ppm	98.2 %	Reactive Orange 13	Shah et al. (2012)
Bacillus sp	Static 40 °C, 120 hours	100 ppm	95 %	Reactive Black 5 azo	Wang et al. (2013)
	Static 35 °C, 4 hours	200 ppm	100 %	Reactive Red	Padmanaban et al. (2016)
Lysinibacillus sp	Static 30 °C, 24 hours	50 ppm	100 %	Remazol Red azo	Saratale et al. (2013)
*Morganell*a sp	Static anaerobic 30 °C, 24 hours	10 ppm	97 %	Reactive Black B azo	Pathak et al. (2014)
Aeromonas hydrophilia	Static 35 °C, 24 hours	100 ppm	76 %	Reactive Black 5	El Bouraie and El Din (2016)

Table 22. Decolourisation of Dyes Using Fungal Species.

Adapted from Sen et al. (2016).

Yeast	Method of Decolourisation	Experimental Conditions	Initial Dye Concentration	Efficacy of Colour Removal	Dyestuff	Source
Candida tropicalis	Adsorption	28 °C 2 days, 120 rpm	10 ppm	85.3 %	Basic Violet 3	Das et al. (2011)
Candida utilis	Adsorption	25 °C 10 days, 140 rpm	50 ppm	82 %	Remazol Turquoise Blue-G	Gönen and Aksu (2009)
Pichia fermentans	Adsorption	28 °C 2 days, 120 rpm	10 ppm per dye type	100 % 95 % 70 %	Acid Blue 93 Direct Red 28 Basic Violet 3	Das et al. (2010)
Rhodotorula mucilaginosa	Adsorption	30 °C 6 days, 100 rpm	389 ppm	96 %	Remazol Blue	Ertuğrul et al. (2008)
Trichosporon akiyoshidainum	Adsorption and aerobic degradation	26 – 35 °C 9 days, 250 rpm	200 ppm per dye type	100 % 100 % 100 %	Reactive Blue 221 Reactive Red 141 Reactive Black 5	Pajot et al. (2011)
Candida rugopelliculosa	Anaerobic degradation	28 °C 48 h, 120 rpm	2000 ppm	90 %	Reactive Blue 13	Liu et al. (2011)

Yeast	Method of Decolourisation	Experimental Conditions	Initial Dye Concentration	Efficacy of Colour Removal	Dyestuff	Source
Galactomyces geotrichum	Anaerobic degradation	30 °C 24 h, 120 rpm	10 ppm per dye type	88 %	Remazol Red Golden Yellow HER Rubine GFL Scarlet RR Methyl Red Brown 3 REL Brilliant Blue	Waghmode et al. (2011)
Candida Tropicalis	Anaerobic degradation	28 °C 30 h, 120rpm	50 ppm per dye type	100 % 100 % 90 %	Acid Blue 93 Direct Red 28 Basic Violet 3	Das et al. (2011)
Candida sp., Williopsis californica	Aerobic degradation	25 °C 24 h, 250 rpm	200 ppm per dye type	96 %	Reactive Yellow 84 Reactive Black 5 Reactive Blue 221 Reactive Red 141	Grassi et al. (2011)
Candida albicans	Adsorption	35 °C 72 h, 150 rpm	100 ppm	73.2 %	Direct Violet 51	Vitor and Corso (2008)

7.8 Use of Bacterial Consortia for Colour Removal

As several bacterial and/or fungal species are often required to efficiently break down a dyestuff, some studies have focused on a consortium approach, using various co-existing microbial species (Suvilampi et al., 2003). Table 23 presents an overview of these studies. These consortia are often found in natural aquatic eco- systems and water- treatment techniques such as activated sludge and constructed wetlands (Suvilampi et al., 2003). The microbes in these consortia are often a mix of structures (e.g. branched, filamentous, spherical, oval, mushroom, sheet, irregular) (Wu et al., 2012) and adapt their activities depending on the surrounding conditions (Khatoon et al., 2007). Sen (2016) states that this gives more stability against environmental fluctuations therefore improving the efficacy and durability of the treatment but also concedes that evaluation of this type of treatment is still at an early stage. Wu (2012) suggests that when conditions are favourable, the mix of microbes exhibit enhanced performance (compared to single species treatments) in terms of adsorption and degradation of contaminants and therefore can prove useful in the removal of pollution in wastewater. The consortium is often consolidated by extra polymeric substances (EPS), forming a 'microbial aggregate' and as Wu (2012) postulates, the EPS is usually made up of proteins and carbohydrates. This combination is otherwise known as biofilm or pellicle. Sheng (2010) further explains that the content and physical structure of the EPS is critical in terms of the adsorption ability, surface characteristics and formation of the microbial community. Sharma (2018) suggests that the effluent treatment performance is enhanced by the microbes being considered 'immobile' due to the EPS structure. This can

allow the consortium to be used for multiple treatment cycles, thus reducing the cost of the process.

Table 23. Colour Removal Using Microbial Consortia.

Consortium	Method of Decolourisation	Experimental Conditions	Efficacy of Colour Removal	Dyestuff	Source
Alpha, Beta, Gamma proteobacteria	Aerobic degradation	pH 7 27 °C, 24 h	100 %	Reactive Blue 59	Kolekar et al. (2012)
Penicillium sp., *Exiguobacterium sp., Pseudomonas sp.*	Anaerobic degradation	pH 7 30 °C, 48 h	97 % 100 %	Reactive Dark Blue Reactive Orange	Jadhav et al. (2010)
Fungi and Bacteria	Aerobic degradation	-	94 % 65 %	Acid Red 249 Reactive Red M3BE	Yang et al. (2009)
Irpex lacteus Mixed bacterial communities	Not stated	pH 7.0 9 – 20 days	98.4 % 98.5 %	Reactive Orange 16 Remazol Brilliant Blue R	Novotný et al. (2011)
Pseudomonas aeruginosa, Rhodobacter sphaeroides, Proteus mirabilis, Bacillus circulans	Anaerobic / Aerobic	pH 7.0 37 °C, 24 h	>90 %	Remazol Black	Dafale et al. (2008)
Pseudomonas aeruginosa, Bacillus flexus, Staphylococcus lentus	Aerobic	pH 7.0 37 °C, 16 h	93.7 %	Acid Blue 113	Shanmugam and Mahadevan (2015)
Pseudomonas stutzeri, cineobacter baumannii		pH 8.0 37 °C, 5 days	97 % 95 %	Congo Red Gentian Violet	Kuppusamy et al. (2017)

Consortium	Method of Decolourisation	Experimental Conditions	Efficacy of Colour Removal	Dyestuff	Source
Diverse community including *Streptococcus, Lactobacillus, Clostridium, Enterococcus*	Aerobic	pH 7.5 – 9.5 35 - 40 °C, 48 h	90.74 %	Direct Blue 2B	Cao et al. (2019)

Additionally, Peinado (2006) claims the EPS helps the consortium withstand the toxic nature of the dyestuffs, thus further improving the durability of the treatment.

Other studies further support these claims. Kurade (2019) described how a consortium of bacteria (*Brevibacillus laterosporus*) and yeast (*Galactomyces geotrichum*) immobilized in various matrices (calcium alginate (CA), polyvinyl alcohol (PVA), polyurethane foam and a stainless-steel sponge) were used to decolourise a reactive azo dye (Remazol Red). This combination showed 100 % colour removal in 11 hours, whilst a similar result took 24 hours to achieve with CA and PVA alone. Kurade (2019) also studied the action of free cells on the decolorization of Remazol Red but found those cells which were 'immobile' to be much more effective, postulating that this was due to the adsorption action of the matrices in which they were held.

Azo dyes such as Direct Blue have been treated by microbial communities in a batch-fed reactor, with a study by Cao (2019) showing a consortium consisting of *Bordetella, Gammaproteobacteria*, *Betaproteobacteria*, *Enterococcus, Bacilliacinetobacter, Escherichia-Shigella, and Lysinibacillus* to be effective, with a result of over 90 % decolourisation. Cao (2019) concluded that this technique could have decolourisation applications for a range of azo dyes.

7.9 Use of Bacterial Cellulose for Dyehouse Effluent Treatment

BC has been explored in water treatment applications as an adsorbent and nano-filter due to its enhanced strength, surface area, water absorbency and lack of contaminants

compared to its plant cellulose counterpart (Wang, 2019). Additionally, its non-toxicity and bio-adsorption ability makes it suitable as a water purifying membrane (Menon et al., 2017).

Mohite and Patil (2014b) produced BC using *Gluconacetobacter hanseii* grown in H&S medium and measured the efficiency of the 'pure' BC structure (any residual microbes present after the BC pellicle formation were removed by rinsing with NaOH) in removing Aniline Blue azo dye. The dye was shown to adsorb onto the surface of the nanofibres and a colour reduction rate of 80 % was recorded after 60 min when the dye solution was measured with a spectrophotometer. Additionally, Mohite and Patil (2014b) found BC had the potential to adsorb lead, cadmium, and nickel ions.

Whilst the structure of a bacterial cellulose can act as a nano-filter (section 7.7.1), functionalization of the cellulosic structure can lead to enhanced filtration and adsorption properties. Functionalisation using carboxyl, phosphonate and sulphonate groups has led to selective uptake and adsorption of contaminants '*such as metal ions, dyes and microbes for water purification'* (Wang, 2019).

Carboxylated nanocellulose has shown promise in the removal of lead (Abou-Zeid et al., 2018), (Saito and Isogai, 2005), silver (Saito and Isogai, 2005; Karim et al., 2017), lanthanum (Saito and Isogai, 2005), copper (Sehaqui et al., 2014; Karim et al., 2017), cadmium (Sharma et al., 2018), iron (Karim et al., 2017) and calcium ions (Abou-Zeid et al., 2018). More specifically in terms of dyestuffs, carboxylated cellulose has shown good adsorption of cationic dyes such as Methylene Blue (Leung et al., 2011; Batmaz et al., 2014) Crystal Violet and Malachite Green (Qiao et al., 2015).

Succinylation of the cellulose has been indicated as an efficient adsorbent, attracting lead, cadmium nickel and chromium ions which were subsequently harvested and recycled (Yu et al., 2016). Additionally, functionalization with positively charged groups has shown some success in attracting anionic metallic ions such as arsenates and chromates (Hasani et al., 2008). Nanocellulose functionalized with amino groups are useful for the adsorption of anionic dyes such as Congo Red (Pei et al., 2013; Jin et al., 2015; Zhu et al., 2016), Acid Green (Pei et al., 2013), Acid Red, Reactive Light Yellow (Jin et al., 2015) and Cationic Base Yellow (Zhu et al., 2016).

7.10 The Potential Use of a Kombucha SCOBY as a Colour Remover

The previous sections have described the use of BC as an adsorbent for dyestuffs and the use of various bacterial consortia as effluent treatments, but no evidence can be found in the literature to suggest that these have been tried in tandem.

A BC pellicle developed from a Kombucha SCOBY inoculum contains the BC nanofibrils discussed previously in microfilter approaches to colour removal (section 7.7.1), and comprises EPS (extra polymeric substances) but additionally hosts a community of bacteria and yeasts (section 3.3.3). Some of these species have previously been explored as colour removers, such as *Pseudomonas sp., Bacillaceae* and *Candida sp.,* but not in combination (Table 21, Table 22).

Sequencing performed as part of this thesis revealed that *Komagataeibacter xylinus* is the dominant species in BC pellicles developed from Kombucha SCOBYs, in agreement with

existing literature (Marsh et al., 2014; Gaggìa et al., 2019). No evidence could be found in the literature of this species being used to remove colour from dyehouse effluent. Additionally, the Kombucha SCOBY consortium has never been examined to extract colour from dye solutions, although other bacteria / yeast consortium approaches have been considered by some studies with positive results (Table 23).

Whilst there have been some studies exploring the most effective ways of imparting colour onto BC pellicles (section 6.8.2), all of these have focussed on the colour of the pellicle, rather than the (lack) of colour remaining in the dye solution after the process. For example, Kim et al. (2022) studied the effects of a BC pellicle as an adsorbent for Methylene Blue, but this work assessed the efficiency of the dye adsorption rate in relation to dye concentration. It used pellicles that had been pre-dyed with coffee extract and then treated with aluminium potassium sulphate, copper sulphate or iron sulphate which are commonly known as 'mordants' in the textile dyeing industry and are traditionally used to improve dye adherence to a textile. This work did show the potential of BC to adsorb Methylene Blue, a synthetic dye used on cotton, wool and silk (Oladoye et al., 2022) with the aid of mordants. However, no analysis of exhaustion rate was provided and, the modification of the BC using the mordanting chemicals did introduce an additional potential environmental pollution hazard, thus negating some of the benefits of the colour removal from the solution. Similarly, Shim and Kim (2018) and Costa (2019) assessed the effectiveness of dye uptake of the BC pellicles with synthetic and natural dyes respectively but did not analyse the remaining dye solution.

As there is no existing work investigating the potential of the BC nanofibrillar structure mixed with the microbial community, as found in a Kombucha SCOBY pellicle, this part of the thesis aims to examine the potential use of the Kombucha SCOBY pellicle as a colour reducer of dyehouse effluent. The work will not examine microbial metabolic degradation mechanisms but will focus preliminary explorations of the efficacy of the pellicle to reduce colour from dye solution and note any potential additional effects of the microbial community in colour reduction.

7.11 Experimental Details

7.11.1 Dye Treatment Selection

The effects of the nanofibrillar BC structure, the live bacterial and yeast community, and a combination of both on the dye uptake of pellicles was examined. In brief, these were prepared as follows:

- Nanofibrillar BC pellicle only – pellicles were cleaned using NaOH to lyse any bacterial or yeast cells (in line with methods documented in literature (Table 5).
- Live bacterial and yeast community only - a sample of liquid growth medium used to prepare solid pellicles (as described in section 7.11.3), which could be considered a liquid phase of the Kombucha SCOBY consortium.
- Combination nanofibrillar BC and live bacterial and yeast community - pellicles taken directly from the growing medium without any subsequent treatment.

7.11.2 Dye Class Selection

In textile processing, cellulosic fibres are commonly (but not exclusively) dyed with reactive classes of dyes. These dyes are water-soluble and form covalent bonds with the cellulose fibre, imparting a degree of colour fastness (durability) to the textile. As BC is of cellulosic base, the reactive dye class was deemed appropriate for further examination as it would be likely to have affinity for the cellulose nanofibrils in the pellicle. Commercially, the application of colour using reactive dyes also requires auxiliary chemicals to be added to the dyebath to optimise colour uptake (as discussed in section 7.4). However, for the purposes of this experiment, the dye solution was examined without the addition of the auxiliaries because effluent colour is often the factor of most concern in effluent discharge, as previously discussed in section 7.5. Procion Reactive Navy MX (Reactive Blue 4) was chosen as an example of a reactive dye for exploration as this was easily accessible through university stores.

Acid dyes are used in the colouration of protein (wool and silk) and nylon fibres. Due to the molecular structure of these dyes, they are not used to dye cellulose because they are not able to form chemical bonds with the fibre. If acid dyes are used with cellulose, light staining occurs which is quickly removed by washing because the lack of chemical bonding means the dye molecule has no permanent adherence to the fibre. Acid Brilliant Blue (Acid Blue 9) was used for exploration (due to ease of access through university stores) as an example of an acid dye to assess the effect of BC pellicle and the associated microbial community on this class of dye.

7.11.3 Pellicle Preparation

In brief, 800 mL of sterile black tea with sugar, and 800 mL H&S medium were prepared as discussed in part one of this thesis. 200 mL medium was decanted into a 500 mL sterile pot (4 pots each of black tea with sugar and H&S) and inoculated with 20 mL of Kombucha SCOBY liquid. The lids of the pots were loosely attached, and the pots placed in a 30 °C incubator for 30 days. After the incubation period, pellicles had developed on the surface of the sterile media and were removed from the pots.

Once removed, one pellicle per growth medium was treated by either:

i) The pellicle was cut into 1 g pieces and stored in sterile water until needed (untreated control).

ii) The surface liquid media was removed by pressing the pellicle between filter paper sheets. The pellicle was cut into pieces weighing 1 g each. The pieces were placed on a petri dish and dried in a 60 °C fan oven for 24 hours. The dried pieces were stored in a petri dish in a desiccator until needed (dry).

iii) The pellicle was placed in a conical flask, containing 300 mL 0.1 M NaOH. The conical flask was placed in a water bath and heated to 80 °C for one hour. The flask was removed from the water bath, allowed to cool and the pellicle removed. The surface liquid of the pellicle was removed by pressing the pellicle between filter paper sheets. The pellicles were then cut into pieces weighing 1 g each. The pieces were placed on petri dishes and dried in a 60 °C fan oven for 24

hours. The dried pieces were stored in a petri dish in a desiccator until needed (NaOH dry).

iv) The pellicle was placed in a conical flask, containing 300 mL 0.1 M NaOH. The conical flask was placed in a water bath and heated to 80 °C for one hour. The flask was removed from the water bath, allowed to cool and the pellicle removed. The pellicle was cut into 1 g pieces and stored in sterile water until needed (NaOH wet).

This resulted in four sets of prepared pellicles per growth medium.

The liquid growth media from the pots in which the pellicles were developed was also reserved for subsequent inoculation into dye solutions.

7.11.4 Dye Solution Preparation

To create the dye solutions for testing with the Kombucha SCOBY pellicle and liquid inocula, 3 L of dye solution per dye (Reactive Navy and Acid Blue) was prepared by dissolving 20 ppm powdered dyestuff in distilled water. The absorbance of the dye solution was measured using a Jenway 6305 spectrophotometer (Fischer Scientific, Leicestershire, UK).

15 mL of dyestuff was decanted into universal tubes and the lids tightly screwed on until ready for use. Three pots per inoculum type per sample timepoint were prepared (a total of 120 pots per dye type).

7.11.5 Inoculation

Each universal tube of dyestuff was inoculated with either 1 g pellicle or 1 mL of reserved growth medium, as described in Table 24. The lids of the universal tubes were replaced loosely to support the aerobic activity of the microbial consortium. Each sample was replicated in triplicate. Five sets of triplicate samples were prepared per dyestuff type and placed in a 30 °C incubator. One set per dyestuff was removed after one, two, four, six, and eight weeks to assess the effect of both the inoculum and incubation time on the colour intensity of the liquid.

Table 24. Details of Inoculum Preparation.

Inoculum	Growth Medium	Preparation	Reference
Solid pellicle	H&S	Surface liquid removed and dried at 60 °C for 24 hours and stored in a desiccator.	H&S (dry)
		Placed in distilled water	H&S (wet)
		Treated with NaOH (0.1M) for 1 hour at 80 °C. Dried at 60 °C for 24 hours and stored in a desiccator.	H&S (NaOH dry)
		Treated with NaOH (0.1M) for 1 hour at 80 °C. Placed in distilled water.	H&S (NaOH wet)
	Black tea with sugar	Surface liquid removed and dried at 60 °C for 24 hours and stored in a desiccator.	Tea (dry)
		Placed in distilled water	Tea (wet)
		Treated with NaOH (0.1M) for 1 hour at 80 °C. Dried at 60 °C for 24 hours and stored in a desiccator.	Tea (NaOH dry)
		Treated with NaOH (0.1M) for 1 hour at 80 °C. Placed in distilled water.	Tea (NaOH wet)
Liquid medium	H&S	Decanted from growth pot	H&S liquid
	Black tea with sugar	Decanted from growth pot	Tea liquid

7.11.6 Sample Evaluation

At the end of incubation period the pellicles were removed and placed with sterile forceps onto a petri dish, photographed, visual observations recorded, and the pellicles allowed to dry in ambient conditions.

Remaining liquid in each of the universal pots was photographed and visual observations recorded. The liquid was then sampled, placed into a cuvette and light absorbance measured on a Jenway 6305 spectrophotometer (Fischer Scientific, Leicestershire, UK) at 597 nm for Acid Blue and at 583 nm for Reactive Navy dye samples. Each measurement was performed in duplicate, from opposite sides of the cuvette. The Beer - Lambert law was used to translate absorbance data into concentration (parts per million) dyestuff remaining in the dye solution as follows:

$$C = {}^{A}/_{\varepsilon b}$$

Where: A = absorbance; ε = molar absorptivity; b = length of light path; C = dye concentration.

On the spectrophotometer used, the light path length was 10mm. The molar absorptivity (ε) had been previously calculated by plotting the measured absorptivity against a range of known concentration of each dyestuff and calculating the gradient of the slopes. R^2

indicates ‘goodness of fit’ of the linear slope to the plotted points. In both cases, this is close to 1, indicating the slope is an accurate representation of the trend of the plotted measurements (Figure 35, Figure 36).

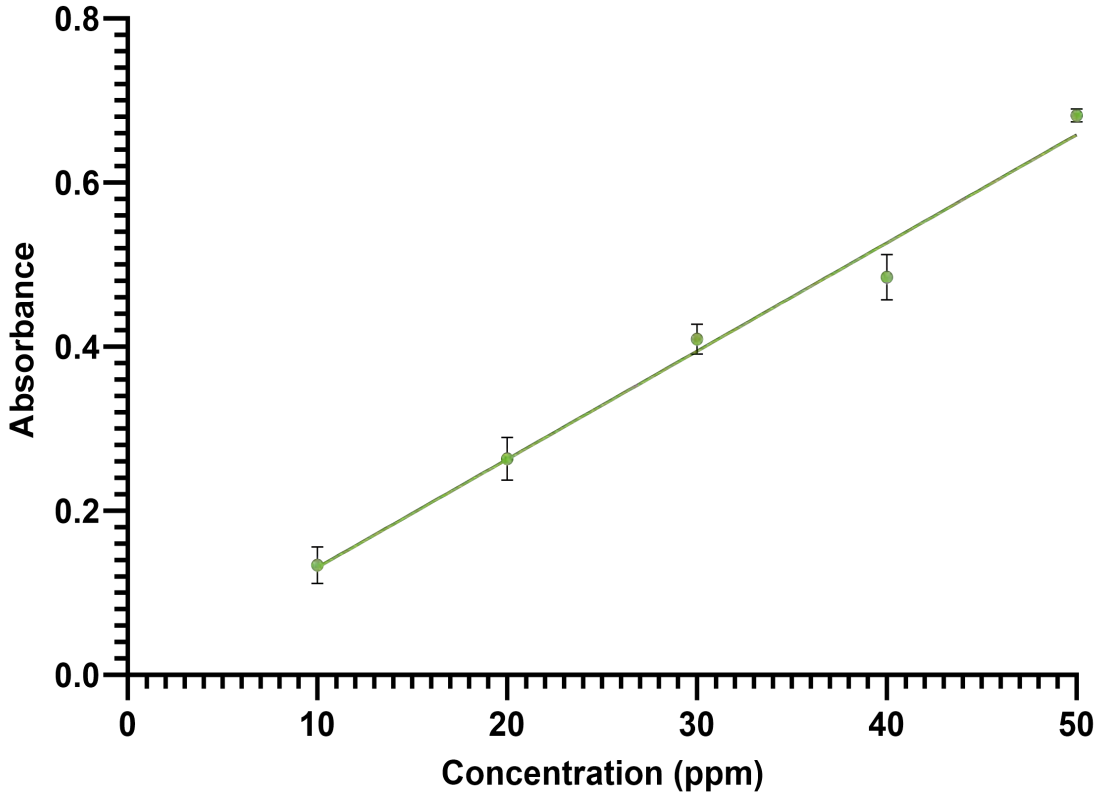


Figure 35. Acid Blue Solution Absorbance vs Concentration at 597 nm. $\varepsilon = 0.01318$, $R^2 = 0.9770$.

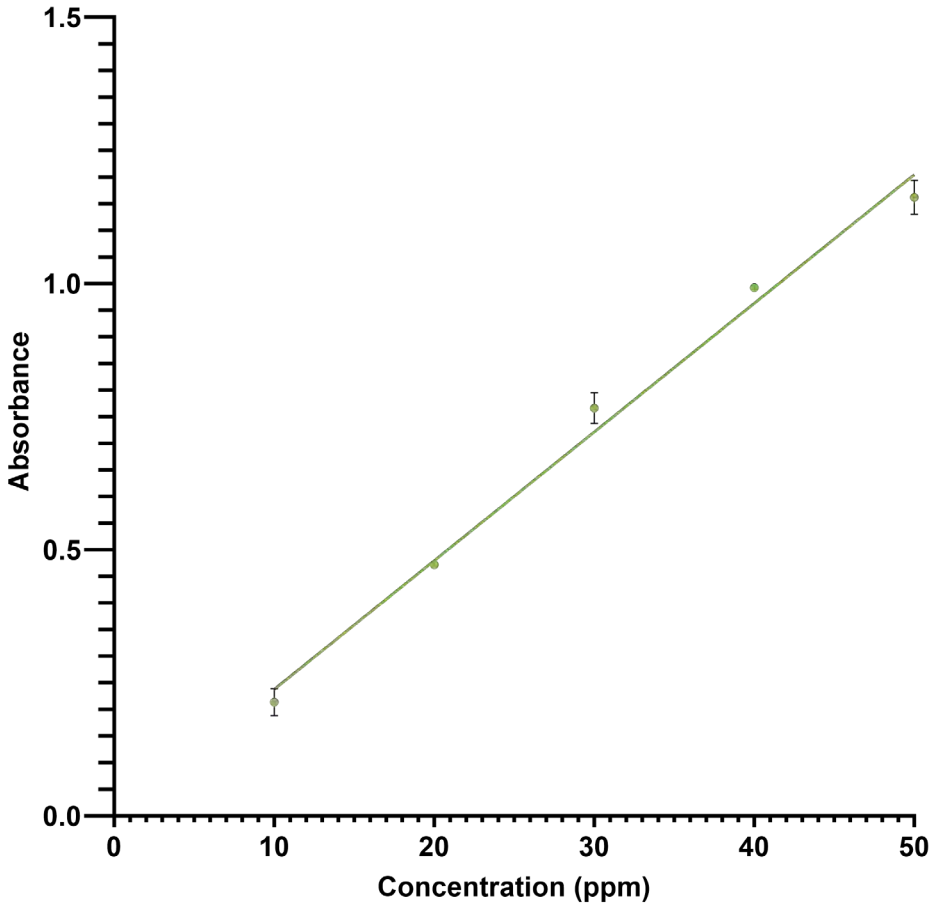


Figure 36. Reactive Navy Solution Absorbance vs Concentration at 583 nm. ε = 0.02416, R^2 = 0.9881.

The photographs of the pellicles and liquids were processed and analysed using Image J open-source software visual analysis tool (www.imagej.net) to facilitate visual comparison of hue changes.

7.11.7 Results and Discussion

As previously stated, these experiments were designed as a preliminary exploration into the potential of the BC pellicles (a mix of BC microfibrils and a microbial consortium) as a treatment to remove colour from dyehouse effluent. Whilst the data collected has been analysed, it should be considered preliminary data only, and used as a foundation for future further experimental design.

7.11.7.1 Colour Loss in Dye Solutions

Using the Beer Lambert law, absorbance was used to determine colour loss, in line with industrial techniques used and those noted in previous studies (Oturkar et al., 2011; Kalyani et al., 2009; Environment Agency, 2009). When measured on the spectrophotometer, the calculated concentration of the prepared and untreated dye liquid at the start of the experiment was 16.59 ppm for Acid Blue (absorbance = 0.147 @ 597 nm) and 12.98 ppm (absorbance = 0.297 @ 583 nm) for Reactive Navy. This was below the anticipated level of 20 ppm (acid blue absorbance = 0.267 @ 597 nm, reactive navy absorbance = 0.483 @ 583 nm). This discrepancy was attributed to experimental error in measuring the quantities of dyestuff when making the solutions (section 7.11.4). The calculated dye concentration was used as the benchmark against which to measure the effects of the various inocula over time.

Following the capture of digital images of each container, the most commonly occurring hue in the pixels of the images was identified using ImageJ software (Figure 37 and Figure 38).

Absorbance measurements showed that the dye solutions with the lowest concentration of colour remaining were observed after 14 days in the case of every inoculum, with the exception of H&S NaOH dry in Reactive Navy (Figure 39, Figure 40).

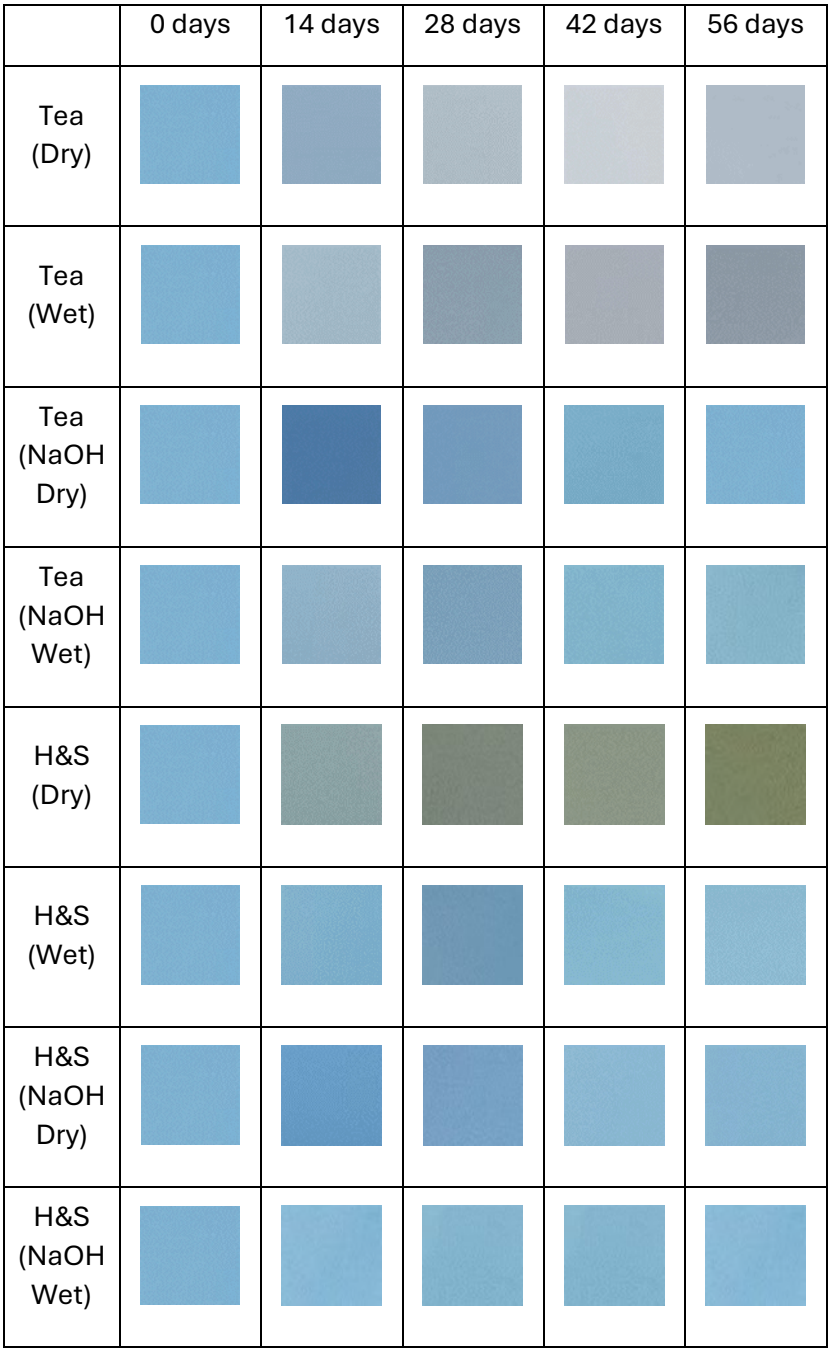


Figure 37. Colour (Hue and Intensity) Change of Acid Blue Dye Solution After Pellicle Inoculation.

Images Produced Using Image J Software (www.imagej.net).

	0 days	14 days	28 days	42 days	56 days
Tea (Dry)					
Tea (Wet)					
Tea (NaOH Dry)					
Tea (NaOH Wet)					
H&S (Dry)					
H&S (Wet)					
H&S (NaOH Dry)					
H&S (NaOH Wet)					

Figure 38. Colour (Hue & Intensity) Change of Reactive Navy Dye Solution After Pellicle Inoculation.

Images Produced Using Image J Software (www.imagej.net).

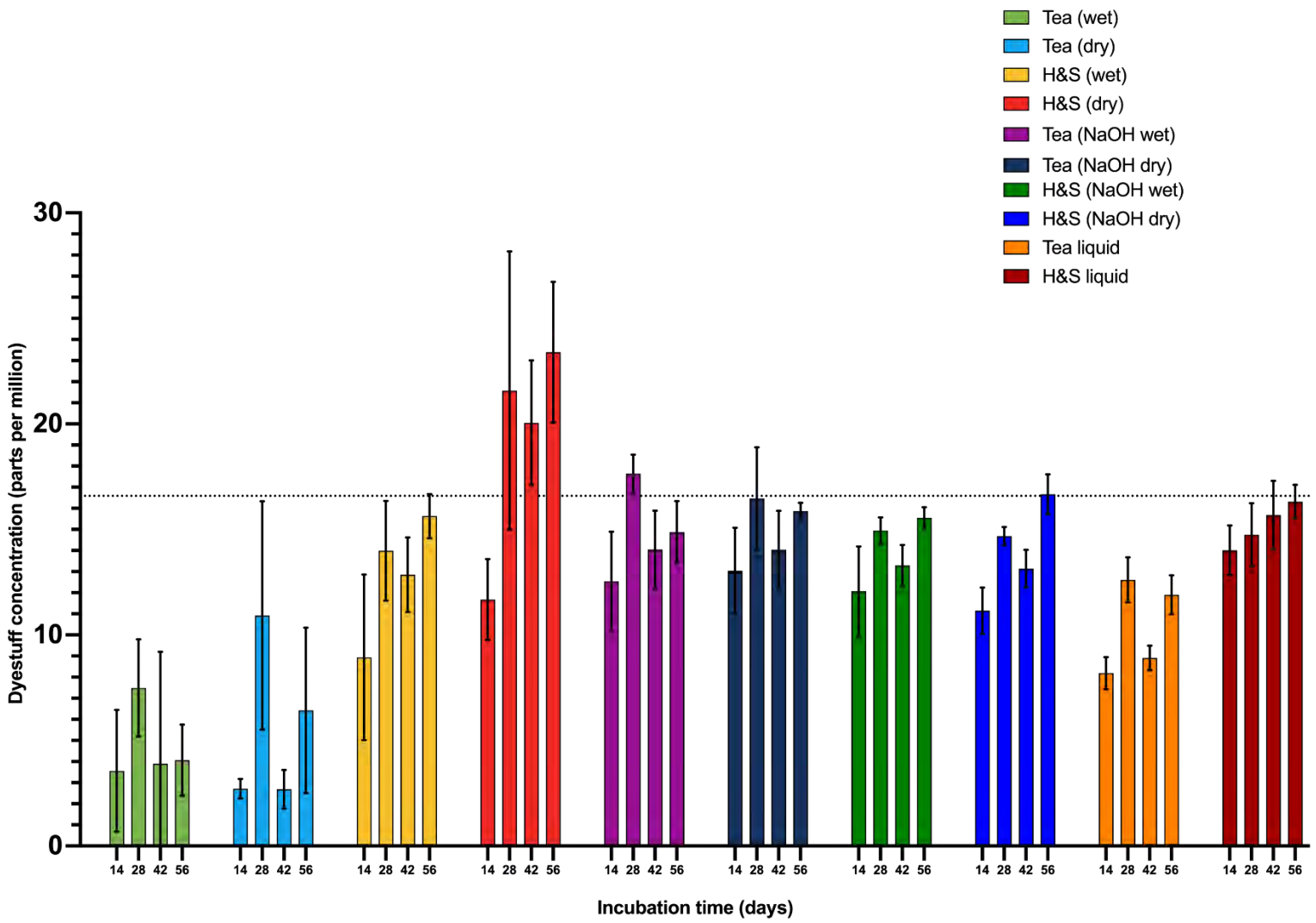


Figure 39. Dyestuff Concentration (ppm) Acid Blue.

Dotted Line Shows Measured Concentration of Initial Dyestuff Prior to Inoculation. Error bars = standard deviation.

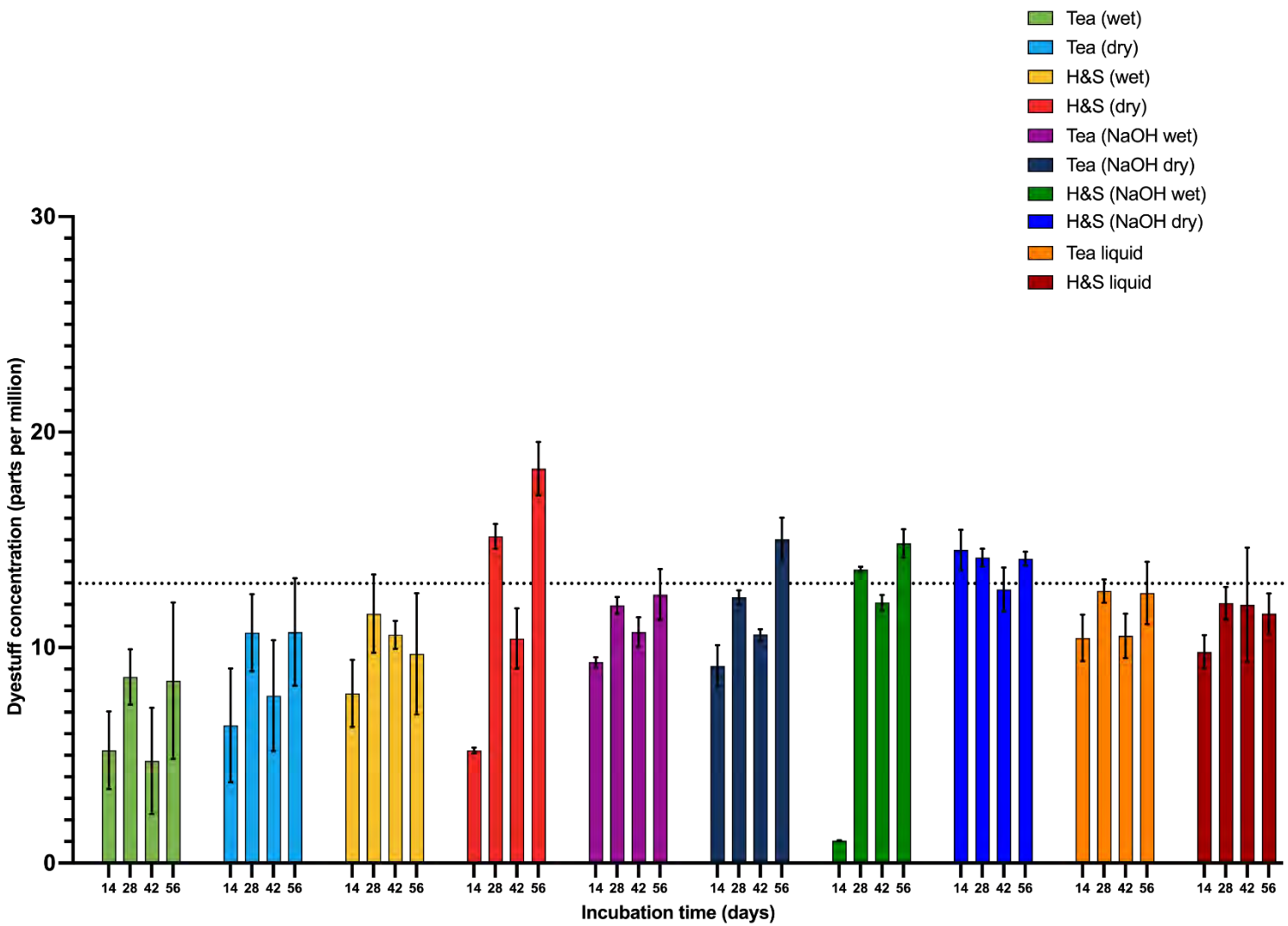


Figure 40. Dyestuff Concentration (ppm) Reactive Navy.

Dotted Line Shows Measured Concentration of Initial Dyestuff Prior to Inoculation. Error bars = standard deviation.

7.11.7.1.1 Acid Blue

Acid Blue solution inoculated with pellicles developed in tea broth (tea pellicles) that had not been treated with NaOH visually displayed a reduction of colour intensity and hue (Figure 37). As these pellicles had not been treated with NaOH, they were deemed to contain active microbial communities. This colour reduction observation was supported by the measurements of dyestuff concentration (Figure 39) where a significant difference ($p < 0.0001$) can be seen in the solutions inoculated with tea pellicles compared to all other inocula types; less colour remained in these solutions. The change in colour concentration ranged from 79 % reduction in tea (dry) after 7 days to 61 % in tea (wet) after 56 days.

This finding showed that the treatment was less effective than for the single fungal species approaches used in Table 22, where up to 100 % colour removal in Acid Blue dyestuff solution was achieved using *Candida tropicalis* (Das et al., 2010). As there has been relatively little study on the effect of microbes on the reduction of colour in acid dye solutions, evidence could not be found in the literature of a single strain bacterial approach to reducing the colour of acid dye.

The results of the study in this thesis revealed less of an effect of a consortium approach than those reported by Yang et al. (2009) who achieved a colour reduction of 94 % on Acid Red (Table 23). However, the colour reduction in the Yang et al. (2009) study focussed on the effects of the fungal communities in the consortium used.

As the tea pellicles had not been treated with NaOH and therefore still contained active microbial communities, the findings in this part of the thesis indicate that it is the pellicle

and the microbial communities working in tandem to reduce colour and hue intensity in the acid dye solution.

Anomalies were seen in several of the Acid Blue inoculations at 28 days where there were spikes in the graphs, indicating an apparent increase in dyestuff concentration (Figure 39). This was not possible as no extra dyestuff had been added, so these are attributed to a degree of microbial contamination in some of the samples and therefore turbidity in the liquid. This is likely to have affected both the behaviour of the colour in the solution and the spectral readings. It is also worthy of note that these anomalous results also displayed high standard deviations (illustrated by the errors bars in Figure 39) showing a high variability in the measurements.

This does indicate a weakness in this method of data collection. Any microbial contamination of the samples will affect the light absorbance behaviour of the liquid by changing its colour or turbidity and potentially not accurately reflect colour reduction (Figure 41). Further work would be required to identify the microbial contaminants and their origin, assess their growth, and evaluate their environmental toxicity and thus their impact in colour treatment for effluent.

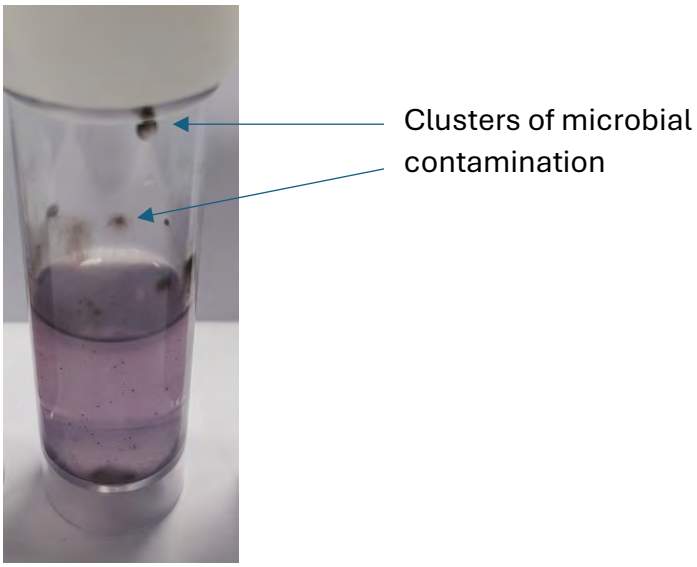


Figure 41. Dye Solution Inoculated with H&S (wet) at 14 Days Incubation Displaying Microbial Contamination and Turbidity of Dye Solution.

In contrast to the tea pellicle inocula, the pellicles developed in H&S liquid medium (H&S pellicles) did not reduce colour in the Acid Blue dye solutions to the same degree. Visually, the H&S pellicle inoculated solutions showed little change across the sampled timepoints, except for H&S (dry) which showed a shade change to a green / brown hue (Figure 37). It is postulated that residue (microbial cells or extra polymeric substances as described in section 7.8) on the surface of the pellicle inoculum could be responsible for this colour change in the dye solution, as the greatest visual changes were noted where the pellicle inocula had not been treated with NaOH (the NaOH treatment process is used to lyse any residual cells from the surface of the pellicle). As acid dyes are not used to impart colour on cellulose, it was not expected that acid dye solutions would experience a reduction in colour when treated with a BC pellicle. However, in the study in this thesis, Reactive Navy solutions

treated with H&S pellicles not treated with NaOH behaved in a similar way to Acid Blue solutions treated in the same way giving further grounds for speculation that it is residual microbial cells or other contamination on the surface of the pellicle are responsible for this visual change (Figure 41).

7.11.7.1.2 Reactive Navy

Reactive Navy dye solutions visually displayed a change when inoculated with different pellicle types (Figure 38). In this study, the best colour reduction both visually (Figure 38) and according to light absorbance measurements (Figure 40) was achieved in solutions inoculated with tea pellicles not treated with NaOH (63.5 % - tea (dry) - 42 days). This is a lower change than reported in other studies. There have been considerably more publications on the effect of bacterial species on the colour reduction of reactive dyes (than those on acid), with rates of 87 % (Gomare et al., 2009) to 100 % (Chang and Kuo, 2000) on Reactive Golden Yellow and Reactive Red 22 respectively.

Treatment of reactive dyes with fungal species have reported a 100 % reduction in colour on Reactive Blue dye solution (Pajot et al., 2011) using *Trichosporon akiyoshidainum*; this strain was not identified as present in the Kombucha SCOBY community used in this thesis. No studies can be found in literature of the effects of the yeast species present in the Kombucha SCOBY (identified in Chapter 3) on reactive dyes.

As observed with Acid Blue, there was a marked change in hue for the Reactive Navy dye solutions treated with H&S pellicles not treated with NaOH, from blue at the start of the

experiment to a brown shade at 14, 28, 42 and 56 days (Figure 38). These solutions also showed discolouration with a shade change to green / brown, further supporting the hypothesis that microbial residue or extra polymeric substance on the pellicle inocula is responsible for this shade change. The effect of the residue is supported by the appearance of the dye solutions treated with H&S pellicles that had been rinsed with NaOH; hue change was not apparent in these (Figure 38). Other studies have reported a change in hue of black reactive dye solution (from black to purple) when treated with yeast species (Martorell et al., 2017), so some effects of the yeast community in the inoculum cannot be discounted. In order to verify the effects of microbial residue, further microbiological testing on the dye solutions would be required. This would need to assess the effect of each microbial strain found in the consortium on the shade change of each dyestuff. To facilitate this, each microbial strain would need to be isolated and identified and the dyestuff treated with each isolate independently with shade changes observed and measured across a period of time, using similar methods to those described in this thesis.

7.11.7.2 Observed Differences Between Wet and Dry Pellicle Inocula

In the dyeing of cellulosic fibres, dye uptake is generally improved by the 'wetting out' or pre swelling of the fibres before the dye is introduced as this increases the surface area of the fibre and improves diffusion of the dye into the fibre structure (Chen et al., 2011; Mao et al., 2014). When viewing the data collected in this study, it is noted that this principle applies to the measurements of the colour remaining in the liquids; wet pellicles used as inocula reduced colour intensity of dye solutions more than those used dry, with wet tea pellicle

inocula (not treated with NaOH) significantly more effective ($p < 0.0001$) (Figure 39, Figure 40).

The effect was not observed to such a degree in the pellicles treated with NaOH. NaOH is commonly used in the dyeing of cellulose to swell the fibres (this is referred to as mercerisation when used on cotton fibre (Clark, 2011; Chakraborty, 2014) and therefore it was anticipated that treating the pellicles with NaOH would improve their uptake of dye. However, this was not the case; no significant improvements in colour reduction were observed in NaOH treated pellicles compared to those untreated. For example, in Acid Blue, tea (dry) pellicles reduced colour by 79 % compared to tea (dry NaOH) 15 %; in Reactive Navy the maximum reduction was achieved by tea (dry), 63.5 %, whilst tea (dry NaOH) achieved 29.5 %. In the case of this experiment, NaOH was primarily used to lyse any residual bacterial cells within the pellicle (as detailed in existing literature, Table 8), thus the effects of a BC pellicle without a residual bacterial community (NaOH treated) could be compared to one with an active community (untreated). It has been suggested that treatment with NaOH could affect the molecular structure of BC by increasing its crystalline area (Suryanto et al., 2019; Younesi et al., 2019) and thus reduce its ability to take up dye, which could explain the differences observed in this study. However, the absence of residual microbes (due to the NaOH treatment) cannot be disregarded in analysing the decreased ability of the NaOH treated pellicles to reduce colour intensity in the dye solution.

The results further suggest that it is a combination of the BC pellicle developed in tea and the active microbial community that have the highest efficacy in colour reduction in dye solution in the cases of both Acid Blue and Reactive Navy. In accordance with these results,

Suvilampi (2003) states that the most effective colour reduction occurs when a variety of bacterial and fungal species are used to treat dye solutions. Additionally, this concurs with the findings of Wang et al. (2009b), Hayat et al. (2015) and Masi et al. (2019) who all agree a biological approach to the removal of colour from effluent is most effective when combined with other approaches, such as adsorption.

7.11.7.3 Colour Absorbance by Pellicles

After removal from the dye solution, as the pellicles dried, they became wrinkled (i.e. did not have a flat surface) therefore collection of light absorbance data using a spectrophotometer was not possible because inconsistent results were achieved due to the uneven surface of the pellicles.

Visual analysis (by eye) was conducted, with Reactive Navy and Acid Blue tea pellicles darkest in appearance, supporting the findings (above) of the reduction in colour of the dye solutions. Additionally, images of the pellicles were processed using ImageJ software (section 7.11.7.1) and the results presented in Figure 42 and Figure 43.

In general, pellicles were more heavily stained with colour from the Reactive Navy compared to those submerged in the Acid Blue solution, in contrast with the measurements of dye remaining in the liquids when the pellicles were removed (Figure 39, Figure 40) (colour intensity was less in Acid Blue solution compared to Reactive Navy solution after pellicle treatment). However, the dyes are different in terms of hue (shade); Acid Blue visually appears a ‘weaker’ or ‘paler’ shade than that of Reactive Navy and this could account for the

visual appearance of the pellicles submerged in Acid Blue solution appearing less pronounced.

As Acid dyes are unable to form chemical bonds with the BC fibres (compared to reactive dyes which form covalent bonds with the hydroxyl groups found in the cellulosic structure), it is suggested that the dye molecules are trapped within the fibrillar network of the pellicle and therefore there may be less colour bound to the pellicles.

As discussed in section 7.11.2, Reactive dyes are used commonly in the textile industry to dye cellulosic fibres and form covalent bonds with the fibre, and it is therefore anticipated that the dye in solution could bond to the cellulosic fibres within the pellicle. Another common feature of reactive dyeing is the hydrolysis of the dye molecules in the dye solution; some dye molecules can preferentially bond with water, rather than the fibre (Best, 2012). If the reactive dye molecules in solution are able to bond with the cellulosic fibres in the pellicle and any hydrolysed dye molecules are trapped within the pellicle structure, two mechanisms of colour capture are available and potentially a more effective method of dye removal from solution. As discussed above, (section 7.11.7.2) it is the combination of microbial and physical approaches (e.g. adsorption) that have been found to be most effective at colour removal from effluent (Wang et al., 2009a; Hayat et al., 2015; Masi et al., 2019).

With the exception of H&S (dry) pellicles, colour adsorption was observed in all pellicles submerged in Reactive Navy dye solution. However, as noted in the discussion of the dye

solution colour changes, the H&S pellicle colours could have been affected by contamination (section 7.11.7.1.1).

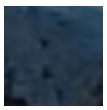
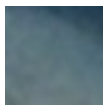
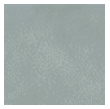
	0 days	14 days	28 days	42 days	56 days
Tea (Dry)					
Tea (Wet)					
Tea (NaOH Dry)					
Tea (NaOH Wet)					
H&S (Dry)					
H&S (Wet)					
H&S (NaOH Dry)					
H&S (NaOH Wet)					

Figure 42. Colour (Hue & Intensity) Change of Pellicle Inocula Removed from Acid Blue Dye Solution.

Images Produced Using Image J Software (www.imagej.net).

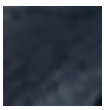
	0 days	14 days	28 days	42 days	56 days
Tea (Dry)					
Tea (Wet)					
Tea (NaOH Dry)					
Tea (NaOH Wet)					
H&S (Dry)					
H&S (Wet)					
H&S (NaOH Dry)					
H&S (NaOH Wet)					

Figure 43. Colour (Hue & Intensity) Change of Pellicle Inocula Removed from Reactive Navy Dye Solution.

Images Produced Using Image J Software (*www.imagej.net*).

7.11.7.4 Effects of Liquid Inocula

Colour change was observed in the dye solutions treated with the liquid growth media (microbial community) alone (Figure 44, Figure 45).

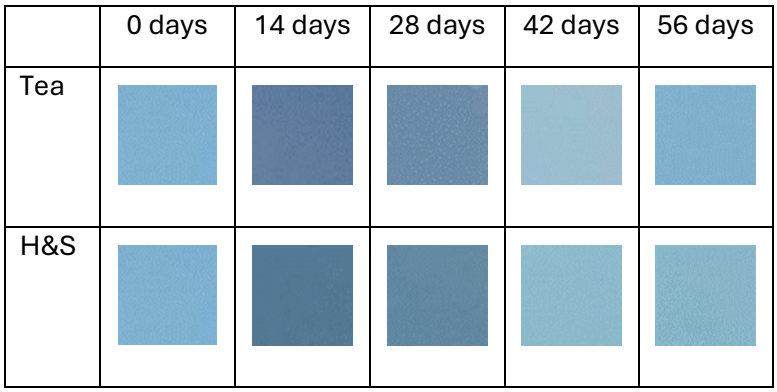


Figure 44. Colour (Hue & Intensity) Change of Acid Blue Dye Solution After Growth Liquid Inoculation.

Images Produced Using Image J Software (*www.imagej.net*).

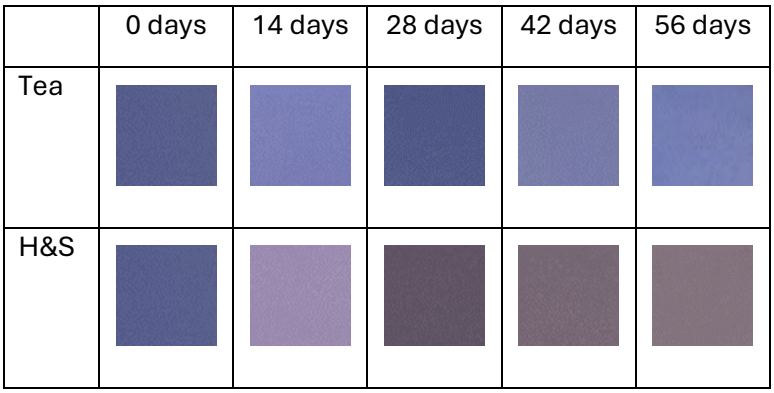


Figure 45. Colour (Hue & Intensity) Change of Reactive Navy Dye Solution After Growth Liquid Inoculation.

Images Produced Using Image J Software (*www.imagej.net*).

Acid Blue dye solutions were affected in a similar way by tea-based and H&S-based liquid inocula. Initially, visual observations (both by eye and using ImageJ software) noted an increase in colour intensity in Acid Blue dye solution; after 42 days a decrease in intensity was observed for both tea-based and H&S-based liquid inocula (Figure 44). This agrees with light absorbance measurements of the dye solutions; clear trends in colour intensity across all sample points were difficult to detect (Figure 39). The light absorbance measurements indicate that the tea-based liquid inoculum (50.7 % colour reduction after 14 days) was more significantly ($p < 0.0001$) effective at reducing colour than the H&S-based (15 % reduction after 14 days) inoculum in Acid Blue dye solution. This effect was likely due to the biological degradation of the dye molecule (section 7.7.3) and beyond the scope of further invetigation in this thesis.

Effects on colour for Reactive Navy dye solution were similar for both tea-based and H&S-based liquid inocula; whilst changes in hue were visually observed, visual reductions of colour intensity were not noted (Figure 45). When treated with H&S-based liquid inocula, the change of the dye solution hue over time (from blue to brown) was similar to that observed when Reactive Navy dye solution was treated with a H&S solid pellicle not treated with NaOH (Figure 45). Absorbance data for tea-based and H&S-based liquid inocula showed no significant trends in colour intensity reduction across the period of the experiment for Reactive Navy (Figure 40) .

These observations indicate that whilst suspended microbial and yeast communities could have an impact on colour intensity reduction in Acid Blue dye solution, there is little impact on Reactive Navy in terms of intensity of colour, albeit the hue is changed which could

indicate some microbial degradation effect on the dye molecular structure. Ali (2010) and Mishra (2018) agree that hue change can occur due to microbial breakdown of the dye structure and whilst this leads to colour change, it does not necessarily lead to loss of colour intensity and could even produce toxic by-products.

The dyes in this study were of anthraquinone (Reactive Navy) and azo (Acid Blue) molecular structure, both of which have shown a degree of resistance to breakdown by microbial processes in the natural environment (Banat et al., 1996; Holkar et al., 2016). Nevertheless, in other studies, it has been noted that *Pseudomonas sp*. and *Candida sp*. are effective in colour reduction of reactive dye solution. *Pseudomonas sp*. reduced colour intensity by 80 - 99 % in Reactive Red solution (Kalyani et al., 2009; Sheth and Dave, 2009) and *Candida sp*. reduced colour intensity by 90 % in Reactive Blue solution (Liu et al., 2011). *Pseudomonas sp*. and *Candida sp*. were both identified in the communities of bacteria present in the Kombucha SCOBY used in this study (Chapter 3). However it may be that a particular strain of these species is most effective, which were used in previous studies but not found in the consortium used in this work, but as the reviewed literature did not state specific strains, it is difficult to specifically conclude this. Nevertheless, a change in hue, rather than a reduction in colour intensity is not useful for effluent treatment, regardless of possible molecular breakdown, as colour intensity reduction is critical before liquid waste can be discharged into the environment (section 7.5).

There is no previous work in the current literature examining the effects of the microbial communities described in this thesis which specifically focusses on acid dyes. However, it should be remembered that dyes are classed according to two methods, mode of

application (e.g. acid, reactive) and molecular structure (e.g. azo, anthraquinone). Whilst microbial effects have been discussed from the angle of molecular structure, acid dyes are also worthy of further exploration because some trends of colour intensity reduction of Acid Blue solution were observed, particularly when the dye solution was treated with solid tea pellicles.

7.12 Summary

- BC pellicles were prepared using a Kombucha SCOBY as an inoculum and either sterile H&S lab manufactured or sterile black tea with sugar as growth media. After incubation at 30 °C for 30 days the BC pellicles were either rinsed in water and dried, or washed with NaOH and dried. The resultant pellicles were used to treat dye solutions of Acid Blue and Reactive Navy to assess the effects of these on the concentrations of colour remaining in the solutions after set periods of time, i.e. the amount of dye absorbed by the pellicles.
- The observations were made over 56 days but greatest amount of colour intensity reduction from the solutions was noted at 14 days, indicating this could be the optimal time for treatment of dye solutions with Kombucha SCOBY derived pellicles.
- The pellicles developed in tea (and not treated with NaOH thus containing an active microbial community) reduced colour intensity to the greatest degree from the dye solutions. The pellicles with the least effect were those developed in H&S medium

and treated with NaOH. Wet pellicles used as inocula, rather than dry, also had the greatest effects of reducing colour intensity.

- In dye solutions inoculated with liquid growth media, there was limited effect in terms of colour intensity in Reactive Navy solutions. However, there was a decrease in intensity in Acid Blue solution inoculated with tea liquid growth medium indicating possible dye degradation due to microbial activity.
- The greatest reduction in intensity was that of the 'active' tea derived pellicles (not treated with NaOH), suggesting that it is the combination of the microbial community and the BC pellicle itself that are most effective at colour removal from acid dye solution.
- Whilst BC developed from a Kombucha SCOBY has not been specifically investigated as a colour remover of acid dyes in previous studies, *Pseudomonas sp.* and *Candida sp.* have both been explored in previous work with varying degrees of success (Table 21, Table 22). Both of these genera were found in the sequencing work of the Kombucha SCOBY conducted in chapter 3 of this thesis, suggesting that their presence may have affected the reduction of colour in Acid Blue dye solution.
- In exisiting literature, the effect of microbial removal of colour from acid dye solutions is limited compared to that of reactive dyes; this study has shown that the effect of a microbial consortium in a BC pellicle on removal of both types of dye is worthy of further exploration.

Chapter 8: Conclusions and Recommendations for Future Work

This thesis has explored the use of BC as a sustainable material for the textile industry. BC pellicles offer a diverse range of properties such as enhanced moisture absorbency and abrasion resistance and the potential to be cheaply and relatively easily manufactured without contaminants and or the intervention of synthetic chemicals. Thus, they have potential to be considered a 'next generation' textile and improve the environmental credentials of the textile industry.

The thesis first investigated the production of BC pellicles using both pure culture and a Kombucha SCOBY (symbiotic culture of bacteria and yeasts) as inocula and evaluating the most suitable type of growth medium and conditions. Sterile black tea with sugar produced the greatest yields of BC pellicles by weight, although the lab manufactured medium, H&S, produced pellicles more consistently, albeit with a lower yield. This section of the thesis discovered that it was the pellicles developed from the microbial consortium Kombucha SCOBY inoculum that were the most consistently reproducible when the liquid growth broth (rather than the solid pellicle) was used to inoculate subsequent generations, illustrating the potential sustainable manufacture of such material. Additionally, when used to inoculate black tea with sugar growth medium, this method could be used to reproduce pellicles in a public space, with minimal effects of contamination compared to those observed with lab manufactured (H&S) growth medium. This is an exciting contribution to knowledge as it further illustrates the potential of BC pellicles to be produced in non-sterile

conditions, using easily accessible and relatively cheap growth media, and a constant source of inoculum, opening the possibility to manufacture in relatively uncontrolled environments.

Furthermore, the utilisation of a citizen science method to collect data regarding the replication of BC pellicles (via a school science club and science festival event) demonstrated that engagement was significantly enhanced when scientific enquiry is either contextualised with or embedded in creative discourse. The integration not only yielded greater interest and participation from the public (non-experts) but also highlighted the potential of combining science with arts-based subjects to develop a more dynamic and engaging learning experience. These findings could be used to support the development of STEAM curricula, illustrating how creative approaches can deepen scientific enquiry and learning. This could provide a pathway to further develop the findings of this study in terms of community-based data collection and interdisciplinary engagement.

The thesis then examined the uses and applications of BC. Already widely used in the medical, beauty and food industries, these applications make use of the exceptional hydrophilic characteristics of the BC and largely use pure culture (bacteria) to produce the pellicles in carefully controlled conditions. Additionally, many of these applications are for single use products which impacts both cost and environmental impacts of such items. Whilst there has been some exploration of BC in clothing applications, these have been restricted to conceptual pieces and have yet to reach the mainstream market. In contribution to knowledge, part two of the thesis explored some of the reasons why this is the case from an apparel textile performance point of view; at the time of writing there is no

evidence that BC has been tested as an apparel textile in current literature aside from the outcomes of this thesis. Apart from high hydrophilicity causing potential issues with wearer comfort, the pellicles were also considerably weaker than animal leather in terms of tensile strength. BC has been labelled 'vegetable leather' by some researchers; the reduced tensile strength compared to that of animal leather limits its application in some clothing applications, particularly those that fit more closely to the body. However, this part of the thesis revealed the exceptional abrasion properties of BC, where it outperformed animal leathers of a similar weight and thickness. This discovery suggests BC could be suitable for applications such as protective clothing if the issues with hydrophilicity could be addressed. One remedy to this could be the application of synthetic finishes, although this would compromise the 'environmentally friendly' credentials of the pellicles, which are produced using natural (i.e. tea and sugar) and renewable (i.e. Kombucha SCOBY) resources.

As the textile industry has a problem with coloured dyehouse effluent discharge polluting the natural environment, the next part of the thesis examined the role of BC in the treatment of this, utilising its exceptional hydrophilic properties. It was hypothesised that hydrophilic BC pellicles and their inherent active microbial communities (when developed from a bacterial and yeast consortium) have potential in removal of colour from dyehouse effluent. An opinion in current literature is that the more effective method of decolourisation of dye solutions is using microbes under anaerobic conditions, but this method frequently produces toxic by-products (Zabłocka-Godlewska et al., 2018). Solis (2012) agrees but proposes that more research into microbes active under aerobic conditions is required, as the by-products of their action of dyestuffs are usually inert. The BC pellicle and the active

microbial community (all aerobic) had more effect on reducing the colour intensity in Acid Blue solutions compared to Reactive Navy, whilst changing the hue of Reactive Navy with limited effect on colour intensity. This was contrary to expected results; Reactive Navy is used to dye cellulosic fibre, and it was anticipated that the BC pellicle would therefore attract the dye molecules from solution to a greater degree than those from Acid Blue solution, resulting in a greater reduction in colour intensity of Reactive Blue solution at the end of the experiment. This final section of the thesis contributes to knowledge by indicating that the microbial consortium and the BC nanofibrillar structure in the pellicle work in tandem to reduce colour intensity in Acid dye solution via both microbial metabolic and pellicle absorbance mechanisms.

This work could be explored further; the focus of most studies to date have been to evaluate the cleanliness of the water after treatment (Holkar et al., 2016), without considering the dyestuffs or other additives captured by the various systems. The reuse or recycling of these dyestuffs and additives is an area worthy of exploration in line with the '*technical, environmental, and economical performance of textile industries'* and the adoption of cleaner production strategies and sustainable approaches indicated by the Waste Resources Action Plan (WRAP) (2021). It should also be noted that not all the extracted products have the potential for reuse; for example, reactive dyes left in effluent can become non-reactive due to the hydrolysis process undertaken as part of the dyeing mechanism and therefore cannot be recycled (Hassan and Carr, 2018). Much of the work in the field has focussed on the degradation of reactive dyes, possibly due to their ease of mode of application (a neutral dyebath) and their prevalence in the modern industry in the dyeing of

cellulosic based fibres. Acid dyes are not used in the colouration of cellulosics but are widely used in the dyeing of protein fibres (e.g. silk and wool) and synthetics (e.g. nylon). A novel and unexpected finding in this thesis has illustrated how a BC nanofibrillar structure and microbial consortium in a pellicle can be used in tandem to capture acid dye from solution. Whilst this could be viewed as a method of using dyestuff waste to dye BC pellicles for use in other textile applications, with implied impacts on both cost and reduction of waste, further work could explore the extraction of these dyes from the pellicles, with a view to recycling and reuse.

In summary, further work should focus on:

- The exploration of embedding creative practice more deeply into citizen science projects and the assessment of the impact on the quality of data collected in the context of communicating sustainable practice and environmental responsibility to a non-expert audience.
- To consider BC as a true sustainable replacement to animal leather in clothing, the attributes of hydrophilicity and reduced tensile strength need to be addressed without compromising the non-toxic characteristics of untreated BC pellicles. In the shorter term, BC could be incorporated into clothing as patches in key areas to provide enhanced protection; methods of encapsulation of the pellicle would need to be explored to prevent degradation of properties due to environmental exposure.
- Investigation and clarification of the effectiveness of the BC nanofibrillar structure, microbial consortium, BC nanofibrillar structure and microbial consortium

combined, and individual specific bacterial / yeast strains found in the consortium on the adsorption (and therefore dye solution decolourisation) of a range of dye classes. Assessment of the by-products of this mechanism is also necessary to ensure that the resultant solution is safe for discharge into the natural environment.

- Evaluation of the BC pellicle after being used to treat dye solution as a colour extraction method, with a view to either removing the dye for subsequent re-use, re-using the BC pellicle to absorb more dye, or using the BC pellicle as a textile material which has been dyed using by-products of earlier textile manufacture. This could find a place for BC in the revolution of the textile industry.

Chapter 9: References

Abaci, N., Senol Deniz, F. S. & Orhan, I. E. (2022) 'Kombucha - An ancient fermented beverage with desired bioactivities: A narrowed review', *Food Chemistry: X,* 14.

Abell. S., K., Lederman, N., G. (2011) Handbook of Research in Science Education, Routledge, New York.

Aberg, J. (2023). *Materiom : Kombucha material textured and dyed*, UK. [Online] [Accessed 23/03/2024] https://materiom.org/recipe/655

Abou-Zeid, R. E., Dacrory, S., Ali, K. A. & Kamel, S. (2018) 'Novel method of preparation of tricarboxylic cellulose nanofiber for efficient removal of heavy metal ions from aqueous solution', *International Journal of Biological Macromolecules,* 119, pp. 207-214.

Abral, H., Fajri, N., Mahardika, M., Handayani, D., Sugiarti, E. & Kim, H.-J. (2020) 'A simple strategy in enhancing moisture and thermal resistance and tensile properties of disintegrated bacterial cellulose nanopaper', *Journal of materials research and technology,* 9(4), pp. 8754-8765.

Agustin, Y. E. & Padmawijaya, K. S. (2018) 'Effect of Acetic Acid and Ethanol as Additives on Bacterial Cellulose Production by Acetobacter xylinum', *IOP Conference Series: Earth and Environmental Science,* 209(1), pp. 12045.

Ahlström, L.-H., Sparr Eskilsson, C. & Björklund, E. (2005) 'Determination of banned azo dyes in consumer goods', *Trends in Analytical Chemistry,* 24(1), pp. 49-56.

Al-Tohamy, R., Ali, S. S., Li, F., Okasha, K. M., Mahmoud, Y. A. G., Elsamahy, T., Jiao, H., Fu, Y. & Sun, J. (2022) 'A critical review on the treatment of dye-containing wastewater: Ecotoxicological and health concerns of textile dyes and possible remediation approaches for environmental safety', *Ecotoxicology and environmental safety,* 231, pp. 113160-113160.

Ali, H. (2010) 'Biodegradation of Synthetic Dyes—A Review', *Water, Air, & Soil Pollution,* 213(1), pp. 251-273.

Almeida, I. F., Pereira, T., Silva, N. H. C. S., Gomes, F. P., Silvestre, A. J. D., Freire, C. S. R., Sousa Lobo, J. M. & Costa, P. C. (2014) 'Bacterial cellulose membranes as drug delivery systems: An in vivo skin compatibility study', *European Journal of Pharmaceutics and Biopharmaceutics,* 86(3), pp. 332-336.

Almeida, L. (2015). *Functional finishes for textiles : improving comfort, performance and protection*. Cambridge: Woodhead Publishing.

Amnuaikit, T., Chusuit, T., Raknam, P. & Boonme, P. (2011) 'Effects of a cellulose mask synthesized by a bacterium on facial skin characteristics and user satisfaction', *Medical devices,* 4, pp. 77.

Antolak, H., Piechota, D. & Kucharska, A. (2021) 'Kombucha Tea-A Double Power of Bioactive Compounds from Tea and Symbiotic Culture of Bacteria and Yeasts (SCOBY)', *Antioxidants,* 10(10).

Aouni, A., Fersi, C., Ben Sik Ali, M. & Dhahbi, M. (2009) 'Treatment of textile wastewater by a hybrid electrocoagulation/nanofiltration process', *Journal of Hazardous Materials,* 168(2), pp. 868-874.

Arıkan, M., Mitchell, A. L., Finn, R. D. & Gürel, F. (2020) 'Microbial composition of Kombucha determined using amplicon sequencing and shotgun metagenomics', *Journal of Food Science,* 85(2), pp. 455-464.

Ashjaran, A., Yazdanshenas, M. E., Rashidi, A., Khajavi, R. & Rezaee, A. (2013) 'Overview of bio nanofabric from bacterial cellulose', *Journal of the Textile Institute,* 104(2), pp. 121-131.

Ashrafi, A., Jokar, M. & Mohammadi Nafchi, A. (2017) 'Preparation and characterization of biocomposite film based on chitosan and Kombucha tea as active food packaging', *International journal of biological macromolecules,* 108, pp. 444.

Aswini, K., Gopal, N. O. & Uthandi, S. (2020) 'Optimized culture conditions for bacterial cellulose production by Acetobacter senegalensis MA1', *BMC biotechnology,* 20(1), pp. 46-46.

Ayed, L. & Hamdi, M. (2015) 'Manufacture of a beverage from cactus pear juice using “tea fungus” fermentation', *Annals of Microbiology,* 65(4), pp. 2293-2299.

Aßhauer, K. P., Wemheuer, B., Daniel, R. & Meinicke, P. (2015) 'Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data', *Bioinformatics (Oxford, England),* 31(17), pp. 2882-2884.

Babuponnusami, A. & Muthukumar, K. (2014) 'A review on Fenton and improvements to the Fenton process for wastewater treatment', *Journal of Environmental Chemical Engineering,* 2(1), pp. 557-572.

Bai, Y., Liu, R., Liu, Y., Li, E., Li, X. & Yuan, G. (2019) 'Graphene/Carbon Nanotube/Bacterial Cellulose assisted supporting for polypyrrole towards flexible supercapacitor applications', *Journal of Alloys and Compounds,* 777, pp. 524-530.

Baker, M. (2016) '1,500 scientists lift the lid on reproducibility', *Nature (London),* 533(7604), pp. 452-454.

Balaz, B., Mooney, P., Novakova, E., Bastin, L. & Arsanjani, J. (2021) 'Data Quality in Citizen Science', in Vohland, K. (ed.) *The Science of Citizen Science*: Springer, pp. 139-157.

Baldrian, P. & Gabriel, J. (2003) 'Lignocellulose degradation by Pleurotus ostreatus in the presence of cadmium', *FEMS Microbiology Letters,* 220(2), pp. 235-240.

Banat, I. M., Nigam, P., Singh, D. & Marchant, R. (1996) 'Microbial decolorization of textile-dye-containing effluents: A review', *Bioresource Technology,* 58(3), pp. 217-227.

Banks, F. & Barlex, D. (2021). *Teaching STEM in the secondary school : helping teachers meet the challenge* (Second Edition. ed.). New York: Routledge.

Basta, A. H. & El-Saied, H. (2009) 'Performance of improved bacterial cellulose application in the production of functional paper', *Journal of applied microbiology,* 107(6), pp. 2098-2107.

Batmaz, R., Mohammed, N., Zaman, M., Minhas, G., Berry, R. M. & Tam, K. C. (2014) 'Cellulose nanocrystals as promising adsorbents for the removal of cationic dyes', *Cellulose,* 21(3), pp. 1655-1665.

Battikh, H., Chaieb, K., Bakhrouf, A. & Ammar, E. (2013) 'Antibacterial and antifungal activities of black and green Kombucha teas', *Journal of Food Biochemistry,* 37(2), pp. 231-236.

Best, J. (2012). *Colour design : theories and application*. Cambridge: Woodhead.

Bianchet, R. T., Vieira Cubas, A. L., Machado, M. M. & Siegel Moecke, E. H. (2020) 'Applicability of bacterial cellulose in cosmetics – bibliometric review', *Biotechnology reports,* 27, pp. e00502-e00502.

Biofabricate (2021) *Understanding Biomaterial Innovations*, UK: Biofabricate. [Online] [accessed 25/10/2023] https://www.biofabricate.co/resources

Birkeland, I. J., Burton, R., Parra, C. & Siivonen, K. (2018). *Cultural sustainability and the nature-culture interface : livelihoods, policies, and methodologies*. Abingdon: Routledge.

Blackburn, R. S. (2005). *Biodegradable and sustainable fibres*. Cambridge: Woodhead.

Blanco Parte, F. G., Santoso, S. P., Chou, C.-C., Verma, V., Wang, H.-T., Ismadji, S. & Cheng, K.-C. (2020) 'Current progress on the production, modification, and applications of bacterial cellulose', *Critical Reviews in Biotechnology,* 40(3), pp. 397-414.

BNP Media (2011). Passing the Test of Time: Leathers Natural Abrasion Resistance*, Industrial Safety and Hygiene News,* 45 (2), pp. 44.

Bodin, A. K., Bäckdahl, H., Fink, H., Gustafsson, L., Risberg, B. & Gatenholm, P. (2007) 'Influence of cultivation conditions on mechanical and morphological properties of bacterial cellulose tubes', *Biotechnology and Bioengineering,* 97(2), pp. 425.

Boekhout, T., Mayser, P., Guého-Kellermann, E. & Velegraki, A. (2010). *Malassezia and the Skin : Science and Clinical Practice*. Berlin, Heidelberg: Springer.

Bolt Threads (2023) Bolt Threads, UK, [online] [accessed 10/03/2023], www.boltthreads.com

Braune, S., Lendlein, A. & Jung, F. (2018) 'Developing standards and test protocols for testing the hemocompatibility of biomaterials'. *In* Siedlecki, C. A. (ed.) (2018) *Hemocompatability of Biomaterials for Clinical Applications.* USA: Elsevier Ltd, pp. 51-76.

Brown, R. M., Willison, J. H. M. & Richardson, C. L. (1976) 'Cellulose biosynthesis in acetobacter xylinum - visualization of site of synthesis and direct measurement of invivo process', *Proceeding of the National Academy of Sciences of the United States of America,* 73(12), pp. 4565-4569.

Brundtland, G. H. (1987). *Our common future*. Oxford: Oxford University Press.

Bryman, A. (2008). 'Barriers to Integrating Quantitative and Qualitative Research' *Journal of Mixed Methods Research,* 1(1), pp.8–22.

Budhiono, A., Rosidi, B., Taher, H. & Iguchi, M. (1999) 'Kinetic aspects of bacterial cellulose formation in nata-de-coco culture system', *Carbohydrate Polymers,* 40(2), pp. 137-143.

Bueno, F., Chouljenko, A. & Sathivel, S. (2021) 'Development of coffee Kombucha containing Lactobacillus rhamnosus and Lactobacillus casei: Gastrointestinal simulations and DNA microbial analysis', *Food Science and Technology,* 142, pp 110980.

Burrows, A. and Borowczak, M. (2022). Integrated STEM and STEM Partnerships: Teaching and Learning. Basel: MDPI - Multidisciplinary Digital Publishing Institute.

Bäckdahl, H., Esguerra, M., Delbro, D., Risberg, B. & Gatenholm, P. (2008) 'Engineering microporosity in bacterial cellulose scaffolds', *Journal of Tissue Engineering and Regenerative Medicine,* 2(6), pp. 320-330.

Cai, Z. & Yang, G. (2011) 'Optical nanocomposites prepared by incorporating bacterial cellulose nanofibrils into poly(3-hydroxybutyrate)', *Materials Letters,* 65(2), pp. 182-184.

Çakar, F., Katı, A., Özer, I., Demirbağ, D. D., Şahin, F. & Aytekin, A. Ö. (2014) 'Newly developed medium and strategy for bacterial cellulose production', *Biochemical Engineering Journal,* 92, pp. 35-40.

Campano, C., Merayo, N., Negro, C. & Blanco, Á. (2018) 'Low-fibrillated bacterial cellulose nanofibers as a sustainable additive to enhance recycled paper quality', *International Journal of Biological Macromolecules,* 114, pp. 1077-1083.

Cao, J., Sanganyado, E., Liu, W., Zhang, W. & Liu, Y. (2019) 'Decolorization and detoxification of Direct Blue 2B by indigenous bacterial consortium', *Journal of Environmental Management,* 242, pp. 229-237.

Castro, C., Zuluaga, R., Putaux, J.-L., Caro, G., Mondragon, I. & Gañán, P. (2011) 'Structural characterization of bacterial cellulose produced by Gluconacetobacter swingsii sp. from Colombian agroindustrial wastes', *Carbohydrate Polymers,* 84(1), pp. 96-102.

Celik, K. B., Cengiz, E. C., Sar, T., Dursun, B., Ozturk, O., Akbas, M. Y. & Demir-Cakan, R. (2018) 'In-situ wrapping of tin oxide nanoparticles by bacterial cellulose derived carbon nanofibers and its application as freestanding interlayer in lithium sulfide based lithium-sulfur batteries', *Journal of Colloid And Interface Science,* 530, pp. 137-145.

Četojević-Simin, D. D., Velićanski, A. S., Cvetković, D. D., Markov, S. L., Mrđanović, J. Ž., Bogdanović, V. V. & Šolajić, S. V. (2012) 'Bioactivity of Lemon Balm Kombucha', *Food and Bioprocess Technology,* 5(5), pp. 1756-1765.

Chakraborty, J. N. (2014). *Fundamentals and practices in colouration of textiles* (Second edition. ed.). India: Woodhead.

Chakravorty, S., Bhattacharya, S., Bhattacharya, D., Chatzinotas, A., Chakraborty, W. & Gachhui, R. (2016) 'Kombucha tea fermentation: Microbial and biochemical dynamics', *International Journal of Food Microbiology,* 220, pp. 63-72.

Chan, C. K., Shin, J. & Jiang, S. X. K. (2018) 'Development of Tailor-Shaped Bacterial Cellulose Textile Cultivation Techniques for Zero-Waste Design', *Clothing and Textiles Research Journal,* 36(1), pp. 33-44.

Chan, H. Y., Choi, H., Hailu, M. F., Whitford, M. & Duplechain DeRouen, S. (2020) 'Participation in structured STEM-focused out-of-school time programs in secondary school: Linkage to postsecondary STEM aspiration and major', *Journal of Research in Science Teaching,* 57(8), pp. 1250-1280.

Chang, J.-S., Chou, C., Lin, Y.-C., Lin, P.-J., Ho, J.-Y. & Lee Hu, T. (2001) 'Kinetic characteristics of bacterial azo-dye decolorization by Pseudomonas luteola', *Water Research,* 35(12), pp. 2841-2850.

Chang, J.-S. & Kuo, T.-S. (2000) 'Kinetics of bacterial decolorization of azo dye with Escherichia coli NO3', *Bioresource Technology,* 75(2), pp. 107-111.

Chao, Y., Ishida, T., Sugano, Y. & Shoda, M. (2000) 'Bacterial cellulose production by Acetobacter xylinum in a 50-L internal-loop airlift reactor', *Biotechnology and Bioengineering,* 68(3), pp. 345-352.

Chatzisymeon, E., Xekoukoulotakis, N. P., Coz, A., Kalogerakis, N. & Mantzavinos, D. (2006) 'Electrochemical treatment of textile dyes and dyehouse effluents', *Journal of Hazardous Materials,* 137(2), pp. 998-1007.

Chauhan, A. & Jindal, T. (2020). *Microbiological methods for environment, food and pharmaceutical analysis*. Cham: Springer.

Chawla, P. R., Bajaj, I. B., Survase, S. A. & Singhal, R. S. (2009) 'Microbial cellulose: Fermentative production and applications', *Food Technology and Biotechnology,* 47(2), pp. 107-124.

Chen, C. & Liu, B. Y. (2000) 'Changes in major components of tea fungus metabolites during prolonged fermentation', *Journal of Applied Microbiology,* 89(5), pp. 834-839.

Chen, G., Huang, M. h., Chen, L. & Chen, D. h. (2011) 'A batch decolorization and kinetic study of Reactive Black 5 by a bacterial strain Enterobacter sp. GY-1', *International Biodeterioration & Biodegradation,* 65(6), pp. 790-796.

Chi, H. K., Hoon, H. S., Joon, K. Y., Cheol, C. J. & Keun, L. C. (2016). *Method for manufacturing a cosmetic bio-cellulose mask pack sheet and use thereof*. United States Patent US 20130244977A1.

Choi, S. M., Rao, K. M., Zo, S. M., Shin, E. J. & Han, S. S. (2022) 'Bacterial Cellulose and Its Applications', *Polymers,* 14(6), pp. 1080.

Christie, R. M., Royal Society of, C. & Books24x, I. (2001). *Colour chemistry*. Cambridge: Royal Society of Chemistry.

Citizen Science (2023) *Citizen Science* [online] [accessed 13 / 08 / 2023] https://eu-citizen.science/

Clark, M. (2011). *Handbook of textile and industrial dyeing. Volume 1, Principles, processes and types of dyes,* Cambridge: Woodhead.

Coelho, R. M. D., Almeida, A. L. d., Amaral, R. Q. G. d., Mota, R. N. d. & Sousa, P. H. M. d. (2020) 'Kombucha: Review', *International journal of gastronomy and food science,* 22, pp. 100272.

Cole, J. R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R. J., Kulam-Syed-Mohideen, A. S., McGarrell, D. M., Marsh, T., Garrity, G. M. & Tiedje, J. M. (2009) 'Ribosomal Database Project: improved alignments and new tools for rRNA analysis', *Nucleic acids research,* 37(suppl-1), pp. D141-D145.

Costa, A. F. d. S., de Amorim, J. D. P., Almeida, F. C. G., de Lima, I. D., de Paiva, S. C., Rocha, M. A. V., Vinhas, G. M. & Sarubbo, L. A. (2019) 'Dyeing of bacterial cellulose films using plant-based natural dyes', *International Journal of Biological Macromolecules,* 121, pp. 580-587.

Cristea, D. & Vilarem, G. (2006) 'Improving light fastness of natural dyes on cotton yarn', *Dyes and Pigments,* 70(3), pp. 238-245.

Czaja, W., Krystynowicz, A., Bielecki, S. & Brown, R. M. (2006) 'Microbial cellulose—the natural power to heal wounds', *Biomaterials,* 27(2), pp. 145-151.

Czaja, W. K., Young, D. J., Kawecki, M. & Brown Jr, R. M. (2007) 'The future prospects of microbial cellulose in biomedical applications', *Biomacromolecules,* 8(1), pp. 1-12.

da Silva, C. J. G., de Medeiros, A. D. L. M., de Amorim, J. D. P., do Nascimento, H. A., Converti, A., Costa, A. F. S. & Sarubbo, L. A. (2021) 'Bacterial cellulose biotextiles for the future of sustainable fashion: a review', *Environmental chemistry letters,* 19(4), pp. 2967-2980.

Dafale, N., Wate, S., Meshram, S. & Nandy, T. (2008) 'Kinetic study approach of remazol black-B use for the development of two-stage anoxic–oxic reactor for decolorization/biodegradation of azo dyes by activated bacterial consortium', *Journal of Hazardous Materials,* 159(2), pp. 319-328.

Das, D., Charumathi, D. & Das, N. (2010) 'Combined effects of sugarcane bagasse extract and synthetic dyes on the growth and bioaccumulation properties of Pichia fermentans MTCC 189', *Journal of Hazardous Materials,* 183(1), pp. 497-505.

Das, D., Charumathi, D. & Das, N. (2011) 'Bioaccumulation of the synthetic dye Basic Violet 3 and heavy metals in single and binary systems by Candida tropicalis grown in a sugarcane bagasse extract medium: Modelling optimal conditions using response surface methodology (RSM) and inhibition kinetics', *Journal of Hazardous Materials,* 186(2-3), pp. 1541-1552.

Davis, J. R., (2003). *Handbook of materials for medical devices* (1st ed.). Materials Park: ASM International.

Dayal, M. S., Goswami, N., Sahai, A., Jain, V., Mathur, G. & Mathur, A. (2013) 'Effect of media components on cell growth and bacterial cellulose production from Acetobacter aceti MTCC 2623', *Carbohydrate Polymers,* 94(1), pp. 12-16.

DeCoito, I., Fazio, X. and Gichuru, J. (2024). Global perspectives on STEM education theory and practice. Cham: Springer.

De Meyer, K. et al. (2021). Transforming the stories we tell about climate change: from 'issue' to 'action', *Environmental research letters*, 16(1), pp.15002.

De Moor, T., Rijpma, A. & Prats López, M. (2019) 'Dynamics of Engagement in Citizen Science: Results from the "Yes, I do!"-Project', *Citizen science : theory and practice,* 4(1), pp. 1-17.

De Vrieze, J., Pinto, A. J., Sloan, W. T. & Ijaz, U. Z. (2018) 'The active microbial community more accurately reflects the anaerobic digestion process: 16S rRNA (gene) sequencing as a predictive tool', *Microbiome,* 6(1), pp. 63-13.

Delahunty, T. J. & Ríordáin, M. I. N. (2023) *Perspectives in contemporary STEM education research : research methodology and design*, New York, Routledge.

Delate, K., Heller, B. & Shade, J. (2021) 'Organic cotton production may alleviate the environmental impacts of intensive conventional cotton production', *Renewable agriculture and food systems,* 36(4), pp. 405-412.

Desantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P. & Andersen, G. L. (2006) 'Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB', *Applied and Environmental Microbiology,* 72(7), pp. 5069-5072.

Dickinson, J. L., Bonney, R., Louv, R. & Fitzpatrick, J. W. (2012). *Citizen Science : Public Participation in Environmental Research*. Ithaca: Comstock.

Diez-Ozaeta, I. & Astiazaran, O. J. (2022) 'Recent advances in Kombucha tea: Microbial consortium, chemical parameters, health implications and biocellulose production', *International Journal of Food Microbiology,* 377, pp. 109783-109783.

Dima, S. O., Panaitescu, D. M., Orban, C., Ghiurea, M., Doncea, S. M., Fierascu, R. C., Nistor, C. L., Alexandrescu, E., Nicolae, C. A., Trica, B., Moraru, A. & Oancea, F. (2017) 'Bacterial Nanocellulose from Side-Streams of Kombucha Beverages Production: Preparation and Physical-Chemical Properties', *Polymers,* 9(8), pp. 374.

Domskiene, J., Sederaviciute, F. & Simonaityte, J. (2019) 'Kombucha bacterial cellulose for sustainable fashion', *International Journal of Clothing Science and Technology,* 31(5), pp. 644-652.

Dong, X., Gu, Z., Hang, C., Ke, G., Jiang, L. & He, J. (2019) 'Study on the salt-free low-alkaline reactive cotton dyeing in high concentration of ethanol in volume', *Journal of Cleaner Production,* 226, pp. 316-323.

dos Santos, R. F., Ramlow, H., Dolzan, N., Machado, R. A. F., de Aguiar, C. R. L. & Marangoni, C. (2018) 'Influence of different textile fibers on characterization of dyeing wastewater and final effluent', *Environmental Monitoring and Assessment,* 190(11), pp. 1-12.

Drew, D. & Yehounme, G. (2023) *The apparel industry's environmental impact in 6 graphics*. [Online] [Accessed 25/08/2023] https://www.wri.org/insights/apparel-industrys-environmental-impact-6-graphics

Du, H., Liu, W., Zhang, M., Zhang, X., Si, C. & Li, B. (2019) 'Cellulose nanocrystals and cellulose nanofibrils based hydrogels for biomedical applications', *Carbohydrate Polymers,* 209, pp. 130-144.

Dufresne, A. (2012). *Nanocellulose: From Nature to High Performance Tailored Materials* (Vol. 1). Berlin/Boston: Walter de Gruyter GmbH.

Dufresne, C. & Farnworth, E. (2000) 'Tea, Kombucha, and health: a review', *Food Research International,* 33(6), pp. 409-421.

Dutta, D. & Gachhui, R. (2006) 'Novel nitrogen-fixing Acetobacter nitrogenifigens sp. nov., isolated from Kombucha tea', *International Journal of Systematic and Evolutionary Microbiology,* 56(8), pp. 1899-1903.

ECSA, (2015) *European Citizen Science Association: Ten Principles of Citizen Science.*, Berlin: EU-Citizen Science [Online] [Accessed 19/07/2023] https://zenodo.org/records/5127534#.YR98rkBCRhE

El Bouraie, M. & El Din, W. S. (2016) 'Biodegradation of Reactive Black 5 by Aeromonas hydrophila strain isolated from dye-contaminated textile wastewater', *Sustainable Environment Research,* 26(5), pp. 209-216.

Ellen MacArthur Foundation, (2017). *A new textiles economy: Redesigning fashion's future*. [Online] [Accessed 15/05/2023] https://www.ellenmacarthurfoundation.org/a-new-textiles-economy

Eloe-Fadrosh, E. A., Ivanova, N. N., Woyke, T. & Kyrpides, N. C. (2016) 'Metagenomics uncovers gaps in amplicon-based detection of microbial diversity', *Nature Microbiology,* 1(4), pp. 15032-15032.

Elsasser, V. H. & Sharp, J. R. (2022). *Textiles : concepts and principles* (Fourth edition ed.). New York: Fairchild Books.

Environment Agency UK, (2009). *The Textile Sector*. Bristol, UK: UK Government. [Online] [Accessed 20/06/2021] https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/298040/geho0209bpjc-e-e.pdf

Ertuğrul, S., Bakır, M. & Dönmez, G. (2008) 'Treatment of dye-rich wastewater by an immobilized thermophilic cyanobacterial strain: Phormidium sp', *Ecological Engineering,* 32(3), pp. 244-248.

Esa, F., Tasirin, S. M. & Rahman, N. A. (2014) 'Overview of Bacterial Cellulose Production and Application', *Agriculture and Agricultural Science Procedia,* 2, pp. 113-119.

Esguerra, M., Fink, H., Laschke, M. W., Jeppsson, A., Delbro, D., Gatenholm, P., Menger, M. D., Risberg, B., Göteborgs, Sahlgrenska, A., (2010) 'Intravital fluorescent microscopic evaluation of bacterial cellulose as scaffold for vascular grafts', *Journal of Biomedical Materials Research. Part A,* 93(1), pp. 140.

European Parliament, (2023). *The impact of textile production and waste on the environment (infographics).* [Online] (Accessed 20/06/2023] https://www.europarl.europa.eu/news/en/headlines/society/20201208STO93327/the-impact-of-textile-production-and-waste-on-the-environment-infographics.

Fang, D. D. (2018). *Cotton Fiber: Physics, Chemistry and Biology* (1st 2018. ed.). Cham: Springer International Publishing.

Fernandes, M., Gama, M., Dourado, F. & Souto, A. P. (2019) 'Development of novel bacterial cellulose composites for the textile and shoe industry', *Microbial Biotechnology,* 12(4), pp. 650-661.

Fersi, C. & Dhahbi, M. (2008) 'Treatment of textile plant effluent by ultrafiltration and/or nanofiltration for water reuse', *Desalination,* 222(1), pp. 263-271.

Fijałkowski, K., Żywicka, A., Drozd, R., Kordas, M. & Rakoczy, R. (2016) 'Effect of Gluconacetobacter xylinus cultivation conditions on the selected properties of bacterial cellulose', *Polish Journal of Chemical Technology,* 8, pp. 117-123.

Fink, H., Faxälv, L., Molnár, G. F., Drotz, K., Risberg, B., Lindahl, T. L. & Sellborn, A. (2010) 'Real-time measurements of coagulation on bacterial cellulose and conventional vascular graft materials', *Acta Biomaterialia,* 6(3), pp. 1125.

Fletcher, K. (2008). *Sustainable fashion and textiles: design journeys*. London: Earthscan.

Fletcher, K. & Grose, L., (2012). Fashion and Sustainability: Design for Change. London, Laurence King Publishing.

Fourt, L. & Hollies, N. R. S. (2002). *Clothing : comfort and function*. Ann Arbor: UMI Books on Demand.

Frensley, T., Crall, A., Stern, M., Jordan, R., Gray, S., Prysby, M., Newman, G., Hmelo-Silver, C., Mellor, D. & Huang, J. (2017) 'Bridging the Benefits of Online and Community Supported Citizen Science: A Case Study on Motivation and Retention with Conservation-Oriented Volunteers', *Citizen Science : Theory and Practice,* 2(1), pp. 4.

Gaggìa, F., Baffoni, L., Galiano, M., Nielsen, D. S., Jakobsen, R. R., Castro-Mejía, J. L., Bosi, S., Truzzi, F., Musumeci, F., Dinelli, G. & Di Gioia, D. (2019) 'Kombucha beverage from green, black and rooibos teas: A comparative study looking at microbiology, chemistry and antioxidant activity', *Nutrients,* 11(1), pp. 1.

Gallegos, A. M. A., Herrera Carrera, S., Parra, R., Keshavarz, T. & Iqbal, H. M. N. (2016) 'Bacterial cellulose: a sustainable source to develop value-added products – a review', *Bioresources,* 11(2), pp. 5641-5655.

Gao, C., Yan, T., Du, J., He, F., Luo, H. & Wan, Y. (2014) 'Introduction of broad spectrum antibacterial properties to bacterial cellulose nanofibers via immobilising l[micro]-polylysine nanocoatings', *Food Hydrocolloids,* 36, pp. 204.

García, C. & Prieto, M. A. (2019) 'Bacterial cellulose as a potential bioleather substitute for the footwear industry', *Microbial Biotechnology,* 12(4), pp. 582-585.

Gardetti, M. A. & Muthu, S. S. (2019). *Organic cotton : is it a sustainable solution?* Singapore: Springer.

Gardetti, M. A. & Torres, A. L. (2013). *Sustainability in fashion and textiles: values, design, production and consumption*. Sheffield: Greenleaf Publishing.

Gill, E. (2015) 'Leave your kids behind - this ball pool's for the grown ups'. The Manchester Evening News. [Online] 29/09/2015 [accessed 25/06/2023] https://www.manchestereveningnews.co.uk/whats-on/family-kids-news/ball-pool-for-adults-manchester-10155991

Global Fashion Agenda. (2023) *The GFA monitor*. [Online] [Accessed 02/02/2024] https://globalfashionagenda.org/resource/the-gfa-monitor-2023/

Goh, W. N., Rosma, A., Kaur, B., Fazilah, A., Karim, A. A. & Bhat, R. (2012) 'Fermentation of black tea broth (Kombucha): I. Effects of sucrose concentration and fermentation time on the yield of microbial cellulose', *International food research journal,* 19(1), pp. 109.

Gomare, S. S., Tamboli, D. P., Kagalkar, A. N. & Govindwar, S. P. (2009) 'Eco-friendly biodegradation of a reactive textile dye Golden Yellow HER by Brevibacillus laterosporus MTCC 2298', *International Biodeterioration & Biodegradation,* 63(5), pp. 582-586.

Gordon, S. & Hsieh, Y.-l. (2007). *Cotton : science and technology*. Cambridge: Woodhead.

Grassi, E., Scodeller, P., Filiel, N., Carballo, R. & Levin, L. (2011) 'Potential of Trametes trogii culture fluids and its purified laccase for the decolorization of different types of recalcitrant dyes without the addition of redox mediators', *International Biodeterioration & Biodegradation,* 65(4), pp. 635-643.

Gullo, M., Verzelloni, E. & Canonico, M. (2014) 'Aerobic submerged fermentation by acetic acid bacteria for vinegar production: Process and biotechnological aspects', *Process Biochemistry (1991),* 49(10), pp. 1571-1579.

Guo J., Lai, W., Ming, X., Xie-Men, X., Yang, W., Zhang, S. & Zhang, T. (2019). *Ecological biocellulose mask matrix and method of preparing it*. United States Patent 201910273809.4.

Gönen, F. & Aksu, Z. (2009) 'Predictive expressions of growth and Remazol Turquoise Blue-G reactive dye bioaccumulation properties of Candida utilis', *Enzyme and Microbial Technology,* 45(1), pp. 15-21.

Ha, J. H., Shehzad, O., Khan, S., Lee, S. Y., Park, J. W., Khan, T. & Park, J. K. (2008) 'Production of bacterial cellulose by a static cultivation using the waste from beer culture broth', *Korean Journal of Chemical Engineering,* 25(4), pp. 812-815.

Han, J., Shim, E. & Kim, H. R. (2018) 'Effects of cultivation, washing, and bleaching conditions on bacterial cellulose fabric production', *Textile Research Journal*, 89 (6), pp.1094-1104.

Happy Kombucha (2022) *Happy Kombucha* [online] [accessed 11 / 01 / 2022] https://happyKombucha.co.uk/

Harrelkas, F., Azizi, A., Yaacoubi, A., Benhammou, A. & Pons, M. N. (2009) 'Treatment of textile dye effluents using coagulation–flocculation coupled with membrane processes or adsorption on powdered activated carbon', *Desalination,* 235(1), pp. 330-339.

Harrison, K. & Curtin, C. (2021) 'Microbial composition of SCOBY starter cultures used by commercial Kombucha brewers in North America', *Microorganisms (Basel),* 9(5), pp. 1060.

Hasani, M., Cranston, E. D., Westman, G. & Gray, D. G. (2008) 'Cationic surface functionalization of cellulose nanocrystals', *Soft Matter,* 4(11), pp. 2238-2244.

Hayat, H., Mahmood, Q., Pervez, A., Bhatti, Z. A. & Baig, S. A. (2015) 'Comparative decolorization of dyes in textile wastewater using biological and chemical treatment', *Separation and Purification Technology,* 154, pp. 149-153.

He, J., Zhao, H., Li, X., Su, D., Zhang, F., Ji, H. & Liu, R. (2018) 'Superelastic and superhydrophobic bacterial cellulose/silica aerogels with hierarchical cellular structure for oil absorption and recovery', *Journal of Hazardous Materials,* 346, pp. 199-207.

Henninger, C. E., Alevizou, P. J. & Oates, C. J. (2016) 'What is sustainable fashion?', *Journal of Fashion Marketing and Management,* 20(4), pp. 400-416.

Hervy, M., Blaker, J. J., Braz, A. L. & Lee, K.-Y. (2018) 'Mechanical response of multi-layer bacterial cellulose nanopaper reinforced polylactide laminated composites', *Composites Part A,* 107, pp. 155-163.

Hestrin, S. & Schramm, M. (1954) 'Synthesis of cellulose by Acetobacter xylinum. II. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose', *The Biochemical Journal,* 58(2), pp. 345.

Hoenich, N. (2006) 'Cellulose for medical applications: past, present, and future', *Bioresources,* 1(2), pp. 270-280.

Holkar, C. R., Jadhav, A. J., Pinjari, D. V., Mahamuni, N. M. & Pandit, A. B. (2016) 'A critical review on textile wastewater treatments: Possible approaches', *Journal of Environmental Management,* 182, pp. 351-366.

Hosseini, H., Teymouri, M., Saboor, S., Khalili, A., Goodarzi, V., Poudineh Hajipoor, F., Khonakdar, H. A., Shojaei, S., Asefnejad, A. & Bagheri, H. (2019) 'Challenge between sequence presences of conductive additives on flexibility, dielectric and supercapacitance behaviors of nanofibrillated template of bacterial cellulose aerogels', *European Polymer Journal,* 115, pp. 335-345.

Hu, W., Chen, S., Liu, L., Ding, B. & Wang, H. (2011) 'Formaldehyde sensors based on nanofibrous polyethyleneimine/bacterial cellulose membranes coated quartz crystal microbalance', *Sensors & Actuators: B. Chemical,* 157(2), pp. 554-559.

Hu, Z., Li, S., Cheng, P., Yu, W., Li, R., Shao, X., Lin, W. & Yuan, D. (2016) 'N,P-co-doped carbon nanowires prepared from bacterial cellulose for supercapacitor', *Journal of Materials Science,* 51(5), pp. 2627-2633.

Huang, J. (2006) 'Sweating guarded hot plate test method', *Polymer testing,* 25(5), pp. 709-716.

Huang, J. & Qian, X. (2008) 'Comparison of test methods for measuring water vapor permeability of fabrics', *Textile Research Journal,* 78(4), pp. 342-352.

Huang, Y., Zhu, C., Yang, J., Nie, Y., Chen, C. & Sun, D. (2014) 'Recent advances in bacterial cellulose', *Cellulose,* 21(1), pp. 1-30.

Hungund, B. S. & Gupta, S. G. (2010) 'Production of bacterial cellulose from Enterobacter amnigenus GH-1 isolated from rotten apple', *World Journal of Microbiology & Biotechnology,* 26(10), pp. 1823-1828.

Hussein, A. & Scholz, M. (2018) 'Treatment of artificial wastewater containing two azo textile dyes by vertical-flow constructed wetlands', *Environmental Science and Pollution Research,* 25(7), pp. 6870-6889.

Iguchi, M., Yamanaka, S. & Budhiono, A. (2000) 'Bacterial cellulose—a masterpiece of nature's arts', *Journal of Materials Science,* 35(2), pp. 261-270.

Imran, M., Crowley, D. E., Khalid, A., Hussain, S., Mumtaz, M. W. & Arshad, M. (2015) 'Microbial biotechnology for decolorization of textile wastewaters', *Reviews in Environmental Science and Bio/Technology,* 14(1), pp. 73-92.

Islam, Md.N. & Amstel, A. van. (2021). Bangladesh II : climate change impacts, mitigation and adaptation in developing countries. Cham: Springer.

Jadhav, J. P., Kalyani, D. C., Telke, A. A., Phugare, S. S. & Govindwar, S. P. (2010) 'Evaluation of the efficacy of a bacterial consortium for the removal of color, reduction of heavy metals, and toxicity from textile dye effluent', *Bioresource Technology,* 101(1), pp. 165-173.

Jahan, F., Kumar, V., Rawat, G. & Saxena, R. K. (2012) 'Production of microbial cellulose by a bacterium isolated from fruit', *Applied Biochemistry and Biotechnology,* 167(5), pp. 1157-1171.

Janke, C., Magiera, M. M., Rathfelder, N., Taxis, C., Reber, S., Maekawa, H., Moreno-Borchart, A., Doenges, G., Schwob, E., Schiebel, E. & Knop, M. (2004) 'A versatile toolbox for PCR-based tagging of yeast genes: new fluorescent proteins, more markers and promoter substitution cassettes', *Yeast,* 21(11), pp. 947-962.

Janssen, P. H. (2006) 'Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes', *Applied and Environmental Microbiology,* 72(3), pp. 1719-1728.

Jarrell, J., Cal, T. & Bennett, J. W. (2000) 'The Kombucha consortia of yeasts and bacteria', *Mycologist,* 14(4), pp. 166-170.

Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S. & Sathishkumar, M. (2014) 'A review on Kombucha tea—microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus', *Comprehensive Reviews in Food Science and Food Safety,* 13(4), pp. 538-550.

Jefferson, K. K. (2004). *What drives bacteria to produce a biofilm?* England: Elsevier.

Jia, Y., Yu, H., Zhang, Y., Dong, F. & Li, Z. (2016) 'Cellulose acetate nanofibers coated layer-by-layer with polyethylenimine and graphene oxide on a quartz crystal microbalance for use as a highly sensitive ammonia sensor', *Colloids and Surfaces B: Biointerfaces,* 148, pp. 263-269.

Jin, L., Sun, Q., Xu, Q. & Xu, Y. (2015) 'Adsorptive removal of anionic dyes from aqueous solutions using microgel based on nanocellulose and polyvinylamine', *Bioresource Technology,* 197, pp. 348-355.

Jiong, H., Ming, H., Aimei, L., Jing, T. & Qing, T. (2018). *Preparation method of the biological facial mask composed of sericin / hyaluronic acid-cellulose bacteria*. United States Patent 2018810710124.7.

Juncu, G., Stoica-Guzun, A., Stroescu, M., Isopencu, G. & Jinga, S. I. (2016) 'Drug release kinetics from carboxymethylcellulose-bacterial cellulose composite films', *International Journal of Pharmaceutics,* 510(2), pp. 485-492.

Kalia, S., Kaith, B. S. & Kaur, I. (2011). *Cellulose Fibers: Bio- and Nano-Polymer Composites : Green Chemistry and Technology*. Berlin: Springer.

Kalyani, D. C., Patil, P. S., Jadhav, J. P. & Govindwar, S. P. (2008) 'Biodegradation of reactive textile dye Red BLI by an isolated bacterium Pseudomonas sp. SUK1', *Bioresource Technology,* 99(11), pp. 4635-4641.

Kalyani, D. C., Telke, A. A., Dhanve, R. S. & Jadhav, J. P. (2009) 'Ecofriendly biodegradation and detoxification of Reactive Red 2 textile dye by newly isolated Pseudomonas sp. SUK1', *Journal of Hazardous Materials,* 163(2), pp. 735-742.

Kamiński, K., Jarosz, M., Grudzień, J., Pawlik, J., Zastawnik, F., Pandyra, P. & Kołodziejczyk, A. M. (2020) 'Hydrogel bacterial cellulose: a path to improved materials for new eco-friendly textiles', *Cellulose (London),* 27(9), pp. 5353-5365.

Karahan, A. G., Akoğlu, A., Çakır, İ., Kart, A., Çakmakçı, M. L., Uygun, A. & Göktepe, F. (2011) 'Some properties of bacterial cellulose produced by new native strain Gluconacetobacter sp. A06O2 obtained from Turkish vinegar', *Journal of Applied Polymer Science,* 121(3), pp. 1823-1831.

Karim, Z., Hakalahti, M., Tammelin, T. & Mathew, A. P. (2017) 'In situ TEMPO surface functionalization of nanocellulose membranes for enhanced adsorption of metal ions from aqueous medium, *RSC Advances*, 7(9), pp. 5232-5241.

Kelley, T. R., Knowles, J. G. (2016) 'A Conceptual Framework for Integrated STEM Education', I*nternational Journal of STEM Education*, 3 (1).

Kennedy, T. J., Odell, M. R. L. (2014) 'Engaging Students In Stem Education', *Science Education International*, 25 (246).

Kent, S. (2023) *Bolt Threads Pause Operations of Leather-Alternative Mylo*, The Business of Fashion. [Online] [Accessed 12/11/2023] https://www.businessoffashion.com/news/sustainability/bolt-threads-mylo-alternative-leather-mushroom-pause-operations/.

Kew, S.J. (2024). The path to net zero for the fashion industry : five strategies for decarbonisation. Abingdon, Oxon ; Routledge.

Khan, H., Kadam, A. & Dutt, D. (2020) 'Studies on bacterial cellulose produced by a novel strain of Lactobacillus genus', *Carbohydrate polymers,* 229, pp. 115513-115513.

Khan, S. B. & Kamal, T. (2022). *Bacterial cellulose : synthesis, production, and applications* (First edition. ed.). Boca Raton: CRC Press.

Khandegar, V. & Saroha, A. K. (2013) 'Electrocoagulation for the treatment of textile industry effluent – A review', *Journal of Environmental Management,* 128, pp. 949-963.

Khatoon, H., Yusoff, F., Banerjee, S., Shariff, M. & Bujang, J. S. (2007) 'Formation of periphyton biofilm and subsequent biofouling on different substrates in nutrient enriched brackishwater shrimp ponds', *Aquaculture,* 273(4), pp. 470-477.

Khehra, M. S., Saini, H. S., Sharma, D. K., Chadha, B. S. & Chimni, S. S. (2005) 'Decolorization of various azo dyes by bacterial consortium', *Dyes and Pigments,* 67(1), pp. 55-61.

Khine, M. S., Areepattamannil, S. (2019) *'STEAM Education Theory and Practice'*, Cham, Springer International Publishing.

Kim, H. & Kim, H. R. (2022) 'Production of coffee-dyed bacterial cellulose as a bio-leather and using it as a dye adsorbent', *PloS one,* 17(3), pp. e0265743-e0265743.

Kishor, R., Purchase, D., Saratale, G. D., Saratale, R. G., Ferreira, L. F. R., Bilal, M., Chandra, R. & Bharagava, R. N. (2021) 'Ecotoxicological and health concerns of persistent coloring pollutants of textile industry wastewater and treatment approaches for environmental safety', *Journal of environmental chemical engineering,* 9(2), pp. 105012.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M. & Glöckner, F. O. (2013) 'Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies', *Nucleic acids research,* 41(1), pp. e1-e1.

Kocabas, A.M. et al. (2009). Adoption of European Union's IPPC Directive to a textile mill: Analysis of water and energy consumption. Journal of environmental management, 91(1), pp.102–113.

Kolekar, Y. M., Nemade, H. N., Markad, V. L., Adav, S. S., Patole, M. S. & Kodam, K. M. (2012) 'Decolorization and biodegradation of azo dye, reactive blue 59 by aerobic granules', *Bioresource Technology,* 104, pp. 818-822.

Kongruang, S. (2008) 'Bacterial cellulose production by acetobacter xylinum strains from agricultural waste products', *Applied Biochemistry and Biotechnology,* 148(1), pp. 245-256.

Kotlyakov, V. M. (1991) 'The Aral sea basin a critical environmental zone', *Environment : science and policy for sustainable development,* 33(1), pp. 4-38.

Kruk, M., Trząskowska, M., Ścibisz, I. & Pokorski, P. (2021) 'Application of the "SCOBY" and Kombucha tea for the production of fermented milk Drinks', *Microorganisms,* 9(1).

Krystynowicz, A., Czaja, W., Wiktorowska-Jezierska, A., Gonçalves-Miśkiewicz, M., Turkiewicz, M. & Bielecki, S. (2002) 'Factors affecting the yield and properties of bacterial cellulose', *Journal of Industrial Microbiology & Biotechnology,* 29(4), pp. 189-195.

Kuczynski, J., Lauber, C. L., Walters, W. A., Parfrey, L. W., Clemente, J. C., Gevers, D. & Knight, R. (2012) 'Experimental and analytical tools for studying the human microbiome', *Nature reviews. Genetics,* 13(1), pp. 47-58.

Kumar, N., Kumar, R., Singh, R., Bishnoi, N. R. & Bishnoi, K. (2009) 'Response surface methodology approach for optimization of biosorption process for removal of Cr (VI), Ni (II) and Zn (II) ions by immobilized bacterial biomass sp. Bacillus brevis', *Chemical Engineering Journal,* 146(3), pp. 401-407.

Kuppusamy, S., Sethurajan, M., Kadarkarai, M. & Aruliah, R. (2017) 'Biodecolourization of textile dyes by novel, indigenous Pseudomonas stutzeri MN1 and Acinetobacter baumannii MN3', *Journal of Environmental Chemical Engineering,* 5(1), pp. 716-724.

Kurade, M. B., Waghmode, T. R., Xiong, J.-Q., Govindwar, S. P. & Jeon, B.-H. (2019) 'Decolorization of textile industry effluent using immobilized consortium cells in upflow fixed bed reactor', *Journal of Cleaner Production,* 213, pp. 884-891.

Kurosumi, A., Sasaki, C., Yamashita, Y. & Nakamura, Y. (2009) 'Utilization of various fruit juices as carbon source for production of bacterial cellulose by Acetobacter xylinum NBRC 13693', *Carbohydrate Polymers,* 76(2), pp. 333-335.

Kurtzman, C. P., Robnett, C. J. & Basehoar-Powers, E. (2001) 'Zygosaccharomyces Kombuchaensis, a new ascosporogenous yeast from 'Kombucha tea'', *FEMS yeast research,* 1(2), pp. 133-138.

Kuswandi, B., Jayus, Larasati, T. S., Abdullah, A. & Heng, L. Y. (2012) 'Real-time monitoring of shrimp spoilage using on-package sticker sensor based on natural dye of curcumin', *Food Analytical Methods,* 5(4), pp. 881-889.

Kuswandi, B., Jayus, Oktaviana, R., Abdullah, A. & Heng, L. Y. (2014) 'A novel on-package sticker sensor based on methyl red for real-time monitoring of broiler chicken cut freshness', *Packaging Technology and Science,* 27(1), pp. 69-81.

Kuswandi, B., Maryska, C., Jayus, Abdullah, A. & Heng, L. Y. (2013) 'Real time on-package freshness indicator for guavas packaging', *Journal of Food Measurement and Characterization,* 7(1), pp. 29-39.

Laavanya, D., Shirkole, S. & Balasubramanian, P. (2021) 'Current challenges, applications and future perspectives of SCOBY cellulose of Kombucha fermentation', *Journal of cleaner production,* 295, pp. 126454.

Land-Zandstra, A., Agnello, G. & Gültekin, Y. S. (2021) 'Participants in citizen science', in Vohland, K. (ed.) *The Science of Citizen Science.*: Springer.

Lansiquot, R.D. ed. (2016). Technology, theory, and practice in interdisciplinary STEM programs : connecting STEM and non-STEM approaches. New York: Palgrave Macmillan.

Launch. (2019). *Suzanne Lee: Biocouture - Launch* [Online] [Accessed 12/06/2020] https://www.launch.org/innovators/suzanne-lee/

Lee, K.-Y. (2018). *Nanocellulose and Sustainability: Production, Properties, Applications, and Case Studies*. UK: CRC.

Lee, S. (2023) 'Suzanne Lee: Grow your own clothes TED Talk'. TED Summit. [Online] [Accessed 13/07/2023] https://www.ted.com/talks/suzanne_lee_grow_your_own_clothes?language=en

Lee, Y. A. (2016) 'Case study of renewable bacteria cellulose fiber and biopolymer composites in sustainable design practices', in Muthu, S. & Gardetti, M. (eds.) *Sustainable Fibres for Fashion Industry*. Singapore: Springer, pp. 114 - 162.

Legnani, C., Barud, H. S., Caiut, J. M. A., Calil, V. L., Maciel, I. O., Quirino, W. G., Ribeiro, S. J. L. & Cremona, M. (2019) 'Transparent bacterial cellulose nanocomposites used as substrate for organic light-emitting diodes', *Journal of Materials Science: Materials in Electronics*, pp. 1-6.

Leung, A. C. W., Hrapovic, S., Lam, E., Liu, Y., Male, K. B., Mahmoud, K. A. & Luong, J. H. T. (2011) 'Characteristics and properties of carboxylated cellulose nanocrystals prepared from a novel one-step procedure', *Small,* 7(3), pp. 302-305.

Li, X., Wan, W.-K. & Panchal, C. J. (2011). T*ransparent bacterial cellulose nanocomposite hydrogels*. United States Patent US8940337B2.

Lin, S.-P., Loira Calvar, I., Catchmark, J. M., Liu, J.-R., Demirci, A. & Cheng, K.-C. (2013a) 'Biosynthesis, production and applications of bacterial cellulose', *Cellulose,* 20(5), pp. 2191-2219.

Lin, W.-C., Lien, C.-C., Yeh, H.-J., Yu, C.-M. & Hsu, S.-h. (2013b) 'Bacterial cellulose and bacterial cellulose–chitosan membranes for wound dressing applications', *Carbohydrate Polymers,* 94(1), pp. 603-611.

Liu, K. & Catchmark, J. M. (2019) 'Enhanced mechanical properties of bacterial cellulose nanocomposites produced by co-culturing Gluconacetobacter hansenii and Escherichia coli under static conditions', *Carbohydrate Polymers,* 219, pp. 12-20.

Liu, X., Zhang, J., Jiang, J., Li, R., Xie, Z. & Li, S. (2011) 'Biochemical degradation pathway of reactive blue 13 by Candida rugopelliculosa HXL-2', *International Biodeterioration & Biodegradation,* 65(1), pp. 135-141.

Lončar, E., Djurić, M., Malbaša, R., Kolarov, L. J. & Klašnja, M. (2006) 'Influence of working conditions upon Kombucha conducted fermentation of black tea', *Food and Bioproducts Processing,* 84(3), pp. 186-192.

Lu, Z., Zhang, Y., Chi, Y., Xu, N., Yao, W. & Sun, B. (2011) 'Effects of alcohols on bacterial cellulose production by Acetobacter xylinum 186', *World Journal of Microbiology & Biotechnology,* 27(10), pp. 2281-2285.

Ludwicka, K., Jedrzejczak-Krzepkowska, M., Kubiak, K., Kolodziejczyk, M., Pankiewicz, T. & Bielecki, S. (2016) 'Medical and Cosmetic Applications of Bacterial NanoCellulose', in Gama, M., Dourado, F. & Bielecki, S. (eds.) *Bacterial NanoCellulose*. London: Elsevier, pp. 145 - 165.

Luo, H., Xie, J., Wang, J., Yao, F., Yang, Z. & Wan, Y. (2018) 'Step-by-step self-assembly of 2D few-layer reduced graphene oxide into 3D architecture of bacterial cellulose for a robust, ultralight, and recyclable all-carbon absorbent', *Carbon,* 139, pp. 824-832.

Luo, H., Xie, J., Xiong, L., Zhu, Y., Yang, Z. & Wan, Y. (2019) 'Fabrication of flexible, ultra-strong, and highly conductive bacterial cellulose-based paper by engineering dispersion of graphene nanosheets', *Composites Part B,* 162, pp. 484-490.

Maas, R. & Chaudhari, S. (2005) 'Adsorption and biological decolourization of azo dye Reactive Red 2 in semicontinuous anaerobic reactors', *Process Biochemistry,* 40(2), pp. 699-705.

MadewithReishi (2023) *MadewithReishi,* [online] [accessed 04/04/2022] www.madewithresishi.com

Malbaša, R. V., Lončar, E. S., Vitas, J. S. & Čanadanović-Brunet, J. M. (2011) 'Influence of starter cultures on the antioxidant activity of Kombucha beverage', *Food Chemistry,* 127(4), pp. 1727-1731.

Malik, R., Ramteke, D. S. & Wate, S. R. (2007) 'Adsorption of malachite green on groundnut shell waste based powdered activated carbon', *Waste Management,* 27(9), pp. 1129-1138.

Mao, Z., Yu, H., Wang, Y., Zhang, L., Zhong, Y. & Xu, H. (2014) 'States of water and pore size distribution of cotton fibers with different moisture ratios', *Industrial & Engineering Chemistry Research,* 53(21), pp. 8927-8934.

Marins, J. A., Soares, B. G., Fraga, M., Müller, D. & Barra, G. M. O. (2014) 'Self-supported bacterial cellulose polyaniline conducting membrane as electromagnetic interference shielding material: effect of the oxidizing agent', *Cellulose,* 21(3), pp. 1409-1418.

Marmon, M. (2019). 'The Emergence of Creativity in STEM: Fostering an Alternative Approach for Science, Technology, Engineering and Mathematics Instruction Through the Use of the Arts'. In: Khine, M. S. (ed.) *STEAM Education*. Switzerland: Springer Nature.

Marsh, A. J., O'Sullivan, O., Hill, C., Ross, R. P. & Cotter, P. D. (2014) 'Sequence-based analysis of the bacterial and fungal compositions of multiple Kombucha (tea fungus) samples', *Food Microbiology,* 38, pp. 171-178.

Martorell, M. M., Pajot, H. F. & Figueroa, L. I. C. d. (2017) 'Biological degradation of Reactive Black 5 dye by yeast Trichosporon akiyoshidainum', *Journal of Environmental Chemical Engineering,* 5(6), pp. 5987-5993.

Mas, P., Tran, T., Verdier, F., Martin, A., Alexandre, H., Grandvalet, C. & Tourdot-Maréchal, R. (2022) 'Evolution in composition of Kombucha consortia over three consecutive years in production context', *Foods,* 11(4), pp. 614.

Masi, F., Rizzo, A., Bresciani, R., Martinuzzi, N., Wallace, S. D., Van Oirschot, D., Macor, F., Rossini, T., Fornaroli, R. & Mezzanotte, V. (2019) 'Lessons learnt from a pilot study on residual dye removal by an aerated treatment wetland', *Science of the Total Environment,* 648, pp. 144-152.

Masmoudi, G., Trabelsi, R., Ellouze, E. & Amar, R. B. (2014) 'New treatment at source approach using combination of microfiltration and nanofiltration for dyeing effluents reuse', *International Journal of Environmental Science and Technology,* 11(4), pp. 1007-1016.

Mastascusa, E.J., Snyder, W.J. and Hoyt, B.S. (2011). Effective instruction for STEM disciplines : from learning theory to college teaching. San Francisco, CA: Jossey-Bass.

Matthews, B. and Ross, L. (2010). Research Methods. 1st ed. Harlow: Pearson Education UK.

McCullough, E. A., Kwon, M. & Shim, H. (2003) 'A comparison of standard methods for measuring water vapour permeability of fabrics', *Measurement Science & Technology,* 14(8), pp. 1402-1408.

McKay, G., Porter, J. F. & Prasad, G. R. (1999) 'The removal of dye colours from aqueous solutions by adsorption on low-cost materials', *Water, Air, and Soil Pollution,* 114(3), pp. 423-438.

McKinsey (2023) *Fashion on climate: how the fashion industry can urgently act to reduce its greenhouse gas emissions*. [Online] [Accessed 12/10/2023] https://www.mckinsey.com/~/media/mckinsey/industries/retail/our%20insights/fashion%20on%20climate/fashion-on-climate-full-report.pdf.

McLoughlin, J. & Sabir, T. (2017). *High-performance apparel: materials, development, and applications*. Oxford: Woodhead Publishing.

Medlar, A., Aivelo, T. & Löytynoja, A. (2014) 'Séance: reference-based phylogenetic analysis for 18S rRNA studies', *BMC evolutionary biology,* 14(1), pp. 235-235.

Menon, M., Selvakumar, R., Sureshkumar, P. & Ramakrishna, S. (2017) 'Extraction and modification of cellulose nanofibers derived from biomass for environmental application', *RSC,* 7(68), pp. 4275-42773.

Mishra, S. & Maiti, A. (2018) 'The efficacy of bacterial species to decolourise reactive azo, anthroquinone and triphenylmethane dyes from wastewater: a review', *Environmental Science and Pollution Research,* 25(9), pp. 8286-8314.

Mishra, S., Singh, P. K., Pattnaik, R., Kumar, S., Ojha, S. K., Srichandan, H., Parhi, P. K., Jyothi, R. K. & Sarangi, P. K. (2022) 'Biochemistry, synthesis, and applications of bacterial cellulose: A review', *Frontiers in Bioengineering and Biotechnology,* 10, pp. 780409-780409.

Modern Synthesis (2023) Modern Synthesis [online] [accessed 01/03/2021] www.modernsynthesis.com

Mohammadkazemi, F., Azin, M. & Ashori, A. (2015) 'Production of bacterial cellulose using different carbon sources and culture media', *Carbohydrate Polymers,* 117, pp. 518-523.

Mohite, B. V. & Patil, S. V. (2014a) 'A novel biomaterial: bacterial cellulose and its new era applications: BC and Its New Era Applications', *Biotechnology and Applied Biochemistry,* 61(2), pp. 101-110.

Mohite, B. V. & Patil, S. V. (2014b) 'Bacterial cellulose of Gluconoacetobacter hansenii as a potential bioadsorption agent for its green environment applications', *Journal of Biomaterials Science, Polymer Edition,* 25(18), pp. 2053-2065.

Morgan, J. (2015) 'Hooked on Music: therapy for Alzheimer's disease?', *Lancet Neurology,* 14(1), pp. 37-37.

Morissons. (2020). *Morissons* [Online]. [Accessed 23/01/2020]. www.morrisons.com

Moritz, S., Wiegand, C., Wesarg, F., Hessler, N., Müller, F. A., Kralisch, D., Hipler, U.-C. & Fischer, D. (2014) 'Active wound dressings based on bacterial nanocellulose as drug delivery system for octenidine', *International Journal of Pharmaceutics,* 471(1-2), pp. 45-55.

Muthu, S. S. (2018). *Detox Fashion : Sustainable Chemistry and Wet Processing* (1st edition. ed.). Singapore: Springer.

Muthu, S. S. & Gardetti, M. A. (2020). *Sustainability in the textile and apparel industries : sustainable textiles, clothing design and repurposing*. Cham: Springer.

Naritomi, T., Kouda, T., Yano, H. & Yoshinaga, F. (1998) 'Effect of ethanol on bacterial cellulose production from fructose in continuous culture', *Journal of Fermentation and Bioengineering,* 85(6), pp. 598-603.

Nawaz, M. S. & Ahsan, M. (2014) 'Comparison of physico-chemical, advanced oxidation and biological techniques for the textile wastewater treatment', *Alexandria Engineering Journal,* 53(3), pp. 717-722.

Neera, Ramana, K. V. & Batra, H. V. (2015) 'Occurrence of cellulose-producing gluconacetobacter spp. in fruit samples and Kombucha tea, and production of the biopolymer', *Applied Biochemistry and Biotechnology,* 176(4), pp. 1162-1173.

Nerlich, B., Koteyko, N. & Brown, B. (2010). Theory and language of climate change communication. *Wiley interdisciplinary reviews. Climate change*, 1(1), pp.97–110.

Ng, C.-C. & Shyu, Y.-T. (2004) 'Development and production of cholesterol-lowering Monascus-nata complex', *World Journal of Microbiology & Biotechnology,* 20(9), pp. 875-879.

Nguyen, V. T., Flanagan, B., Gidley, M. J. & Dykes, G. A. (2008) 'Characterization of cellulose production by a Gluconacetobacter xylinus strain from Kombucha', *Current Microbiology,* 57(5), pp. 449-453.

Nisbet, M.C. (2009). Communicating Climate Change: Why Frames Matter for Public Engagement. *Environment : science and policy for sustainable development*, 51(2), pp.12–23.

Niinimäki, K. (2013) 'Sustainable Fashion: New Approaches, Aalto University, Helsinki.

Niinimäki, K., Peters, G., Dahlbo, H., Perry, P., Rissanen, T. & Gwilt, A. (2020) 'The environmental price of fast fashion', *Nature Reviews Earth & Environment,* 1(4), pp. 189-200.

Nishiyama, Y., Langan, P., Wada, M. & Forsyth, V. T. (2010) 'Looking at hydrogen bonds in cellulose', *Acta Crystallographica Section D,* 66(11), pp. 1172-1177.

Novotný, Č., Svobodová, K., Benada, O., Kofroňová, O., Heissenberger, A. & Fuchs, W. (2011) 'Potential of combined fungal and bacterial treatment for color removal in textile wastewater', *Bioresource Technology,* 102(2), pp. 879-888.

Ochu, E. (2012) *Grow a sunflower to solve unfinished Alan Turing experiment*. The University of Manchester. [Online] [Accessed 20/06/2023] https://www.manchester.ac.uk/discover/news/turings-sunflowers-growing-alan-turings-legacy/#:~:text=Over%20500%20sunflowers%20were%20analysed,before%20his%20death%20in%201954.

Okareh, O. T., Ademodi, T. F. & Igbinosa, E. O. (2017) 'Biotreatment of effluent from 'Adire' textile factories in Ibadan, Nigeria', *Environmental Monitoring and Assessment,* 189(12), pp. 1-11.

Oladoye, P. O., Ajiboye, T. O., Omotola, E. O. & Oyewola, O. J. (2022) 'Methylene blue dye: toxicity and potential elimination technology from wastewater', *Results in engineering,* 16, pp. 100678.

Oluwakemi Kehinde, F. & Abdul Aziz, H. (2016) 'Classical optimization of process variables in the treatment of real textile wastewater using clinoptilolite', *Journal of Environmental Chemical Engineering,* 4(1), pp. 1242-1247.

Öner, E. & Sahinbaskan, B. Y. (2011) 'A new process of combined pretreatment and dyeing: REST', *Journal of Cleaner Production,* 19(14), pp. 1668-1675.

Ong, S.-A., Uchiyama, K., Inadama, D., Ishida, Y. & Yamagiwa, K. (2010) 'Treatment of azo dye Acid Orange 7 containing wastewater using up-flow constructed wetland with and without supplementary aeration', *Bioresource Technology,* 101(23), pp. 9049-9057.

Ostadhossein, F., Mahmoudi, N., Morales-Cid, G., Tamjid, E., Navas-Martos, F. J., Soriano-Cuadrado, B., Paniza, J. M. L. & Simchi, A. (2015) 'Development of chitosan/bacterial cellulose composite films containing nanodiamonds as a potential flexible platform for wound dressing', *Materials,* 8(9), pp. 6401-6418.

Oturkar, C. C., Nemade, H. N., Mulik, P. M., Patole, M. S., Hawaldar, R. R. & Gawai, K. R. (2011) 'Mechanistic investigation of decolorization and degradation of Reactive Red 120 by Bacillus lentus BI377', *Bioresource Technology,* 102(2), pp. 758-764.

Ovcharenko, L. P. (2013) 'Metagenomic analysis of domesticated Kombucha multi-microbial culture', *Biopolymers and Cell,* 29, pp. 16.

Özek, H. Z. (2018) 'Development of waterproof breathable coatings and laminates', in Williams, J. (ed.) *Waterproof and Water Repellent Textiles and Clothing*. UK: Woodhead, pp. 25-72.

O'Callaghan, J. L., Willner, D., Buttini, M., Huygens, F. & Pelzer, E. S. (2021) 'Limitations of 16S rRNA gene sequencing to characterize Lactobacillus species in the upper genital tract', *Frontiers in Cell and Developmental Biology,* 9, pp. 641921-641921.

O'Neill, C., Hawkes, F. R., Hawkes, D. L., Lourenço, N. D., Pinheiro, H. M. & Delée, W. (1999) 'Colour in textile effluents – sources, measurement, discharge consents and simulation: a review', *Journal of Chemical Technology & Biotechnology,* 74(11), pp. 1009-1018.

Pacheco, G., Mello, C. V., Chiari-Andréo, B. G., Isaac, V. L. B., Ribeiro, S. J. L., Pecoraro, É. & Trovatti, E. (2018) 'Bacterial cellulose skin masks—Properties and sensory tests', *Journal of Cosmetic Dermatology,* 17(5), pp. 840-847.

Padmanaban, V. C., Geed, S. R., Achary, A. & Singh, R. S. (2016) 'Kinetic studies on degradation of Reactive Red 120 dye in immobilized packed bed reactor by Bacillus cohnii RAPT1', *Bioresource Technology,* 213, pp. 39-43.

Pajot, H. F., Fariña, J. I. & de Figueroa, L. I. C. (2011) 'Evidence on manganese peroxidase and tyrosinase expression during decolorization of textile industry dyes by Trichosporon akiyoshidainum', *International Biodeterioration & Biodegradation,* 65(8), pp. 1199-1207.

Patchan, M. W., Chae, J. J., Lee, J. D., Calderon-Colon, X., Maranchi, J. P., McCally, R. L., Schein, O. D., Elisseeff, J. H. & Trexler, M. M. (2016) 'Evaluation of the biocompatibility of regenerated cellulose hydrogels with high strength and transparency for ocular applications', *Journal of Biomaterials applications,* 30(7), pp. 1049.

Patel, H. & Vashi, R. T. (2015). *Characterization and Treatment of Textile Wastewater*. US: Elsevier.

Pathak, H., Soni, D. & Chauhan, K. (2014) 'Evaluation of in vitro efficacy for decolorization and degradation of commercial azo dye RB-B by Morganella sp. HK-1 isolated from dye contaminated industrial landfill', *Chemosphere,* 105, pp. 126-132.

Pei, A., Butchosa, N., Berglund, L. A., Zhou, Q., Wallenberg Wood Science, C., Centra, Skolan för, b., Skolan för, k., Glykovetenskap, Fiber- och, p. & Kth (2013) 'Surface quaternized cellulose nanofibrils with high water absorbency and adsorption capacity for anionic dyes', *Soft Matter,* 9(6), pp. 2047.

Peinado, R. A., Moreno, J. J., Villalba, J. M., González-Reyes, J. A., Ortega, J. M. & Mauricio, J. C. (2006) 'Yeast biocapsules: A new immobilization method and their applications', *Enzyme and Microbial Technology,* 40(1), pp. 79-84.

Petersen, N. & Gatenholm, P. (2011) 'Bacterial cellulose-based materials and medical devices: current state and perspectives', *Applied Microbiology and Biotechnology,* 91(5), pp. 1277-1286.

Picheth, G. F., Pirich, C. L., Sierakowski, M. R., Woehl, M. A., Sakakibara, C. N., de Souza, C. F., Martin, A. A., da Silva, R. & de Freitas, R. A. (2017) 'Bacterial cellulose in biomedical applications: A review', *International Journal of Biological Macromolecules,* 104(Pt A), pp. 97-106.

Pirsa, S., Shamusi, T. & Kia, E. M. (2018) 'Smart films based on bacterial cellulose nanofibers modified by conductive polypyrrole and zinc oxide nanoparticles', *Journal of Applied Polymer Science,* 135(34), pp. 46617.

Pole, C.J. (Christopher J. and Lampard, R. (2013). Practical social investigation : qualitative and quantitative methods in social research. Abingdon, Oxon: Routledge.

Ponciano, L., Brasileiro, F., Simpson, R. & Smith, A. (2014) 'Volunteers' Engagement in Human Computation for Astronomy Projects', *Computing in Science & Engineering,* 16(6), pp. 52-59.

Popli, S. & Patel, U. D. (2015) 'Destruction of azo dyes by anaerobic–aerobic sequential biological treatment: a review', *International Journal of Environmental Science and Technology,* 12(1), pp. 405-420.

Priyadharshini, T., Nageshwari, K., Vimaladhasan, S., Parag Prakash, S. & Balasubramanian, P. (2022) 'Machine learning prediction of SCOBY cellulose yield from Kombucha tea fermentation', *Bioresource Technology Reports,* 18, pp. 101027.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J. & Glöckner, F. O. (2007) 'SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB', *Nucleic acids research,* 35(21), pp. 7188-7196.

Qiao, H., Zhou, Y., Yu, F., Wang, E., Min, Y., Huang, Q., Pang, L. & Ma, T. (2015) 'Effective removal of cationic dyes using carboxylate-functionalized cellulose nanocrystals', *Chemosphere,* 141, pp. 297-303.

Qu, J. & Ruckman, J. (2006) 'A new calculation method of water vapour permeability at unsteady states', *Journal of the Textile Institute,* 97(5), pp. 449-453.

R. Rebelo, A., Archer, A. J., Chen, X., Liu, C., Yang, G. & Liu, Y. (2018) 'Dehydration of bacterial cellulose and the water content effects on its viscoelastic and electrochemical properties', *Science and Technology of Advanced Materials,* 19(1), pp. 203-211.

Rajwade, J. M., Paknikar, K. M. & Kumbhar, J. V. (2015) 'Applications of bacterial cellulose and its composites in biomedicine', *Applied Microbiology and Biotechnology,* 99(6), pp. 2491-2511.

Rambo, C. R., Recouvreux, D. O. S., Carminatti, C. A., Pitlovanciv, A. K., Antônio, R. V. & Porto, L. M. (2008) 'Template assisted synthesis of porous nanofibrous cellulose membranes for tissue engineering', *Materials Science & Engineering C,* 28(4), pp. 549-554.

Ramezani, Z., Pourmand, N., Behfar, A. & Momeni, A. (2016) 'Removal and recovery of mercury from chlor-alkali petrochemical wastes using γ-Fe2O3 nanoparticles', *Applied Petrochemical Research,* 6(4), pp. 403-411.

Ramkalaon, S. & Sayem, A. S. M. (2021) 'Zero-Waste Pattern Cutting (ZWPC) to tackle over sixty billion square metres of fabric wastage during mass production of apparel', *Journal of the Textile Institute,* 112(5), pp. 809-819.

Rani, M. U. & Appaiah, K. A. A. (2013) 'Production of bacterial cellulose by Gluconacetobacter hansenii UAC09 using coffee cherry husk', *Journal of Food Science and Technology,* 50(4), pp. 755-762.

Regan, H. (2020) 'Asian Rivers are turning black and our colorful closets are to blame', *CNN*.[Online] 28/09/2020 [Accessed 28/11/2020] https://edition.cnn.com/style/article/dyeing-pollution-fashion-intl-hnk-dst-sept/index.html

Reva, O. N., Zaets, I. E., Ovcharenko, L. P., Kukharenko, O. E., Shpylova, S. P., Podolich, O. V., de Vera, J.-P. & Kozyrovska, N. O. (2015) 'Metabarcoding of the Kombucha microbial community grown in different microenvironments', *AMB Express,* 5(1), pp. 1-8.

Riera-Torres, M., Gutiérrez-Bouzán, C. & Crespi, M. (2010) 'Combination of coagulation–flocculation and nanofiltration techniques for dye removal and water reuse in textile effluents', *Desalination,* 252(1), pp. 53-59.

Rosamond, W., Flegal, K., Furie, K., Go, A., Greenlund, K., Haase, N., Hailpern, S. M., Ho, M., Howard, V., Kissela, B., Kissela, B., Kittner, S., Lloyd-Jones, D., McDermott, M., Meigs, J., Moy, C., Nichol, G., O'Donnell, C., Roger, V., Sorlie, P., Steinberger, J., Thom, T., Wilson, M., Hong, Y., (2008) 'Heart disease and stroke statistics--2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee', *Circulation,* 117(4), pp. e25.

Ross, C. (2023). *Fabric Made From Tea!* [Online] [Accessed 17/06/2023] http://www.the-sustainable-fashion-collective.com/2016/08/16/fabric-made-tea

Saito, T. & Isogai, A. (2005) 'Ion-exchange behavior of carboxylate groups in fibrous cellulose oxidized by the TEMPO-mediated system', *Carbohydrate Polymers,* 61(2), pp. 183-190.

Saleh, A. K., El-Gendi, H., Soliman, N. A., El-Zawawy, W. K. & Abdel-Fattah, Y. R. (2022) 'Bioprocess development for bacterial cellulose biosynthesis by novel Lactiplantibacillus plantarum isolate along with characterization and antimicrobial assessment of fabricated membrane', *Scientific Reports,* 12(1), pp. 2181-2181.

Sambo, F., Finotello, F., Lavezzo, E., Baruzzo, G., Masi, G., Peta, E., Falda, M., Toppo, S., Barzon, L. & Di Camillo, B. (2018) 'Optimizing PCR primers targeting the bacterial 16S ribosomal RNA gene', *BMC Bioinformatics,* 19(1), pp. 343-343.

Sanders, M. (2008) 'STEM, STEM Education, STEMmania', *The Technology Teacher*, 68, pp. 20.

Santos, S. C. R. & Boaventura, R. A. R. (2015) 'Treatment of a simulated textile wastewater in a sequencing batch reactor (SBR) with addition of a low-cost adsorbent', *Journal of Hazardous Materials,* 291, pp. 74-82.

Saratale, R. G., Gandhi, S. S., Purankar, M. V., Kurade, M. B., Govindwar, S. P., Oh, S. E. & Saratale, G. D. (2013) 'Decolorization and detoxification of sulfonated azo dye C.I. Remazol Red and textile effluent by isolated Lysinibacillus sp. RGS', *Journal of Bioscience and Bioengineering,* 115(6), pp. 658-667.

Sauermann, H. & Franzoni, C. (2015) 'Crowd science user contribution patterns and their implications', *Proceedings of the National Academy of Sciences - PNAS,* 112(3), pp. 679-684.

Schenzel, K. & Fischer, S. (2001) 'NIR FT Raman Spectroscopy - a Rapid Analytical Tool for Detecting the Transformation of Cellulose Polymorphs', *Cellulose,* 8(1), pp. 49-57.

Schramm, M., Gromet, Z. & Hestrin, S. (1957) 'Synthesis of cellulose by Acetobacter Xylinum. 3. Substrates and inhibitors', *The Biochemical Journal,* 67(4), pp. 669-679.

Schramm, M. & Hestrin, S. (1954a) 'Factors affecting production of cellulose at the air/liquid interface of a culture of Acetobacter xylinum', *Journal of General Microbiology,* 11(1), pp. 123.

Schramm, M. & Hestrin, S. (1954b) 'Synthesis of cellulose by Acetobacter xylinum. I. Micromethod for the determination of celluloses', *The Biochemical Journal,* 56(1), pp. 163.

SCOBYTec (2022), *SCOBYTec* [online] [accessed 23/04/2022] www.scobytec.com

SDL Atlas (2023) SDL Atlas [online] [accessed 25/07/2023] www.sdlatlas.com

Sehaqui, H., de Larraya, U. P., Liu, P., Pfenninger, N., Mathew, A. P., Zimmermann, T., Tingaut, P.(2014) 'Enhancing adsorption of heavy metal ions onto biobased nanofibers from waste pulp residues for application in wastewater treatment', *Cellulose,* 21(4), pp. 2831-2844.

Sen, S. K., Raut, S., Bandyopadhyay, P. & Raut, S. (2016) 'Fungal decolouration and degradation of azo dyes: A review', *Fungal Biology Reviews,* 30(3), pp. 112-133.

Seshadri, S., Bishop, P. L. & Agha, A. M. (1994) 'Anaerobic/aerobic treatment of selected azo dyes in waste-water', *Waste Management,* 14(2), pp. 127-137.

Seto, A., Saito, Y., Matsushige, M., Kobayashi, H., Sasaki, Y., Tonouchi, N., Tsuchida, T., Yoshinaga, F., Ueda, K. & Beppu, T. (2006) 'Effective cellulose production by a coculture of Gluconacetobacter xylinus and Lactobacillus mali', *Applied Microbiology and Biotechnology,* 73(4), pp. 915-921.

Setyawati, M. I., Chien, L.-J. & Lee, C.-K. (2007) 'Expressing Vitreoscilla hemoglobin in statically cultured Acetobacter xylinum with reduced O.sub.2 tension maximizes bacterial cellulose pellicle production', *Journal of Biotechnology,* 132(1), pp. 38.

Shah, P. D., Dave, S. R. & Rao, M. S. (2012) 'Enzymatic degradation of textile dye Reactive Orange 13 by newly isolated bacterial strain Alcaligenes faecalis PMS-1', *International Biodeterioration & Biodegradation,* 69, pp. 41-50.

Shanmugam, B. K. & Mahadevan, S. (2015) 'Metabolism and biotransformation of azo dye by bacterial consortium studied in a bioreaction calorimeter', *Bioresource Technology,* 196, pp. 500-508.

Sharma, P. R., Chattopadhyay, A., Sharma, S. K., Geng, L., Amiralian, N., Martin, D. & Hsiao, B. S. (2018) 'Nanocellulose from spinifex as an effective adsorbent to remove Cadmium(II) from water', *ACS Sustainable Chemistry & Engineering,* 6(3), pp. 3279-3290.

Shaw, D. (2012). *Biocouture: Designer Suzanne Lee on Growing Your Own Clothes.* BBC Technology. [Online] [Accessed 20/09/2018] https://www.bbc.co.uk/news/av/technology-18301669

Shehzadi, M., Afzal, M., Khan, M. U., Islam, E., Mobin, A., Anwar, S. & Khan, Q. M. (2014) 'Enhanced degradation of textile effluent in constructed wetland system using Typha domingensis and textile effluent-degrading endophytic bacteria', *Water Research,* 58, pp. 152-159.

Sheng, G.-P., Yu, H.-Q. & Li, X.-Y. (2010) 'Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review', *Biotechnology Advances,* 28(6), pp. 882-894.

Sherif, M. A. S. K. & Sameshima, K. (2005) 'Evaluation of different carbon sources for bacterial cellulose production', *African Journal of Biotechnology,* 4(6), pp. 478.

Sheth, N. T. & Dave, S. R. (2009) 'Optimisation for enhanced decolourization and degradation of Reactive Red BS C.I. 111 by Pseudomonas aeruginosa NGKCTS', *Biodegradation,* 20(6), pp. 827-836.

Shezad, O., Khan, S., Khan, T. & Park, J. K. (2009) 'Production of bacterial cellulose in static conditions by a simple fed-batch cultivation strategy', *Korean Journal of Chemical Engineering,* 26(6), pp. 1689-1692.

Shi, Z., Zhang, Y., Phillips, G. O. & Yang, G. (2014) 'Utilization of bacterial cellulose in food', *Food Hydrocolloids,* 35, pp. 539-545.

Shim, E. & Kim, H. R. (2019) 'Coloration of bacterial cellulose using in situ and ex situ methods', *Textile Research Journal,* 89(7), pp. 1297-1310.

Shim, E., Su, J., Noro, J., Teixeira, M. A., Cavaco-Paulo, A., Silva, C. & Kim, H. R. (2019) 'Conductive bacterial cellulose by in situ laccase polymerization of aniline', *PloS one,* 14(4), pp. e0214546-e0214546.

Sigaard, A. S. & Laitala, K. (2023) 'Natural and sustainable? consumers' textile fiber preferences', *Fibers,* 11(2), pp. 12.

Sinclair, R. (2015). *Textiles and fashion: materials, design and technology* (Vol. 126). Cambridge: Elsevier/Woodhead Publishing.

Singh, P. (2024). Dye pollution from the textile industry : challenges and opportunities for sustainable development. Singapore: Springer.

Singh, R., Mathur, A., Goswami, N. & Mathur, G. (2016) 'Effect of carbon sources on physicochemical properties of bacterial cellulose produced from Gluconacetobacter xylinus MTCC 7795', *E-Polymers,* 16(4), pp. 331-336.

Solís, M., Solís, A., Pérez, H. I., Manjarrez, N. & Flores, M. (2012) 'Microbial decolouration of azo dyes: A review', *Process Biochemistry,* 47(12), pp. 1723-1748.

Song, H.-J., Li, H., Seo, J.-H., Kim, M.-J. & Kim, S.-J. (2009) 'Pilot-scale production of bacterial cellulose by a spherical type bubble column bioreactor using saccharified food wastes', *Korean Journal of Chemical Engineering,* 26(1), pp. 141-146.

Song, J. E., Su, J., Noro, J., Cavaco-Paulo, A., Silva, C. & Kim, H. R. (2018) 'Bio-coloration of bacterial cellulose assisted by immobilized laccase', *AMB Express,* 8(1), pp. 1-11.

Spinmaster (2023) *Spinmaster* [online] [accessed 01/04/2023] www.spinmaster.com

Sriplai, N., Sirima, P., Palaporn, D., Mongkolthanaruk, W., Eichhorn, S. J. & Pinitsoontorn, S. (2018) 'White magnetic paper based on a bacterial cellulose nanocomposite', *Journal of Materials Chemistry C,* 6(42), pp. 11427-11435.

STEM Abassadors (2024) *STEM Ambassadors* [online] [accessed 01/03/2023] www.stem.org.uk/stem-ambassadors

Stephenson, J. (2023). *Culture and sustainability : exploring stability and transformation with the cultures framework*. Cham: Palgrave Macmillan.

Stohlmann, M., Moore, T. J. & Roehrig, G. H. (2012). 'Considerations for Teaching Integrated STEM Education', *Journal of Pre-College Engineering Education Research*, 2, pp.28-34.

Stoica-Guzun, A., Stroescu, M., Jinga, S. I., Mihalache, N., Botez, A., Matei, C., Berger, D., Damian, C. M. & Ionita, V. (2016) 'Box-Behnken experimental design for chromium(VI) ions removal by bacterial cellulose-magnetite composites', *International Journal of Biological Macromolecules,* 91, pp. 1062-1072.

Stone, R. (1999) 'Coming to Grips with the Aral Sea's Grim Legacy', *Science (American Association for the Advancement of Science),* 284(5411), pp. 30-33.

Sulaeva, I., Henniges, U., Rosenau, T. & Potthast, A. (2015) 'Bacterial cellulose as a material for wound treatment: properties and modifications - a review', *Biotechnology Advances,* 33(8), pp. 1547-1571.

Surma-Ślusarska, B., Presler, S. & Danielewicz, D. (2008) 'Characteristics of bacterial cellulose obtained from acetobacter xylinum culture for application in papermaking', *Fibres & Textiles in Eastern Europe,* 16(4), pp. 108-111.

Suryanto, H., Muhajir, M., Sutrisno, T. A., Mudjiono, Zakia, N. & Yanuhar, U. (2019) 'The mechanical strength and morphology of bacterial cellulose films: the effect of NaOH concentration', *IOP Conference Series: Materials Science and Engineering,* 515(1), pp. 12053.

Suvilampi, J., Lehtomäki, A. & Rintala, J. (2003) 'Comparison of laboratory-scale thermophilic biofilm and activated sludge processes integrated with a mesophilic activated sludge process', *Bioresource Technology,* 88(3), pp. 207-214.

Tang, W., Jia, S., Jia, Y. & Yang, H. (2010) 'The influence of fermentation conditions and post-treatment methods on porosity of bacterial cellulose membrane', *World Journal of Microbiology and Biotechnology,* 26(1), pp. 125-131.

Tanskul, S., Amornthatree, K. & Jaturonlak, N. (2013) 'A new cellulose-producing bacterium, Rhodococcus sp. MI 2: Screening and optimization of culture conditions', *Carbohydrate Polymers,* 92(1), pp. 421-428.

Teoh, A. L., Heard, G. & Cox, J. (2004) 'Yeast ecology of Kombucha fermentation', *International Journal of Food Microbiology,* 95, pp. 119-126.

Tessari, V.F. dos S. and Bonadio, M.C. (2023). Thinking about fashion consumption, consumption and fashion. Revista dObra[s], (37), pp.4–6.

Textor, T., Derksen, L., Bahners, T., Gutmann, J. & Mayer-Gall, T. (2019) 'Abrasion resistance of textiles: gaining insight into the damaging mechanisms of different test procedures', *Journal of Engineered Fibres and Fabrics,* 14.

Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., Locey, K. J., Prill, R. J., Tripathi, A., Gibbons, S. M., Ackermann, G., Navas-Molina, J. A., Janssen, S., Kopylova, E., Vázquez-Baeza, Y., González, A., Morton, J. T., Mirarab, S., Zech Xu, Z., Jiang, L., Haroon, M. F., Kanbar, J., Zhu, Q., Jin Song, S., Kosciolek, T., Bokulich, N. A., Lefler, J., Brislawn, C. J., Humphrey, G., Owens, S. M., Hampton-Marcell, J., Berg-Lyons, D., McKenzie, V., Fierer, N., Fuhrman, J. A., Clauset, A., Stevens, R. L., Shade, A., Pollard, K. S., Goodwin, K. D., Jansson, J. K., Gilbert, J. A., Knight, R., Earth Microbiome Project, C., Naturvetenskapliga, f., Faculty of, S., Göteborgs, u., Gothenburg, U., Institutionen för marina, v. & Department of marine, s. (2017) 'A communal catalogue reveals Earth's multiscale microbial diversity', *Nature,* 551(7681), pp. 457.

Torres, F. G., Arroyo, J. J. & Troncoso, O. P. (2019) 'Bacterial cellulose nanocomposites: An all-nano type of material', *Materials Science & Engineering C,* 98, pp. 1277-1293.

Tran, T., Grandvalet, C., Winckler, P., Verdier, F., Martin, A., Alexandre, H. & Tourdot-Maréchal, R. (2021) 'Shedding light on the formation and structure of Kombucha biofilm using two-photon fluorescence microscopy', *Frontiers in Microbiology,* 12, pp. 725379-725379.

Trovatti, E., Serafim, L. S., Freire, C. S. R., Silvestre, A. J. D. & Neto, C. P. (2011) 'Gluconacetobacter sacchari: An efficient bacterial cellulose cell-factory', *Carbohydrate Polymers,* 86(3), pp. 1417-1420.

Ullah, H., Santos, H. A. & Khan, T. (2016) 'Applications of bacterial cellulose in food, cosmetics and drug delivery', *Cellulose,* 23(4), pp. 2291-2314.

U. N. Environment Programme, (2023) *The environmental costs of fast fashion*. [Online] [Accessed 23/06/2023] https://www.unep.org/news-and-stories/story/environmental-costs-fast-fashion

UNCTAD. (2020). *United Nations conference on Trade and Development: Report maps manufacturing pollution in sub-Saharan Africa and South Asia* [Online] [Accessed 11/07/2023] https://unctad.org/news/report-maps-manufacturing-pollution-in-sub-saharan-africa-and-south-asia

Urbina, L., Corcuera, M. Á., Eceiza, A. & Retegi, A. (2019) 'Stiff all-bacterial cellulose nanopaper with enhanced mechanical and barrier properties', *Materials Letters,* 246, pp. 67-70.

Vazquez, A., Foresti, M., Cerrutti, P. & Galvagno, M. (2013) 'Bacterial cellulose from simple and low cost production media by Gluconacetobacter xylinus', *Journal of Polymers and the Environment,* 21(2), pp. 545-554.

Verran, J., Redfern, J., Cunliffe, A., Romachney, A. & Wood, J. (2023a) 'Hands on Biofilm! Utilising a public audience in a citizen science project to assess yield variability when culturing Kombucha pellicle', *FEMS Microbiology Letters*.

Verran, J., Wood, J., Redfern, J., Moravej, H. & Radclyffe-Thomas, N. (2023b) 'Hands On Biofilm! A multidisciplinary public engagement event using Kombucha tea pellicle as an accessible example of biofilm', *Biofilm,* 6, pp. 100169-100169.

Visjager, J. F., Bansal-Mutalik, R. & Patist, A. (2019) 'Developing the next generation of sustainable biomaterials', *Chemical Engineering Progress,* 115(10), pp. 30-35.

Vitacoco. (2020). *Vita coco* [Online] [Accessed 23rd January 2020] https://www.vitacoco.com/

Vitor, V. & Corso, C. R. (2008) 'Decolorization of textile dye by Candida albicans isolated from industrial effluents', *Journal of Industrial Microbiology & Biotechnology,* 35(11), pp. 1353-1357.

Vohland, K., Land-zandstra, A., Ceccaroni, L., Lemmens, R., Perelló, J., Ponti, M., Samson, R. & Wagenknecht, K. (2021). *The Science of Citizen Science* (1st 2021. ed.). Cham: Springer Nature.

Von Hoven, T., (2002). *'Characterisation of Alligator, Ostrich and Emu Skins and Comparisons to Traditional Leathers'*, PhD Thesis, Louisana State University, (accessed 2 February, 2025).

Vyjayanthi, J. P. & Suresh, S. (2010) 'Decolorization of Drimarene Red dye using palladized bacterial cellulose in a reactor', *Water Environment Research,* 82(7), pp. 601-609.

Waghmode, T. R., Kurade, M. B. & Govindwar, S. P. (2011) 'Time dependent degradation of mixture of structurally different azo and non azo dyes by using Galactomyces geotrichum MTCC 1360', *International Biodeterioration & Biodegradation,* 65(3), pp. 479-486.

Wahid, F., Hu, X.-H., Chu, L.-Q., Jia, S.-R., Xie, Y.-Y. & Zhong, C. (2019) 'Development of bacterial cellulose/chitosan based semi-interpenetrating hydrogels with improved mechanical and antibacterial properties', *International Journal of Biological Macromolecules,* 122, pp. 380-387.

Waltham, T. & Sholji, I. (2001) 'The demise of the Aral Sea - an environmental disaster', *Geology Today,* 17(6), pp. 218-228.

Wang, D. (2019) 'A critical review of cellulose-based nanomaterials for water purification in industrial processes', *Cellulose,* 26(2), pp. 687-701.

Wang, H., Su, J. Q., Zheng, X. W., Tian, Y., Xiong, X. J. & Zheng, T. L. (2009a) 'Bacterial decolorization and degradation of the reactive dye Reactive Red 180 by Citrobacter sp. CK3', *International Biodeterioration & Biodegradation,* 63(4), pp. 395-399.

Wang, J., Gao, C., Zhang, Y. & Wan, Y. (2010) 'Preparation and in vitro characterization of BC/PVA hydrogel composite for its potential use as artificial cornea biomaterial', *Materials Science & Engineering C,* 30(1), pp. 214-218.

Wang, Y. & Baoping, J. (2013) 'Hepatoprotective Effects of Kombucha Tea', *Society of Chemical Industry,* 2014(94), pp. 265-272.

Wang, Y., Tian, R. M., Gao, Z. M., Bougouffa, S. & Qian, P.-Y. (2014) 'Optimal eukaryotic 18S and universal 16S/18S ribosomal RNA primers and their application in a study of symbiosis', *PloS one,* 9(3), pp. e90053.

Wang, Z.-L., Jia, Y.-Y., Shi, Y., Cong, D.-L., Chen, Y.-Y., Jia, S.-R. & Zhou, Y.-L. (2009b) 'Research on characterization and biocompatibility of nano-bacterial cellulose membrane', *Chemical Journal of Chinese Universities,* 30(8), pp. 1553-1558.

Wang, Z. W., Liang, J. S. & Liang, Y. (2013) 'Decolorization of Reactive Black 5 by a newly isolated bacterium Bacillus sp. YZU1', *International Biodeterioration & Biodegradation,* 76, pp. 41-48.

Watkins, S. M. & Dunne, L. E. (2015). *Functional clothing design: from sportswear to spacesuits*. New York: Fairchild Books.

Wei, B., Yang, G. & Hong, F. (2011) 'Preparation and evaluation of a kind of bacterial cellulose dry films with antibacterial properties', *Carbohydrate Polymers,* 84(1), pp. 533-538.

West, S. & Pateman, R. (2016) 'Recruiting and retaining participants in citizen science: what can be learned from the volunteering literature?', *Citizen science : theory and practice,* 1(2), pp. 15.

Williams, E., Cenian, K., Golsteijn, L., Morris, B. & Scullin, M. L. (2022) 'Life cycle assessment of MycoWorks' Reishi™: the first low-carbon and biodegradable alternative leather', *Environmental sciences Europe,* 34(1), pp. 120.

Williams, J. T. (2018). *Waterproof and water repellent textiles and clothing,* Duxford, United Kingdom: Woodhead

Williams, S. & Cannon, R. (1989) 'Alternative environmental roles for cellulose produced by *Acetobacter xylinum*'', *Applied Environmental Microbiology,* 55, pp. 2448-2452.

Willmott, N., Guthrie, J. & Nelson, G. (1998) 'The biotechnology approach to colour removal from textile effluent', *Journal of the Society of Dyers and Colourists,* 114(2), pp. 38-41.

Wood, J. (2019) 'Bioinspiration in Fashion—A Review', *Biomimetics,* 4(1), pp. 16.

Wood, J., Redfern, J. & Verran, J. (2022) 'Developing textile sustainability education in the curriculum: pedagogical approaches to material innovation in fashion', *International Journal of Fashion Design, Technology and Education,* 16 (2) pp. 1-11.

Wood, J., Verran, J. & Redfern, J. (2023) 'Bacterial cellulose grown from Kombucha: Assessment of textile performance properties using fashion apparel tests', *Textile Research Journal*, 93 (13) pp. 4051752311526.

WRAP (2021). *Textiles 2030.* [Online] [Accessed 03/04/2021] https://wrap.org.uk/taking-action/textiles/initiatives/textiles-2030

Wu, J., Zheng, Y., Wen, X., Lin, Q., Chen, X. & Wu, Z. (2014) 'Silver nanoparticle/bacterial cellulose gel membranes for antibacterial wound dressing: investigation in vitro and in vivo', *Biomedical,* 9(3), pp. 035005.

Wu, Y., Li, T. & Yang, L. (2012) 'Mechanisms of removing pollutants from aqueous solutions by microorganisms and their aggregates: A review', *Bioresource Technology,* 107, pp. 10-18.

Xiang, Z., Zhang, J., Liu, Q., Chen, Y., Li, J. & Lu, F. (2019) 'Improved Dispersion of Bacterial Cellulose Fibers for the Reinforcement of Paper Made from Recycled Fibers', *Nanomaterials,* 9(1), pp. 58.

Xiao, L., Mai, Y., He, F., Yu, L., Zhang, L., Tang, H. & Yang, G. (2012) 'Bio-based green composites with high performance from poly(lactic acid) and surface-modified microcrystalline cellulose', *Journal of Materials Chemistry,* 22(31), pp. 15732.

Yamada, Y., Yukphan, P., Lan Vu, H. T., Muramatsu, Y., Ochaikul, D., Tanasupawat, S. & Nakagawa, Y. (2012) 'Description of Komagataeibacter gen. nov., with proposals of new combinations (Acetobacteraceae)', *The Journal of general and applied microbiology,* 58(5), pp. 397.

Yamanaka, S. & Sugiyama, J. (2000) 'Structural modification of bacterial cellulose', *Cellulose,* 7(3), pp. 213-225.

Yang, J. Z., Yu, J. W., Sun, D. P. & Yang, X. J. (2011) 'Preparation of novel Ag/bacterial cellulose hybrid nanofibers for antimicrobial wound dressing', *Advanced Materials Research,* 152-153, pp. 1771-1774.

Yang, Q., Li, C., Li, H., Li, Y. & Yu, N. (2009) 'Degradation of synthetic reactive azo dyes and treatment of textile wastewater by a fungi consortium reactor', *Biochemical Engineering Journal,* 43(3), pp. 225-230.

Yaseen, D. A. & Scholz, M. (2019) 'Textile dye wastewater characteristics and constituents of synthetic effluents: a critical review', *International Journal of Environmental Science and Technology,* 16(2), pp. 1193-1226.

Yeap, K. L., Teng, T. T., Poh, B. T., Morad, N. & Lee, K. E. (2014) 'Preparation and characterization of coagulation/flocculation behavior of a novel inorganic–organic hybrid polymer for reactive and disperse dyes removal', *Chemical Engineering Journal,* 243, pp. 305-314.

Yim, S. M., Song, J. E. & Kim, H. R. (2017) 'Production and characterization of bacterial cellulose fabrics by nitrogen sources of tea and carbon sources of sugar', *Process Biochemistry,* 59, pp. 26-36.

Yoon, S. H., Jin, H.-J., Kook, M.-C. & Pyun, Y. R. (2006) 'Electrically conductive bacterial cellulose by incorporation of carbon nanotubes', *Biomacromolecules,* 7(4), pp. 1280-1284.

Younesi, M., Wu, X. & Akkus, O. (2019) 'Controlled mercerization of bacterial cellulose provides tunability of modulus and ductility over two orders of magnitude', *Journal of the Mechanical Behavior of Biomedical Materials,* 90, pp. 530-537.

Yu, H., Li, P., Li, Y., Song, Y., Wu, Y. & Chen, B. (2016) 'Preparation and luminescent properties of Gd2O3:Eu3+ nanofibres made by electrospinning', *Ceramics International,* 42(1), pp. 1307-1313.

Zabłocka-Godlewska, E., Przystaś, W. & Grabińska-Sota, E. (2018) 'Possibilities of obtaining from highly polluted environments: new bacterial strains with a significant decolorization potential of different synthetic dyes', *Water, Air, & Soil Pollution,* 229(6), pp. 1-13.

Zahrim, A. Y., Tizaoui, C. & Hilal, N. (2011) 'Coagulation with polymers for nanofiltration pre-treatment of highly concentrated dyes: A review', *Desalination,* 266(1), pp. 1-16.

Zeng, X., Small, D. P. & Wan, W. (2011) 'Statistical optimization of culture conditions for bacterial cellulose production by Acetobacter xylinum BPR 2001 from maple syrup', *Carbohydrate Polymers,* 85(3), pp. 506-513.

Zhang, H., Xu, X., Chen, X., Yuan, F., Sun, B., Xu, Y., Yang, J. & Sun, D. (2017) 'Complete genome sequence of the cellulose-producing strain Komagataeibacter nataicola RZS01', *Scientific reports,* 7(1), pp. 4431-8.

Zhang, M., Wu, X., Hu, Z., Xiang, Z., Song, T. & Lu, F. (2019) 'A highly efficient and durable fluorescent paper produced from bacterial cellulose/eu complex and cellulosic fibers', *Nanomaterials,* 9(9), pp. 1322.

Zheng, Y., Chang, Y., Zhang, R., Song, J., Xu, Y., Liu, J. & Wang, M. (2018) 'Two-stage oxygen supply strategy based on energy metabolism analysis for improving acetic acid production by Acetobacter pasteurianus', *Journal of Industrial Microbiology & Biotechnology,* 45(9), pp. 781-788.

Zheng, Y., Yu, S., Shuai, S., Zhou, Q., Cheng, Q., Liu, M. & Gao, C. (2013) 'Color removal and COD reduction of biologically treated textile effluent through submerged filtration using hollow fiber nanofiltration membrane', *Desalination,* 314, pp. 89-95.

Zhu, H., Jia, S., Yang, H., Jia, Y., Yan, L. & Li, J. (2011) 'Preparation and application of bacterial cellulose sphere: a novel biomaterial', *Biotechnology & Biotechnological Equipment,* 25(1), pp. 2233-2236.

Zhu, W., Liu, L., Liao, Q., Chen, X., Qian, Z., Shen, J., Liang, J. & Yao, J. (2016) 'Functionalization of cellulose with hyperbranched polyethylenimine for selective dye adsorption and separation', *Cellulose,* 23(6), pp. 3785-3797.

Zille, A. and Raoux, S. (2021). The urgency of measuring fluorinated greenhouse gas emission factors from the treatment of textile and other substrates. Resources, conservation and recycling, 174, pp.105820.

Appendix 1 – Peer Reviewed Publications

Biofilm 6 (2023) 100169

Contents lists available at ScienceDirect

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Hands On Biofilm! A multidisciplinary public engagement event using kombucha tea pellicle as an accessible example of biofilm

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ABSTRACT

Public engagement with science has become increasingly important for the scientific community. There are many documented public engagement events that focus on aspects of microbiology, but relatively few utilise biofilms as a topic, despite their importance. Kombucha tea pellicles are easy to grow biofilms, facilitating their use within the public domain as examples of these complex communities.

The aim of this work was to deliver a public engagement event that introduced visitors to general concepts about biofilm, and applications around sustainability, using kombucha. The event encouraged visitors to: build a biofilm using model clay; inoculate kombucha tea cultures using different incubation conditions, as part of a citizen science experiment to assess impact on pellicle biofilm yield; create garments and drapes on mini-mannequins using dried kombucha pellicle fabric, and demonstrate the range and importance of fermented foods (including kombucha tea), and 'good bacteria'. Quantitative and qualitative indicators of engagement were built into the activities.

More than 1200 visitors, mainly in family groups, visited the event over a 4-h period. Knowledge of biofilms was low at the beginning of the event. Participation in all activities was high. Indicators of quantitative engagement were impressive, but it was difficult to obtain qualitative evidence other than observations from the delivery team (nineteen members) because of the intensity of the event and volume of visitors.

The event was clearly successful in terms of fulfilment of aims, audience engagement and enthusiasm: the embedded evaluations helped to evidence the impact and reach of the event, enabling confidence in dissemination of good practice in the enhancement of public understanding of the importance of biofilm in general, and kombucha in particular.

1. Introduction

Public engagement with science has been increasingly important for conveying the importance of microbiology to a range of audiences, acting as a route for dissemination of research findings [1], enabling contribution from the public to enhance/support research in 'citizen science' activities [2], raising awareness of important topics of current concern [3–5], and acquainting audiences with more general aspects of microbiology that are important/interesting [6] and indeed wondrous [7].

The biofilm research community is well aware of the importance of biofilm in all walks of life, but acknowledges that more needs to be done to educate and engage non-scientists, for example around 'more educational material, position papers and text books' [8]. The National Biofilm Innovation Centre (NBIC) has begun to collate examples of public engagement activities focusing on biofilms (https://www.biofilms.ac.uk/what-are-biofilms/), and there is a need to ensure that such activities are robust, reproducible and safe, with clearly defined aims, identifiable outcomes and evidence of impact. Without a central store of resources, it is always difficult for researchers to find examples, or inspiration, for their own event planning and delivery – as has been noted previously with regard to AMR [9].

'Hands-on' activities are the gold standard for education and for 'active engagement' [10]. The mantra of engaging with a student's head (cognitive), hands (practical) and heart (values) is easily translated into events designed to engage with a public audience, enabling consideration of the most effective activities to be implemented [11]. However, few events focusing on microbiology -whether as biofilm or otherwise - can facilitate significant 'hands-on' activities, due to health and safety concerns, particularly with potentially high numbers of visitors. Furthermore, there is little opportunity to set up experiments and provide feedback/show results post-incubation, although of course visitors

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can examine preformed biofilm/images or models, use microscopy [12], or experience virtual reality 'walk-through' events [13]. If a biofilm growth experiment is set up (inoculation), then there is a time gap (incubation) before results/observations can be viewed or disseminated. Typically for microbiology hands-on activities that involve inoculation of culture media and monitoring of subsequent growth, results are obtained within 24–48 h – much less that the time required to cultivate biofilm. To overcome difficulties with getting such results to public audiences, we have previously successfully used FlickR to display images of results [12]. Offering face-to-face repeat visits for participants to view results a week (Saturday to Saturday for visitor convenience) after inoculation [4] was not successful.

Kombucha pellicle provides an accessible and relatively 'safe' biofilm that could be used in a public engagement scenario to generate interest in biofilm more generally whilst also enabling some 'hands-on' activities. Kombucha is a fermented, lightly effervescent sweetened black tea drink commonly consumed for its purported health benefits – but it may also be used as a source of biofilm. Kombucha tea can be purchased commercially, but it can also be cultured at home. Homemade Kombucha tea comprises a liquid (the drink) but also a pellicle (biofilm) that grows on the surface of the culture medium (Fig. 1a and b). Both the liquid and the pellicle can be used in subculture to produce more kombucha tea. The global kombucha market is growing exponentially [14], and there is increasing demand for education in the area [15]. It is generally regarded as safe (GRAS) [16], is readily accessible and self-propagating, thus is cost-effective to use, and potentially easily and safely handled. The kombucha pellicle also provides an excellent medium for exploration of biofilm, fermentation and textile technology. Indeed, the pellicle itself has been receiving attention with regard to its textile-like properties when dried, even for its potential use in fashion [17,18], and as a focus for sustainability in the circular economy.

The obvious cross-disciplinary interests that could arise from considering kombucha as a vehicle for public engagement activities (microbiology, nutrition, fermentation, fashion, sustainability) led to the design and delivery of an event that would engage audiences with biofilm, and that included some hands-on experimental activities which incorporated inputs from across disciplines. Repeated exposure to interdisciplinary thought has been shown to enhance learners' critical thinking and understanding of the relationships between perspectives derived from different disciplines [19], thereby preparing students for dealing with complex societal issues [20]. Using sustainability as an overarching theme for this event provided a focus for the complementarity of the disciplines underpinning the activities provided.

The aim of this paper is to describe and critically evaluate the success of a cross-disciplinary public engagement event designed to raise awareness of biofilms in the context of sustainability and nutrition, using kombucha as an example of biofilm. It is hoped that our experiences might help others planning similar events.

2. Methods

The public engagement event was planned for delivery at the 2022

Fig. 1. Mature kombucha culture showing the biofilm pellicle on the surface (a), and when decanted from the surface (b). Sheets of kombucha fabric dyed with food dyes (c). Mini-mannequin draped in kombucha fabric (d).

Manchester Science Festival (UK). The annual festival is one of the most popular in the UK, attracting over 100,000 visitors across a week in October. In order to assist those planning to deliver similar events, details about event planning and delivery are included below. It is essential to have identified a location, a target audience and an advertising schedule as part of planning, to ensure that activities, equipment, personnel and consumables are appropriate.

2.1. Event planning

2.1.1. Location

Permission to host this event during the Manchester Science Festival (www.scienceandindustrymuseum.org.uk/manchester-science-festival/) was sought and obtained ten months in advance, which enabled a successful funding bid from the National Biofilm Innovation Centre. The one-day event (11am – 3pm), 'Hands On Biofilm!', was to be delivered as part of a 'Get Curious' stream, which took place daily throughout the ten days of the festival, in the Science and Industry Museum.

2.1.2. Ethics statement

The project was assessed and approved by the Manchester Metropolitan University Ethics Committee (application number 41586) which included the retention of personal data for purposes of contacting visitors after the event. Risk assessment was carried out with input from the host (museum) to assess not only the risk of the activities, but also the practicalities of delivering in an active Museum site. Liability insurance was provided by Manchester Metropolitan University.

2.1.3. Logistics and activities

The delivery team (authors) held several meetings (approximately monthly, more intensively as the event approached) both face to face and online. Ideas and their feasibility (cost, practicality) and fit to the overall aims were discussed. Some were discarded, others used or extended.

In addition to the five academics who hosted the event, several students were recruited to support the activities, comprising seven final year biology project students, five members of MetMUnch, a social enterprise originally run from Manchester Metropolitan University (https://metmunch.com), one MetMUnch intern and one microbiology postgraduate. These were recruited closer to the event and were briefed in the week prior. In addition, the Museum tallied visitor numbers (along with other in-house evaluation).

Previous experience of such events [4,21,22] indicated that a busy day should be anticipated, thus materials required for the event were prepared/sourced with the assumption of 200+ visitors (probably in family groups). Activities needed to be spread out across a designated space near the entrance to the museum, so it was decided to host five stations arranged in an inverted, wide U-shape, each with specific aims, and each with a measure of quantitative engagement embedded. It was hoped that visitors would access the five stations in a specific sequence (left to right), but it was also recognised that this might not happen – so each station delivered independent activities and messages. It was particularly important to ensure that sufficient consumables were available, especially for hands-on activities. Qualitative evaluation would rely on observational conversations, engagement following email follow-up, and critical reflection from the staff and students delivering the event. A hashtag #handsonbiofilm was used to promote and document the event on social media.

2.2. Event delivery

Each activity had its own designated space (station). The intended sequence of five stations were set up as listed below, arranged in a '7' shape rather than the 'U', due to space constraints in the museum zone: activities began at the foot of the '7'. Biofilm-related presenters wore lab coats, MetMUnch staff wore customised aprons.

a) Welcome

One of the authors (JV) took responsibility for the 'welcome soapbox'. The aim was to encourage engagement with the event, to outline to visitors the various activities taking place, and to provide a postcard with more details (Fig. 2). Stickers were issued at each of the four activities, and if all four stickers were collected on the postcard, then a prize was given. The postcards were to be kept by the visitors so that they could contact the delivery team in future, and use the QR code/FlickR weblink to access a dedicated album where results from the citizen science part of the event would be uploaded. Three hundred postcards were printed. Quantitative evaluation data were obtained from number of postcards distributed, number of stickers collected, and number of visits to the FlickR website.

b) Film

The aim was to inform visitors about biofilm, and to subsequently encourage them to assist in a hands-on experiment using kombucha biofilm.

One of the authors (JR) took overall responsibility for this first visit station, which was delivered primarily by final year undergraduate biology students supported by two of the authors (JV, JR). This station focused on biofilm, and utilised some of the planning for a previous biofilm event planned which was cancelled due to the pandemic.

A rolling gallery of images of biofilms provided a backdrop to the station. Key conversation/information points had been provided to helpers for use in conversation with visitors (what is a biofilm, where do you find biofilm, how do they grow, what do they look like, what does kombucha biofilm look like) in the form of a 'biofilm explainer' sheet sourced from NBIC (www.biofilms.ac.uk). Bunchems (www.spinmasters.co.uk), coloured plastic self-adhesive Velcro-like balls (2 cm diameter) were used as visual aids to demonstrate bacterial interactions. Visitors were invited to contribute to our day-long 'build a biofilm' activity using Model Magic (www.crayola.com) to make cells that were placed in a home-made Perspex box (415 mm height x 300 width x 35 diameter). To give some indication of prior knowledge, visitors were also asked if they knew what biofilms were, placing a white or clear marble into a jar if they did and other colours if they did not.

Specific risks for this station: Bunchems were not to be used by visitors (risk of tangling in hair); trays and handwipes to be used with Model Magic (to prevent spillage/transfer onto museum floor); supervised use of marbles (choke risk). Quantitative data were obtained from numbers of marbles used to indicate familiarity with biofilm, and number of model magic 'cells' made.

c) Fabric

The aim of this part of the day was to get visitors to select inoculation and incubation conditions from choices provided – a further overall aim for the authors was to assess the variability of biofilm yield using different inoculation and incubation conditions, with multiple operators carrying out the process as part of a research project.

One of the authors (JW) took responsibility for setting up the 'fabric' station, which was delivered by postgraduate and undergraduate project students and supported by another author (JR). Research in our laboratories had shown that the yield of kombucha pellicle biofilm could vary, and that different incubation conditions were used by researchers in the field [18,23]. After inoculation and five weeks incubation, the biofilm yield would be assessed (thickness, wet and dry weight). [This citizen science activity has been reported in more detail elsewhere [24].]

The postcard provided some general information about the intended activity, but in addition, an A1 poster on a free-standing easel was used to talk visitors through their choices (liquid or solid [pellicle] inoculum; 30 °C or room temperature incubation; beer, tea, coconut milk or H&S

a

b.

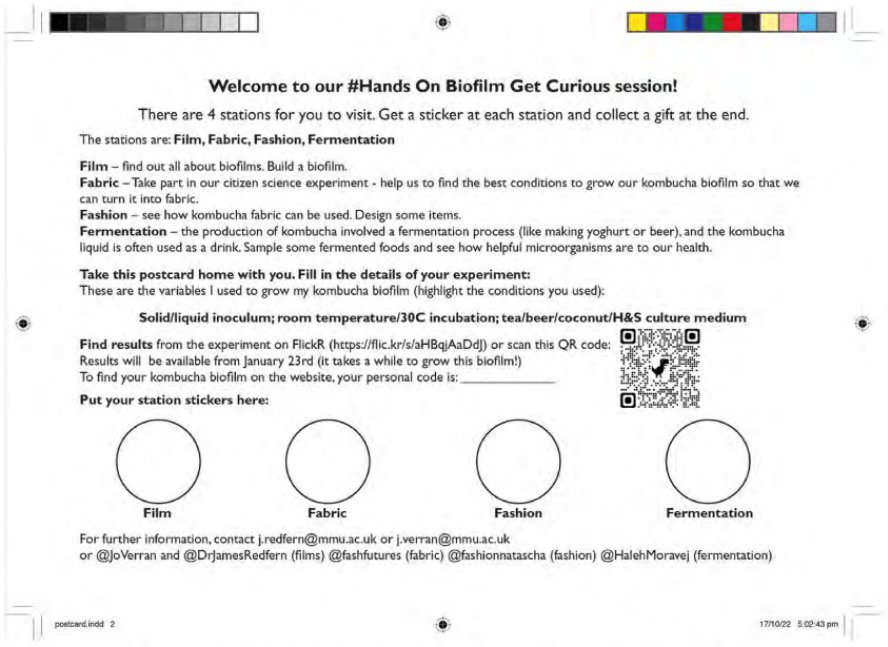

Fig. 2. Postcard given to visitors at the Hands On Biofilm event – front (a) and back (b) views. Images courtesy of Jane Wood and Norton Robinson.

[25].

The work benches at the station displayed examples of pre-grown kombucha cultures/pellicles in 5L Kilner jars. 300 lidded plastic Universal bottles (25 mL) were prepared, each containing 20 mL of one of the culture media. After inoculation (by visitors - supervised or by scientists observed by visitors), each inoculation vessel was marked with a reference number corresponding to that on the postcard, and variables were also noted on the postcard (and stickers collected).

At the end of the event, all inoculated vessels were taken to the laboratory for incubation for eight weeks at the selected temperature. After this time, biofilm thickness was measured, the biofilm decanted from the vessel, photographed (wet and dry), weighed, and analysis made of the effect of variables on yield [17] (.). Results were presented on FlickR at a date specified on the postcards. Email addresses were sought from the visitors, if they were happy to provide them, to facilitate contact and a reminder of the FlickR opening date. Risk assessment focused on spillage/slip prevention. Quantitative data were obtained from number of vessels inoculated, number of emails provided (and number of visits to the FlickR website).

d) Fashion

The aim of the activity was to raise awareness of kombucha as a sustainable material, and the importance of sustainable fashion in the circular economy, through conversation and hands-on activities Two of the authors (JW, NR) took responsibility for and delivered the activities at this station. The focus was on creativity and exploring innovative sustainable materials for fashion. Participants were presented with a variety of materials including kombucha biofilm which had been dried and stained with a range of food dyes to produce fabric (Fig. 1c). To create the komucha biofilm fabrics for the activity, a Kombucha starter culture was purchased from a commercial supplier (www.Happykombucha.com). The starter culture (SCOBY = symbiotic culture of bacteria and yeast) weighed 200g, and was supplied in a sealed package containing 100 ml of liquid (green tea). Tea liquid medium was prepared by steeping 4 tea bags in 4l of boiling water. After 15 min the tea bags were removed, 400g sucrose added and the mixture stirred until all the sucrose had dissolved. The mixture was left to cool to room temperature. A 5l kilner jar (www.kilnerjar.com) was submerged for 15 min in sterilising fluid (www.milton-tm.com), prepared according to manufacturers instructions, and removed directly before use. Once at room temperature, the tea liquid medium was decanted into the kilner jar, along with the Kombucha SCOBY and the 100 ml of green tea from the delivery package. The kilner jar was then stored at room temperature for 30 days. This process was repeated 3 times (3 kilner jars prepared in total).

After 30 days, liquid was decanted from the kilner jars as a liquid inoculum for the event. 250 ml aliquots were decanted into sterile containers, and lids screwed shut in preparation for transportation to the event. At this time, a fresh pellicle had developed on the surface of the tea liquid in each of the kilner jars. This pellicle was removed (leaving the original 'mother' pellicle in situ) and sliced into 2g pieces as a solid inoculum for the event. The 2g pellicle pieces were stored in a lidded sterile container with 100 ml of the liquid inoculum (decanted from the kilner jar).

The fabrics were of differing texture and thickness, due to variations in growing and drying technique (flat dry on bench vs 'stretching' whilst drying, and the addition of coconut oil to some of the fabrics to enhance flexibility). They were dyed using natural food colouring.

Inspired by discovering the tactile qualities of these samples, visitors drew their own designs on paper, which were then converted to clothing by pinning/draping kombucha fabrics onto mini-mannequins (quarter scale size, made at The University of Manchester from foam). No specific risks were identified for this activity. No quantitative data were obtained for this activity.

e) Fermentation

The aim/objective of this station was to inform visitors of the importance of microorganisms in fermented foods and drinks, and in the gut microbiome.

One of the authors (HM) took responsibility for and led the delivery at this station, assisted by a team of undergraduate 'MetMUnchers' and an intern. MetMUnch (www.metmunch.com) is very familiar with this type of event, catering for large numbers of visitors at events with various food-related focus. Examples of fermented foods (Kimchi, kefir, kombucha, sauerkraut) were displayed, and samples were available for tasting. MetMUnch members posted images during the event on social media platforms when time allowed.

Leaflets had been produced describing the microbiological activities taking place: 200 copies were made. For visitors who had collected all four stickers, the prize was a bottle of kombucha drink made specifically for the event (www.tigertea.co.uk). Two hundred bottles of kombucha drinks were sourced, with 50 of each flavour (chai, jasmine, earl grey, mojito) being available.

Risk assessment for this station focused on food consumption and disposal of waste.

Quantitative data were obtained from (approximate) number of samples tasted, number of leaflets distributed and number of prizes handed out.

3. Results and discussion

3.1. Overall observations

Findings from the evaluations carried out at the event are summarised in Table 1. Reflections of the delivery team have been incorporated throughout the results. More than 1200 visitors attended the event (information from the museum), primarily in approximately 300 family groups (observation from stations) – all 300 postcards were distributed throughout the day; all 200 fermentation leaflets were used (with demand for more and promises of digital copies being sent to around twenty additional families). There were 170 kombucha drink bottles given to families who had completed the activities (collecting stickers). The original intention was to have different coloured stickers at each station, to indicate whether any were more, or less, popular. However, the stations were so busy that this organisation could not be maintained: stickers ran out, and the postcards were initialled by those at the stations. The use of 'hands-on' activities proved effective in ensuring visitor engagement and enjoyment. This was not merely indicated by the extensive use of materials, but also by the conversations and discussions taking place alongside the varying tasks set [10]. For example, families discussed among themselves and with the demonstrators which experimental conditions to select at the 'fabric' station, and were keen to carry out the inoculations themselves. The tasting at the 'fermentation' station encouraged conversation and comments on flavour and texture, and the time spent on designing outfits and selecting fabrics at the 'fashion' station facilitated significant engagement (vide supra). These observations reinforce 'minds-on' as well as 'hands-on' activities, which will inevitably enhance memory and learning outcomes [11].

The event was busy throughout the day without any quieter periods. Student helpers were enthusiastic, informative and knowledgeable, but it was difficult for any of the delivery team to take time away from the event for reflection or observation - even for refreshment. If additional observers had been recruited [3], it is likely they would also have been involved in activities rather than as passive watchers.

Despite the detailed planning and preparation for the event we also needed to be prepared for the unexpected (for example table size was unknown in advance, which affected numbers needed; the station layout needed to be altered on site; sticker provision was inadequate; crowd bottlenecks needed management).It is important to have the support of the establishment hosting the event, and an enthusiastic and robust

Table 1
Summary of the four activity stations hosted for the 'HandsOnBiofilm!' public engagement event, including aims, methods for quantitative evaluation and general observations.

Activity Station	Aim	Evaluation method	Data/indicator of engagement	Observations
Film	To inform about biofilm.	Question: do you know what biofilm is? Help us build a biofilm.	9 of 174 respondents knew what biofilm was. 530 separate items contributed to built biofilm.	Numerical indicators of engagement supported observed enthusiastic conversations.
Fabric	To select incubation conditions for growing kombucha biofilm. To identify interest in the project over time. To assess the variability of biofilm yield.	Number of vessels inoculated. Visits to website for results posted 12 weeks after the event. Thickness and weight of biofilm measured.	221 vessels inoculated. 4647 views on FlickR website after email reminder. Not directly related to event evaluation, but an embedded citizen science project.	Numerical indicators of engagement supported observed enthusiastic conversations. Importance of obtaining contact details to enhance subsequent engagement. Citizen science project data supported laboratory findings [17].
Fashion	To raise awareness of kombucha as a sustainable material, and the importance of sustainable fashion in the circular economy.	Noting points raised in conversation.	General notes regarding interest and enthusiasm; specific comments not recorded.	Station too busy to formally note observations. Subjective assessment of interactions revealed enthusiastic engagement.
Fermentation	To inform visitors of the importance of microorganisms in fermented foods and drinks, and in the gut microbiome.	Leaflets handed out. Food and drink samples. Noting points raised in conversation.	All 200 leaflets handed out. All foods sampled. General observations regarding enjoyment of taste; specific comments not recorded.	Station too busy to formally note observations. Subjective assessment of interactions revealed enthusiastic engagement.
Event overall	To encourage engagement with the event	Event information provided. Stickers collected from the four stations.	All 300 postcards handed out. 170 groups completed the activities.	1200 visitors in total, mainly in family groups. Quantitative evaluation data indicate successful engagement. Qualitative evaluation was subjective, but indicative of successful engagement.

delivery team. The amount of staff time and effort put into the event was significant and could not have been factored into the funding sought.

3.2. Social media

It was barely possible to update social media (Twitter, Instagram or Facebook) about the activities during the event because of the intensity and popularity of visitor interaction. Overall, approximately twenty social media posts received around 300 impressions each. A subsequent thread was posted during World Antimicrobial Awareness Week/National Biofilm Week four weeks after the event. Daily posts throughout the week received around 400 impressions each, although the first post garnered almost 3,000. In hindsight, it might have been preferable to prepare a sequence of timed tweets in advance of the day.

In short, social media proved not to be a useful vehicle for dissemination of this event, particularly in real time, hence the best way of accessing more distant audiences interested in biofilms or public engagement may be specifically designed content for social media as well as remaining with peer-reviewed papers, specific website collections and conference presentations across a range of disciplines.

3.3. Film station

The 'film' station was the first that most visitors encountered. For the initial question 'do you know what a biofilm is?', 174 marbles had been put into the appropriate jar, of which nine were white/clear, indicating that very few visitors knew what biofilm was. However, the jar was placed at the end of the station after visitors had encountered some of the activities: perhaps the responses were related to this immediate prior learning. It would have been better if the question were posed at the initial soapbox introduction, if some follow-up activity had been performed at the end, and if some reflective questions had been included in the subsequent email. Nevertheless, a 'new' audience had been reached, for whom biofilm was a novel phenomenon. Often these kinds of events mostly reach people with high prior knowledge, who are already motivated to learn more [26] so the event was of value for enhancing knowledge of an important aspect of microbiology.

The Bunchems were useful as visual aids in demonstrating biofilm formation, but perhaps more reference could have been made to the images on slides, if time and crowds had allowed. The completed Model Magic biofilm comprised 530 separate items/microbial cells (Fig. 3a) not exactly presenting the columnar aspect of a typical biofilm, but reflecting morphological diversity and stimulating engagement. The clay had dried and was very light, but not especially hard, perhaps due to being enclosed in a Perspex box (which nevertheless had to be deconstructed to extract the biofilm). There was a clear diversity of 'microbial' shapes (Fig. 3b–c), as well as some models with less of a microbiological flavour (not presented).

3.4. Fabric station

The citizen science experiment of growing kombucha pellicles under different incubation conditions generated much interest. There were queues of families waiting to choose their incubation conditions. Details are reported elsewhere [17], but 221 bottles were inoculated, and the FlickR website experienced a total of 4,647 photo views, with 109 'photostream' (individual) visits after email reminders were sent to those 114 visitors who had provided contact details (25 views prior to the reminder). A second reminder was posted more widely on Twitter six months after the event to highlight the results posted on FlickR: within a week, there were an additional 800 views/twenty visitors to the site. It was very rewarding to have evidence of a sustained interest and continued engagement in the event: many evaluations stop when the event closes, which makes evidence of continued interest unusual and valuable. The 'best' incubation conditions identified by this citizen science project agreed with those from the research laboratory (tea medium, liquid inoculum, 30 °C or room temperature), supporting the occasionally-disputed fact that citizen science can be on a par with "professional science" [27].

3.5. Fashion station

The fashion station was really popular, with queues forming for the budding designers to create their fashion – this activity was more time-consuming than others available to visitors, which also contributed to the bottleneck (at the hinge of the '7'). The expectation that all designs

3a

3b

3c

Fig. 3. Biofilm models made using Model Magic clay: a) Perspex box containing Model Magic cells as deposited by visitor, b) the diversity of morphological types of 'microbes' made by visitors, c) masses of different cell shapes.

would be converted to clothing on the mini-mannequins could not be met (and had not been promised), but some wonderful costumes were created (Fig. 1d). This station could have utilised more tables and had more staff involved. Drawn and coloured designs could have been photographed and counted, but there was no time for quantitative evaluation. Although fashion is frequently considered a female interest, the station host observed that the visitors did not reflect any such stereotypes. The ability to touch materials and see them transformed into 3D 'garments' was evidently impactful. Visitors were surprised and intrigued about the potential for such innovative materials. The range of outcomes was varied – from sportswear to couture! Most visitors proudly shared their creations with their families and took photos of their sketches and the finished pieces. It is unfortunate that there were no quantitative indicators of engagement at this station (designs could have been collected or photographed, and/or counted), and that qualitative indicators relied on observations of the experts hosting the activities. However, the popularity of the station was evident from the bustle and crowding around the tables, and the conversations demonstrated the value of using different disciplines to consider the overarching sustainability theme [19].

3.6. Fermentation station

The fermentation station was also busy. Some of the aims of the station were more adult-focused, relating to mental health, irritable bowel syndrome issues and food sustainability. However, families and children enjoyed tasting the foods, and appreciated the additional and accessible information provided in the booklet. This combination of fermented food, fundamental microbiology and sustainability have been explored previously in public engagement events [22], illustrating the value of cross-disciplinary collaboration and the 'head, hands, heart' – and gut? - concept of active learning [11].

Kimchi was the most popular of the fermented foods (supplies ran out first), Some families did not want to take away the kombucha tea samples that were the gift for completing the exercise, although others were pleasantly surprised at the taste. In hindsight, it would have been better to give children sachets of Model Magic as their gift. By the end of the day, when fewer families had multiple (individual) copies of the postcard, the remaining kombucha bottles were given out freely.

3.7. Quantitative and qualitative evaluation indicators

Quantitative evaluation indicated considerable engagement with the science by the audiences. Qualitative indicators rely on our observations: the buzz of activity across the event; the diversity of the morphological forms in the Model Magic biofilm; the thoughtful selection of incubation conditions; the time taken to create fashion designs; the ongoing conversations between family members, and between families and the delivery team. More detailed reporting of these conversations was not possible. A possible addition could have been a separate 'vox-pop' kiosk for recording visitors views. It would also have been interesting to know how long individual families stayed: certainly the high number of completed postcards/collected stickers indicated that most families (perhaps an estimate of 170 of 300 in total) visited all stations. This observation reinforces the value of multi-disciplinary inputs to public engagement events.

3.8. Concluding comments

This was a multi-disciplinary event designed to engage family audiences with kombucha biofilm, comprising activities that crossed subject boundaries, demonstrating the value of collaboration and cross-disciplinarity in science and the arts, with an overarching sustainability perspective. There should have been something for every visitor somewhere across the stations, whether it was good bacteria, sustainable fashion, fermentation or biofilm. Quantitative evaluation indicated successful engagement. Subjective observations of the audience enthusiasm and engagement show that positive messages about science, scientists and universities – and biofilms, textiles, fashion and fermentation – were transmitted for that day at least, and likely beyond, when the

citizens got their 'HandsOnBiofilm'.

CRediT authorship contribution statement

Joanna Verran: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft. **Jane Wood:** Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **James Redfern:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Haleh Moravej:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision. **Natascha Radclyffe-Thomas:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: James Redfern, Joanna Verran reports financial support was provided by National Biofilms Innovation Centre.

Data availability

No data was used for the research described in the article.

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References

[1] Wellcome Trust. Factors affecting public engagement by researchers. London: The Wellcome Trust; 2015.
[2] n.d It's microbiology, citizens. Nat Rev Microbiol 2013;11(7):427.
[3] Verran J, Haigh C, Brooks J, Butler JA, Redfern J. Fitting the message to the location: engaging adults with antimicrobial resistance in a World War 2 air raid shelter. J Appl Microbiol 2018;125(4):1008–16.
[4] Redfern J, Bowater L, Crossley M, Verran J. Spreading the message of antimicrobial resistance: a detailed account of a successful public engagement event. FEMS (Fed Eur Microbiol Soc) Microbiol Lett 2018;365(16).
[5] Davis E, Sloan T, Aurelius K, Barbour A, Bodey E, Clark B, et al. Antibiotic discovery throughout the Small World Initiative: a molecular strategy to identify biosynthetic gene clusters involved in antagonistic activity. MicrobiologyOpen 2017;6(3):e00435. n/a.
[6] Redfern J, Burdass D, Verran J. Transforming a school learning exercise into a public engagement event: "The Good, the Bad and the Algae". J Biol Educ 2013;47(4):246–52.
[7] Blackwell D. New harvard exhibit shows the beauty of the microscopic universe around us: SciTechDaily. 2018. Available from: https://scitechdaily.com/new-harvard-exhibit-shows-the-beauty-of-microscopic-universe-around-us/.
[8] Coenye T, Kjellerup B, Stoodley P, Bjarnsholt T. The future of biofilm research – report on the '2019 Biofilm Bash'. Biofilms 2020;2:100012.
[9] Redfern J, Bowater L, Coulthwaite L, Verran J. Raising awareness of antimicrobial resistance among the general public in the UK: the role of public engagement activities. JAC-Antimicrobial Resistance 2020;2(1).
[10] Yannier N, Hudson SE, Koedinger KR, Hirsh-Pasek K, Golinkoff RM, Munakata Y, et al. Active learning: "Hands-on" meets "minds-on". Science 2021;374(6563):26–30.
[11] Sipos Y, Battisti B, Grimm K. Achieving transformative sustainability learning: engaging head, hands and heart. Int J Sustain High Educ 2008;9(1):68–86.
[12] Verran J, Jackson S, Scimone A, Kelly P, Redfern J. Biofilm control strategies: engaging with the public. Antibiotics 2020;9(8):465.
[13] James C. MICROBIhOME. University of Salford; 2019. Available from: http://hub.salford.ac.uk/microbihome/2019/08/06/organic-collaboration-building-from-dream-to-virtual-reality/.
[14] Vantage Market Research. Global kombucha market size & share to surpass $11.2 billion by 2030 | vantage market research: yahoo! Finance. 2023 [Available from: https://finance.yahoo.com/news/global-kombucha-market-size-share-073600467.html?guccounter=1.
[15] Harvard Online. Food fermentation: the science of cooking with microbes harvard online. Harvard Online; n.d. [Available from: https://www.harvardonline.harvard.edu/course/food-fermentation-science-cooking-microbes.
[16] Murphy TE, Walia K, Farber JM. Safety aspects and guidance for consumerson the safe preparation, handling and storage of kombucha — a fermented tea beverage. Food Protect Trends 2018;38:329–37.
[17] Wood J, Redfern J, Verran J. Developing textile sustainability education in the curriculum: pedagogical approaches to material innovation in fashion. International Journal of Fashion Design, Technology and Education 2022:1–11.
[18] Wood J, Verran J, Redfern J. Bacterial cellulose grown from kombucha: assessment of textile performance properties using fashion apparel tests. Textil Res J 2023. 0(0):00405175231152668.
[19] Ivanitskaya L, Clark D, Montgomery G, Primeau R. Interdisciplinary learning: process and outcomes. Innovat High Educ 2002;27(2):95–111.
[20] Schijf JE, van der Werf GPC, Jansen EPWA. Measuring interdisciplinary understanding in higher education. Eur J High Educ.1-19.
[21] Verran J, Crossley M, Carolan K, Jacobs N, Monsters Amos M. Microbiology and mathematics: the epidemiology of a zombie apocalypse. J Biol Educ 2014;48(2):98–104.
[22] Verran J, Redfern J, Moravej H, Adebola Y. Refreshing the public appetite for 'good bacteria': menus made by microbes. J Biol Educ 2018:1–13.
[23] de Miranda JF, Ruiz LF, Silva CB, Uekane TM, Silva KA, Gonzalez AGM, et al. Kombucha: a review of substrates, regulations, composition, and biological properties. J Food Sci 2022;87(2):503–27.
[24] Verran J, Redfern J, Cunliffe A, Romachney A, Wood J. Hands on Biofilm! Utilizing a public audience in a citizen science project to assess yield variability when culturing kombucha pellicle. FEMS (Fed Eur Microbiol Soc) Microbiol Lett 2023:370.
[25] Schramm M, Hestrin S. Factors affecting production of cellulose at the air/liquid interface of a culture of acetobacter xylinum. Microbiology 1954;11(1):123–9.
[26] Kennedy EB, Jensen EA, Verbeke M. Preaching to the scientifically converted: evaluating inclusivity in science festival audiences. Int J Sci Educ, Part B 2018;8(1):14–21.
[27] Burgess HK, DeBey LB, Froehlich HE, Schmidt N, Theobald EJ, Ettinger AK, et al. The science of citizen science: exploring barriers to use as a primary research tool. Biol Conserv 2017;208:113–20.

Figure 1. Poster board used to advise the public involved in the citizen science project on the methodological choices available to them.

incubation duration, mode of inoculum, depth of culture medium, and surface area variability (Wood et al. 2022b). In addition, repeated subculture could affect the pellicle microbiome and therefore the properties of the resultant pellicle (Wood et al. 2022b). Thus, there is a need to identify conditions that best create the most reproducible/reliable yield to enable more in-depth research on the potential of the end textile in a variety of uses.

Previous studies, such as those conducted by Fernandes et al. (2019), García and Prieto (2019), Lee (2023), and Chan et al. (2018), have alluded to the concept of 'growing your own clothes at home', exploring the potential of using bacterial cellulose pellicles in applications such as jackets and shoes. These studies have used a Kombucha SCOBY in green or black tea as a starter to produce larger pellicle sheets to create garment shapes. They have also explored techniques common in the fashion industry such as stitching and shaping, alongside the addition of trims such as zippers, buttons, and ribs. The concept has captured the interest of some mainstream media outlets (Shaw 2012), with 'recipes' for growing being circulated publicly on sites such as Pinterest (Ross 2023) and Materiom (Aberg 2023). Given this interest, the development of bacterial cellulose textiles provides an ideal topic to design and deliver citizen science.

Citizen science is a term that is used in relation to large-scale public participation in research projects (Dickinson et al. 2012). It is a term that is distinct from 'amateur science' in that, whilst the participant does not need specific scientific knowledge (Land-Zandstra et al. 2021), the project is set up by professional researchers with a clear methodology and specific aims in place, often with a view to creating a publishable set of data and findings. It is not a new concept, with the earliest examples noted as Europe-wide bird surveys in the eighteenth century or the creation of the Astronomical Society of the Pacific in 1889 (Dickinson et al. 2012). Historically, citizen science projects have been part of ecological or environmental studies, but with the increase of the reach of the internet, these types of projects have gained popularity in a diverse spectrum of disciplines. Hands-on activities delivered in accessible locations, such as museums, offer an opportunity to engage the public in citizen science.

The aim of this work was to investigate the variability of kombucha pellicle yield using visitors to a public engagement event during the Manchester Science Festival to select the incubation conditions that give the most reproducible yield and to assess participant engagement in a long-term experiment.

Methods

The overall event was designed to raise awareness of the nature and existence of biofilm (Verran et al. 2020), of the importance of 'good germs' in fermented food and drink (Verran et al. 2018) in the human microbiome, and in the development of sustainable biotextiles (specifically kombucha) (Wood 2019).

Structure and layout of the event

The citizen science component of the event was structured so that visitors were introduced first to biofilms in general and then specifically to kombucha. The team delivering this information was primarily composed of five undergraduate and one postgraduate students, with three academic supervisors (JW, JR, and JV). Two five-litre Kilner jars had been inoculated five weeks previously and stored at room temperature so that the pellicle biofilms were visible for observation. Aliquots of the broth from these jars were extracted for use as liquid inoculum in the experiment. For the solid inoculum, the pellicle was cut into ~0.5 cm^2 portions and stored in a covered petri dish until required. A poster board (Fig. 1) was used to help explain the aim of the experiment, whereby participants could select, with guidance, inoculation and incubation variables for the growth of their own kombucha biofilm.

Citizen science experiment—variables and inoculation

These variables were: inoculum—pellicle or liquid; culture medium—coconut water, beer, sweetened black tea, or the medium most used in the literature, H&S medium (Schramm and Hestrin 1954); and incubation temperature (30°C or 'room temperature', measured at 22°C +/− 2°C). Three hundred 25-mL Universal bottles were prepared in advance of the event, each containing 10 mL of a sterile prepared culture medium. Seventy-five bottles were prepared for each medium.

Once these variables had been selected, the participants noted them on a postcard that had been issued on arrival. Each inoculated vessel was given a reference number (participant reference), as well as information on the selected variables. Participants were also invited to share their email addresses so that they could be contacted at the end of the experiment before results were to be presented (8 weeks later). The project was assessed and approved by the Manchester Metropolitan University Ethics Committee (application number 41586), which included the retention of personal data for purposes of contacting visitors after the event. Risk assessment was carried out with input

Figure 2. The bottles containing sterile media (either tea, beer, coconut, or H&S media) ready for inoculation at the start of the event. Visual aids (tea bags, cans of beer, coconuts) were used to help discuss the options with the participants.

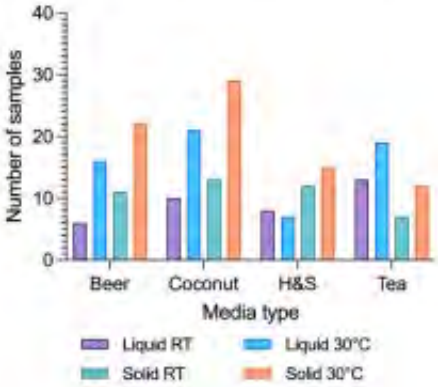

Figure 3. Histogram to show the most common incubation variables selected, including temperature and media composition, by participants in the citizen science project. Solid and liquid refer to the incubation choice.

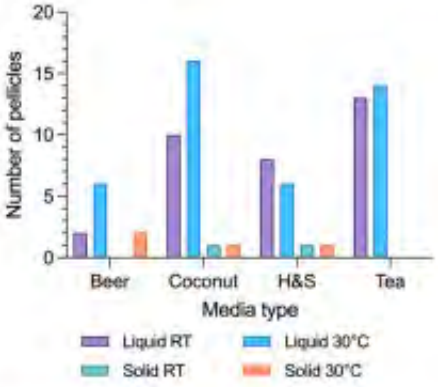

Figure 4. Histogram to show the number of pellicles that resulted from the citizen science experiment, split by original media choice and incubation variables. Solid and liquid refer to the incubation choice. Pellicle production.

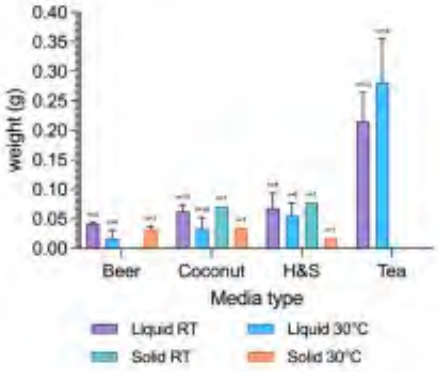

Figure 5. Average pellicle yield (in grammes) that resulted from the citizen science experiment, split by original media choice and incubation variables. Solid and liquid refer to the incubation choice. Pellicle production.

from the host (museum) to assess not only the risk of the activities, but also the practicalities of delivering in an active Museum site. Liability insurance was provided by Manchester Metropolitan University.

Participants either carried out the inoculation process themselves (observed) or watched the delivery team carry out the inoculation process. Lids were screwed on tightly (because of subsequent transport), and at the end of the day were taken to the laboratory for incubation at the designated temperature. After five weeks, it was decided to extend the incubation for an additional three weeks because growth had been relatively slow. Lids were checked to ensure that they were sufficiently loose for aerobic conditions.

Citizen science experiment—incubation and recording results

Thus, after eight weeks, pellicles were harvested. Photographs were taken of all incubation vessels, and the approximate pellicle thickness was measured in mm using a ruler held alongside the

Figure 6. Sample of images presented to participants of the citizen science experiment following upload to a dedicated FlickR page

bottle. Then, pellicles were decanted from the vessels, placed onto filter paper, weighed (g) both wet (immediately after removal) and dry (after 48 hours of drying to a constant weight), and were photographed again. Frequency charts were compiled for each incubation condition (to determine the most popular combinations), and then the conditions that yielded the highest dry quantity of pellicle were identified.

Images and data were posted on FlickR (https://www.flickr.com/photos/196712535@N06/) so that participants could see the results of the study. Emails were sent after the advertised date of data release (to see how many participants remembered the date), and the data were advertised on Twitter one week after that (to see what the general interest for the study might be).

Results and discussion

Overall participation

There were over 1200 visitors to the event overall, primarily in family groups. Subjective observations from the delivery team noted obvious excitement and engagement in the citizen science activity (Fig. 2). Family groups were keen to hear about the experiment and took care thinking about their incubation conditions. There were 221 bottles inoculated during the day from a total of 300 prepared. The most frequently selected medium was coconut (Fig. 3); the most popular inoculum was liquid, and the most popular incubation temperature was 30°C.

Producing kombucha-derived pellicles

In terms of successfully obtaining pellicle (Fig. 4), coconut and tea were most productive (Coconut = 28 and Tea = 27 pellicles grown). Tea was the medium most used to cultivate kombucha (Coelho 2020); coconut water would likely provide a highly nutritious and complex medium that would facilitate growth. Pellicle was not apparent in all inoculated bottles (for example, in the tea medium, the solid inoculum yielded no biofilm). There was also evidence of contamination (cloudy culture medium) in some of the inoculated bottles; usually pellicle was absent in these samples.

When pellicles were dried, those grown in tea at both 30°C and room temperature using a liquid inoculum produced the thickest and heaviest pellicles (Fig. 5). A liquid inoculum would enable cell dispersal (in comparison to a pellicle fragment inoculum) and potentially enhance growth. Interestingly, these data aligned well with the observed 'best'/most reliable incubation conditions as observed previously in our laboratories [tea broth, inoculated with liquid and incubated at 30°C/room temperature (Wood et al. 2023)] and might be useful for people who wish to grow kombucha at home, most often using a tea medium as per kombucha suppliers' recommendations (HappyKombucha.co.uk). As a drink (and therefore brewed for a limited amount of time, ~4 days), Kombucha is commercially available in a variety of liquid media, such as lemonade and passionfruit (e.g. HollandandBarrett.com), agreeing with our findings (and others) that a variety of media can support microbial activity and therefore subsequent pellicle growth. Kombucha beer is also commercially available (gainbrewery.co.uk). For commercially available beverages, the brewing process takes days rather than weeks; thus, a pellicle is not observed in these products. In the domestic setting, no evidence could be found of the use of any culture medium other than tea.

Reproducibility of pellicle production

In terms of reproducibility of yield and control of experimental variables, all of the culture media are complex (undefined)—but the process of making and inoculating the (most commonly used and most reliable) tea medium can at least be defined.

The information gained from this study helps us to speculate on the potential variability of pellicle production in different laboratories using different volumes/surface areas of medium (it would have been useful to inoculate all unused bottles to provide more data, but this was not done).

In brief, with >200 'independent' inoculations, the highest yield was generated using the following conditions: liquid inoculum, 30°C or room temperature incubation, tea medium. Little information is known regarding the effects of vessel volume and liquid surface area on yield.

Citizen science experiment

In terms of a citizen science experiment, it is not easy to retain participant interest in the experiment when the incubation period is so long with no intermediate contact with participants. In a separate study at a school science club, the experiment extended across a half-term, thereby interfering with holidays and examination periods. It was not possible to carry out the pellicle harvesting with the students (vacation), although they were able to inoculate and examine the dried pellicles as well as produce posters on their work (Wood et al. 2022a). Thus, the FlickR site was deemed to be an opportune means of continuing engagement with participants in this study. There was considerable work involved in observing, photographing, and weighing the pellicles, as well as posting images and information on the website (Fig. 6). However, when results were released on FlickR, there were very few hits on the site (27). An email reminder (Supplementary Fig. S1) to 114 participants who provided contact information increased visits to the site significantly, with 4647 views (109 viewers) and with some images receiving more than 70 'hits' over the six-week window of study. Later, a Twitter post was made to flag the event overall, and during a subsequent three-week window, views rose to 5340 (122 individuals).

The interim contact clearly re-invigorated participant interest. The lengthy culture period required for biofilm generation does not sit easily with the provision of feedback to participants and evaluation of their engagement—unlike the culture of bacteria, where data and images can be obtained in a few days, enabling rapid feedback (Verran et al. 2020). The use of FlickR provided a useful record of results and a means of re-engaging audiences, a valuable approach to disseminating experimental results in a citizen science biofilm project.

Conclusion

Data on kombucha pellicle variability caused by varying experimental conditions were obtained, assisting our research in this area. Public engagement with the project was excellent at the point of interaction, but additional strategies are important to sustain interest at a distance over the long incubation period required.

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Supplementary data

Supplementary data are available at *FEMSLE Journal* online.

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References

Abaci N, Sencil Deniz FS, Orhan IE. Kombucha—an ancient fermented beverage with desired bioactivities: a narrowed review. *Food Chem X* 2022;**14**:100302.

Aberg J. *Materiom: Kombucha Material Textured and Dyed*, Josefin Å berg. 2023.

Chan CK, Shin J, Jiang SXK. Development of tailor-shaped bacterial cellulose textile cultivation techniques for zero-waste design. *Cloth Text Res J* 2018;**36**:33–44.

Coelho RMD, Kombucha: review. *Int J Gastron Food Sci* 2020;**22**:100272–2020.

Dickinson JL, Bonney R, Louv R et al. *Citizen Science: Public Participation in Environmental Research*. Comstock Publishing Associates: Ithaca, 2012.

Domskiene J, Sederavičiūtė F, Simonaityte J. Kombucha bacterial cellulose for sustainable fashion. *Int J Cloth Sci Technol* 2019;**31**:644–52.

Ellen MacArthur Foundation. *A New Textiles Economy: Redesigning Fashion's Future*. 2017.

Fernandes M, Gama M, Dourado F et al. Development of novel bacterial cellulose composites for the textile and shoe industry. *Microb Biotechnol* 2019;**12**:650–61.

García C, Prieto MA. Bacterial cellulose as a potential bioleather substitute for the footwear industry. *Microb Biotechnol* 2019;**12**:582–5.

Jayabalan R, Malbaša RV, Lončar ES et al. A review on Kombucha tea-microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Compr Rev Food Sci Food Saf* 2014;**13**:538–50.

Land-Zandstra A, Agnello G, Gültekin YS. Participants in citizen science. In: Vohland K (ed.), *The Science of Citizen Science*. Switzerland: Springer, 2021.

Lee S. Suzanne Lee: *Grow Your Own Clothes | TED Talk*. 2023.

Malbaša RV, Lončar ES, Vitas JS et al. Influence of starter cultures on the antioxidant activity of kombucha beverage. *Food Chem* 2011;**127**:1727–31.

Provin AP, Dutra A, Gouveia I et al. Circular economy for fashion industry: use of waste from the food industry for the production of biotextiles. *Technol Forecast Soc Change* 2021;**169**:120858.

Ross C. *Fabric Made from Tea!* 2023.

Schramm M, Hestrin S. Factors affecting production of cellulose at the air/liquid interface of a culture of acetobacter xylinum. *Microbiology* 1954;**11**:123–9.

Shaw D. *BBC Technology*. London: BBC. 2012.

Verran J, Jackson S, Scimone A et al. Biofilm control strategies: engaging with the public. *Antibiotics* 2020;**9**:465.

Verran J, Redfern J, Moravej H et al. Refreshing the public appetite for 'good bacteria': menus made by microbes. *J Biol Educ* 2018;**53**:1–13.

Wood J. Bioinspiration in fashion—a review. *Biomimetics* 2019;**4**:16.

Wood J, Redfern J, Verran J. Developing textile sustainability education in the curriculum: pedagogical approaches to material innovation in fashion. *Int J Fash Des Technol Educ* 2022a;**16**:1–11.

Wood J, van der Gast C, Rivett D et al. Reproducibility of bacterial cellulose nanofibers over sub-cultured generations for the development of novel textiles. *Front Bioeng Biotechnol* 2022b;**10**:876822.

Wood J, Verran J, Redfern J. Bacterial cellulose grown from kombucha: assessment of textile performance properties using fashion apparel tests. *Text Res J* 2023;**0** 3094–108. 00405175231152668.

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Developing textile sustainability education in the curriculum: pedagogical approaches to material innovation in fashion

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ABSTRACT

The textile industry needs to adopt environmentally sustainable approaches to address ecologically damaging practices. Whilst driven by initiatives such as Textiles 2030, it is current students who will carry this agenda forward. This project investigated pedagogical approaches to develop sustainable textiles for the fashion design curriculum. Pilot studies, using bacterial cellulose (BC) as a material for millinery, revealed members of the public were prepared to experiment with this novel material, and BC was compatible with traditional hat-making techniques. A further study challenged secondary school students, based on an experiential learning model, to grow their own BC biofilm, exploring this as a sustainable apparel fabric. Initial attitudes of reluctance developed into acceptance once engaged in the practical activity. This study illustrates that with appropriate communication and education strategies, the principles of sustainability in fashion, and the acceptability of novel materials, can be engendered in different audiences.

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1. Introduction

Initiatives such as the UN Sustainable Development Goals and Textiles 2030 are bringing the environmental impact of the practices of the apparel, fashion and textile industries into focus (UN, 2022; WRAP, 2021). Whilst many of these initiatives target legislation at a governmental or industrial law level, it is commonly acknowledged that real change in the industry will be implemented by the professionals of the future – current students (Abner & Baytar, 2019; Hiller Connell & and Kozar, 2012).

1.1. Sustainable fashion

The definition of 'sustainable fashion' is an elusive one, and open to interpretation (Henninger, Alevizou, & Oates, 2016; Mukendi, Davies, Glozer, & McDonagh, 2020). However, it is accepted that in fashion terms, an item that could be perceived as 'sustainable' may be described in terms such as 'environmental, social, slow-fashion, reuse, recycling, cruelty-free or anti consumption / production practices' (Mukendi et al., 2020).

1.2. Models for sustainability

Researchers have previously put forward two models for the conceptualisation of sustainable fashion, namely pragmatic and radical change (Burrell & Morgan, 1992). Pragmatic change involves the use of established methods (such as marketing and retail) to push the sustainable agenda, as exemplified by companies such as Patagonia (Patagonia, 2022). Radical change encourages action at a more fundamental level, addressing the accepted practices of the industry (Mukendi et al., 2020). Whilst both approaches are key in the implementation of sustainable practice in the fashion industry, this study focussed on the premise of radical change, challenging the concept of textile creation for apparel.

1.3. Textile materials

Most current fashion and apparel products are dependent on established textiles (e.g. synthetics such as polyester, nylon and elastane, or naturals such as wool, cotton and silk) and there is a fundamental need to understand the properties of the materials used to effectively design and develop products, which is already addressed to some degree in the secondary school and

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undergraduate higher education (HE) curriculum (Haq & Ite, 2022; Hiller Connell & and Kozar, 2012; Landgren & Pasricha, 2011). However, in recent years there have been several research studies focussing on developing textiles from alternative sources such as mycelium, food waste and bacteria (Wood, 2019) in an attempt to address the sustainability agenda. Most of these 'fabrics' are not yet in bulk production, nor are they freely available, thus fashion designers and product developers are not able to use such materials in their work. Nevertheless, the need to understand the properties of these materials is critical if they are to become part of the product developers' and designers' process in the creation of mainstream fashion.

1.4. Bacterial cellulose as an alternative textile

Bacterial cellulose (BC) is one such 'alternative' fabric which has already been explored by some apparel developers (Fernandes, Gama, Dourado, & Souto, 2019; Garcia & Prieto, 2019). It is developed as a biofilm on the surface of a growth medium (e.g. black tea and sugar) that has been inoculated with a bacterium. In the laboratory, BC is grown using lab-prepared media (which have been used previously in BC research), such as Yamanaka or Hestrin & Schramm (H&S) (Schramm, Gromet, & Hestrin, 1957; Yamanaka & Sugiyama, 2000). Other practitioners have adopted a more artisanal approach, using media such as pineapple juice, beer or black tea and sugar as growth support media to develop a BC biofilm for evaluation (Ha et al., 2008; Jarrell, Cal, & Bennett, 2000; Kumbhar, Rajwade, & Paknikar, 2015; Kurosumi, Sasaki, Yamashita, & Nakamura, 2009). *Komagataeibacter xylinus* is one of the most vigorous bacteria in its production of BC biofilm and is commonly used as an inoculant for the media mentioned above. It is found in Kombucha (used in the fermentation of tea drinks) (Chakravorty et al., 2016; Jarrell et al., 2000). Thus, the means for making and studying BC is freely accessible to the public for biofilm experimentation, enabling exploration of concepts of sustainability, usability and acceptability.

1.5. Kolb's experiential learning model

It is widely accepted that there is a variety of learning styles and models (Reid, 2005). Coffield (2005) and Snider (1992) suggests that these are highly dependent on context and that neither students nor topics can be categorised; the models should only be used to develop frameworks and guidance and be adapted as each situation requires. The studies presented in this paper were developed in line with Kolb's Experiential Learning Model (Kolb, 2022) which is based on learning through experience, reflection and further action. Whilst the model is more commonly applied to adult learners, in the context of this paper, the effect of this learning style is also discussed for secondary school students.

Therefore, the aim of this study was to evaluate the application of the experiential learning model with a variety of learners about the use of novel materials within the fashion industry.

2. Methods

Two events were conducted to gather data on the experiential learning model. The first event was a free-to-attend public engagement event, open to the public. The second event was a series of lunchtime clubs held at a local secondary school (children aged 11–12 years). The purpose of the two different events was to expose a variety of potential consumers to BC and evaluate the differences/similarities in their responses. The remainder of this paper provides the details of each event.

2.1. Public engagement event

A public engagement event, 'Hats off to vLeather!'was held as part of Manchester Science Festival (www.scienceandindustrymuseum.org.uk/manchester-science-festival), at the Hat Works Museum of Hatting (www.stockport.gov.uk/topic/hat-works) in Stockport in 2017.

2.1.1. Pre-session preparation

Tea culture medium was prepared by steeping ten tea bags (Yorkshire Black Tea, Bettys & Taylors Group) in 10 l of boiling water for 15 min. The bags were removed, and 1000 g sucrose added to the tea, stirring to dissolve. Once cooled the tea culture medium was placed into plastic containers (L 120 cm × W 60 cm × D 30 cm). The inoculum for the tea was taken from a purchased Kombucha pellicle (www.Happykombucha.com) which had been stored for 30 days at room temperature in a quantity of tea and sugar (as per the manufacturer's instructions). 1000 ml of this liquid inoculum was added to the plastic container and the mixture stirred. The container was loosely covered with lightweight cotton sheeting fabric and stored at room temperature for 40 days to allow a BC biofilm to develop. After this time, a pellicle (i.e. biofilm fabric) had formed on the top of the tea culture medium, which was removed, rinsed with water and flat dried at room temperature for one week.

2.1.2. Attendees

The attendees comprised 20 members of the public (ten per session, two sessions in total) who signed up for the event via the Manchester Science Festival website, on a voluntary basis. All health and safety requirements were implemented by the Hat Works Museum staff.

2.1.3. Session outline

The two individual three-hour practical workshops were held on consecutive Saturday afternoons. Using pre-prepared sheets of BC (as described above), a milliner demonstrated some basic millinery techniques (blocking, steaming, and stitching) to the group. The group was then invited to look at some BC samples and choose the samples most appealing to them to create items of head wear using the demonstrated techniques. Observational notes were taken by the author on the participants activities, reactions to the BC fabric and the millinery pieces created by the participants. The observations were manually analysed to identify qualitative common themes raised by the activities, reactions, and verbal comments. Due to the small number of participants, no statistical evaluation was undertaken.

2.2. Lunchtime science club – Oldham Hulme Grammar School, Manchester

The school runs a science club on alternate Thursday lunchtimes, open to year seven (11- & 12-year-old) students. A 'Grow You Own Fabric' project was delivered across six weeks of the club.

2.2.1. Pre-session preparation

Tea culture medium was prepared by steeping 5 tea bags (Yorkshire brand black tea) in 5 l of boiling water for 15 min. The bag was removed, and 500 g sucrose added to the culture medium, stirring to dissolve. In order to provide a comparison for the tea medium, a second culture medium was prepared in the laboratory by adding 2% glucose, 0.5% bactopeptone, 0.5% yeast extract to 5 l distilled water. This culture medium has been used previously in BC studies and is referred to as Hestrin & Schramm (H&S) medium. The H&S culture medium was prepared by adding 2% glucose, 0.5% bactopeptone, 0.5% yeast extract to 5 l distilled water.

Each of the tea and H&S culture media were placed in separate 1 l duran bottles (i.e five per culture medium) and autoclaved for 10 min at 115°C. Once removed from the autoclave, the media were left to cool to room temperature before use.

500 ml volume 'kilner' style preserving jars (www.kilnerjar.co.uk) were sterilised using sterilising fluid (www.milton-tm.com), prepared according to manufacturers instructions. The jars were submerged in the sterilising solution for 15 min and removed directly before use. Each jar was then filled with either 250 ml sterile tea or 250 ml sterile H&S culture medium (thus creating 20 jars of each from 5 l of prepared culture medium).

The inoculum for each of the tea or H&S culture media was taken from a Kombucha pellicle culture which had been stored for 30 days in a quantity of tea and sugar mixture (as advised by the pellicle manufacturer). A total of 40 × 25 ml sterile 'universal' tubes (25 ml total possible volume) were filled with 10 ml of the inoculum and stored at room temperature.

2.2.2. Participants

The participants were 15 year seven students (a mix of genders) who regularly attended a lunchtime science club on a voluntary basis. The student group had been provided with basic details of the activities to ascertain their interest in the project and asked to commit to attending all 3 sessions across a 6-week period. Ethical procedures covering this event were put into place by the school; the author (JW) was always chaperoned by a DBS checked member of staff.

2.2.3. Session outline

Each session was 20 min long (to fit in with lunch time scheduling).

Each session comprised a brief explanation of the practical activity and discussion with the students about the activity and the themes being addressed (Table 1). The students were asked to note their thoughts/reactions and the sheets were collected at the end of each session for thematic textual analysis. The students were asked not to put their names on the comments sheets to preserve anonymity. Any additional observations from the session were from the lead author. Analysis of the written text and author observations was qualitatively manually coded to identify themes. Where appropriate, the findings were visually presented to illustrate the relevant points.

2.2.4. Session 1

The school tutor and lead author gave a brief overview of the project and discussed the reasons why 'alternative' or 'bio based' textiles, created using microbiology techniques, were being explored.

The class were asked to consider their own clothing and their thoughts on where it came from, what it was made of and what they thought could happen to the clothing at the end of their use for it.

Each student was then provided with two pre-prepared kilner jars, one containing tea culture medium

Table 1. Outline of proposed themes in each session.

Session number	Theme	Question	Mode of answer
1	Initial thoughts on textile impact	Where do our clothes come from? What happens to our clothes when we have finished with them?	In class discussion/ free text
	Expectations and learning outcomes	What do you think you will learn from this project?	In class discussion/ free text
	Initial thoughts on bacteria – based fabrics	What do you think of making clothing/ textiles from bacteria?	In class discussion/ free text
2	Observations of biofilm development	What can you see happening in the jars? Can you measure the thickness of the biofilm? Can you describe the biofilm? What are your feelings about this?	Photography/ free text
3	Observations of biofilm development	What can you see happening in the jars? Can you measure the thickness of the biofilm? Can you describe the biofilm? What are your feelings about this?	Photography/ free text
	Perceived learning outcomes	What do you think you have learned during this project?	Free text

and one containing H&S culture medium, alongside two universal sample pots containing the inoculant mixture. Each student inoculated each kilner jar by pouring in the contents of one of the universal sample pots, closing (but not sealing) the kilner jar after inoculation.

Each jar was labelled with the student's name and placed on a shelf in the biology classroom at room temperature (approx. 22°C).

The students were then asked to note in free text what they thought they would learn from this and the future classes.

2.2.5. Session 2

Two weeks after the first session, each student was asked to examine their jars (opening the lids if required), record their observations of any changes since session one and take photographs with their phones or tablets. Students wrote down their comments and the sheets were collected at the end of the class.

2.2.6. Session 3

Two weeks after the second session, the students attended another lunchtime session to discuss their observations of the changes in the jars, recording their comments using free text and photography.

This session coincided with the end of the school term, so the jars were collected by the author and kept off-site at room temperature (approx. 22°C).

After a further 2 weeks, the jars were photographed by the author and any resultant biofilms (pellicles) were removed. The biofilms were placed on a plastic sheet and allowed to dry on a desktop at room temperature for one week. The images and the resultant dried biofilms were returned to the school tutor to be given to the students on their return to school after 2 weeks. The teacher recorded comments from the students on the dried biofilms and these were verbally communicated to the lead author. The students were asked to reflect on the project and were invited to produce a poster documenting their learning. Three students participated in this exercise.

3. Results

3.1. Public engagement event

The author gave a brief verbal overview to the audience of the problems the textile industry was facing and therefore the need to look at alternative textiles that have less environmental impact. The groups agreed that there was a need to look at alternatives, but during the discussion, the author observed that all participants acknowledged they had little understanding of what this would mean in terms of changing manufacturing practice in the textile industry. The author described the impact of textile effluent on the natural environment and explained how BC sheets were grown, thus eliminating liquid pollution.

However, the use of bacteria in fabric production was not well received, with comments such as '*That's disgusting*' and '*the thought of it … … it makes my skin itchy*'. Whilst some members of the group acknowledge that the BC offered a novel method to fabric production, none of the group expressed an interest in wearing clothing made from such methods.

The milliner then gave a practical demonstration of steaming and blocking using a traditional wool fabric and BC fabric. The participants were invited to take samples of BC to create items of millinery. The reactions to physically handling the fabric were ones of surprise '*it feels a bit like thin leather*' and '*it's more flexible I expected*'. None of the participants expressed any reservations about using the fabric and all were enthusiastic about expressing their creativity through the material. All participants created a

Figure 1. Examples of head wear created from bacterial cellulose.

headwear piece to take home with them (Figure 1). At the end of each event, whilst the participants still felt it was unusual to consider fabrics made from bacteria, all agreed that they had more open minds to consider alternatives to traditional textiles.

3.2. School lunchtime club

3.2.1. Initial reactions

The first lunchtime club involved a brief discussion with the students about their initial comments and feelings regarding the issues of sustainability in the clothing and textiles industry.

3.2.2. Q1: where do our clothes come from/what are they made of?

This question was first discussed generally in the class. Whilst some students did not feel confident to verbally answer the question, some gave very literal answers ('Oldham'/'China') but when asked, they explained that they thought these were the main locations of factories manufacturing clothing. All students were asked to write their comments in answer to the question, with several giving more than one answer. Figure 2 shows an overview of the students' written thoughts regarding the origin of their clothing (where relevant, multiple answers are included in the figure).

There did appear to be a broad knowledge of fibre types in the student group and the broad spectrum of fibres and sources mentioned can be seen in Figure 2. The most common answer was 'polyester'. This is not surprising, polyester is the most common synthetic fibre used in apparel (Objective, 2022). However, this answer could also be attributed to the students looking at the labels in their clothing (they were all wearing polyester school uniform blazers). Wool and cotton featured highly in answer to this question; these are also some of the most commonly used fibres in apparel, jointly accounting for approximately 25% of the global clothing market (Objective, 2022).

3.2.3. Q2: what happens to our clothes when we have finished with them?

This question elicited far fewer responses. Students generally agreed on 5 destinations for used clothing (Figure 3). Some students provided more than one answer to this question – all answers are documented in Figure 3. The majority felt that some of their clothing was thrown away ('trash') and not reused, but also acknowledged that clothing could be donated to either a family member or peer (donated) or passed on to a charity organisation (charity). Interestingly, only around a quarter of the students mentioned upcycling. When this was further discussed with the class, the students were clear in their thoughts that once the clothing had reached the end of its useful life in its current format,

Figure 2. Students' perceptions of fibre type / origins of clothing.

its next destination was trash. There was no understanding of recycling of the garment or the textile in this context. When asked about the response of 'recycling', the students could not define difference in this term from 'charity' and 'donated'. It is therefore assumed that the responses of 'charity', 'donated' and 'recycled' have similar meanings in the student's realm of understanding.

3.2.4. Q3: what do you think you will learn from this project?

This question aimed to explore any preconceptions the students had to using 'alternative' textile sources. They were clear in their expectations with almost all answering in the theme of 'how fabrics are made' or 'how to make eco-friendly fabric' (only one student said they thought BC as an alternative fabric was unworkable). This was encouraging as it showed the students had an interest in learning more about new textile sources.

3.2.5. Q4: what do you think of making clothing/ textiles from bacteria?

Whilst students had expressed an interesting in find out more about alternative fabric production in Q3, Q4 illustrated their reservations regarding using bacteria to create clothing fabrics. Initial responses included

Figure 3. Students' comments on 'end of life' clothing destinations.

concerns around the smell and texture of the fabric, with others showing repulsion regarding the use of microbes in textile generation. This opened a wider discussion with the school's biology teacher who linked the presence of microbes and the term biofilms to topics the students had already covered in the year 7 curriculum (plaque on teeth, food spoilage). This led the students to reconsider their initial thoughts (*'I think we will learn that bacteria can be useful sometimes apart from health'*) and whilst some still had reservations regarding the fabric samples they had seen (*'don't use bacteria, it's uncomfortable, hard and stiff'* and *'it's kind of gross'*), most of these opinions were countered with more positive comments (*' … .the use of bacteria is innovative and clever'; Making clothing out of bacteria is a brilliant idea; making clothes out of biofilm would be a very good for the environment*). The students could make a clear and positive link to the benefits of this type of textile in terms of its impact on the environment, with most students mentioning terms such as *'eco-friendly'*, *'pollution* (reduction)' and *'environment'* in their comments.

3.2.6. First impressions of biofilm development

The second session was designed for the students to monitor the progress of the biofilm development in the jars. In the jars containing the inoculated tea culture medium, all students could see the development of a transparent film on the surface of the culture medium, measuring between 1 and 5 mm in thickness (Figure 4). The students commented on the jelly-like consistency of the biofilm, and many mentioned the vinegar-like smell of the culture medium when the pots were opened.

Many of the jars containing the H&S culture medium had not developed a biofilm and there were instances of contamination with the development of obvious fungal growth in these jars (Figure 5). In these cases, the students were advised not to open the jars (to prevent the release of spores). This again engaged the students in conversation with their biology teacher regarding the development of fungal spores into mould, linking to previously covered year seven curriculum topics.

All the jars were replaced on the classroom shelf and stored in ambient conditions until the next session.

3.2.7. Further biofilm growth

Session 3 was the last formal session with the students. In this session the students made observations on the development of the biofilms. Most of the H&S culture medium jars had developed fungal growth contamination, and these were disposed of appropriately (Figure 5). However, three of the H&S jars had developed biofilms and the students noticed vigorous development

Figure 4. BC biofilm development in tea culture medium. Session 2: A thin, transparent biofilm has developed on the surface of the culture medium. Session 3: Thicker, more opaque biofilms developed with multiple layers noted in some jars.

of these; the biofilms were opaque, white in colour and approximately 10 mm thick (Table 2).

Most tea culture medium biofilms had developed further, with students noting bubbles in the liquid underneath the biofilm, and biofilms measuring approximately 10 mm in thickness (Figure 4). It was also noted that some jars contained multiple thinner biofilms, rather than one thicker sheet (Figure 4).

Before the biofilms were removed from the jars for drying, the students were asked to record in free text what they felt they had learned from the project. All

Figure 5. BC biofilm development in H&S culture medium.

the students agreed the fabric could be considered environmentally friendly (*'the biofilm is eco-friendly and can be broken down', 'I think it is smart that we have come up with a way to make an eco-friendly material for clothes'*). They all commented that they had realised the need for changes in the textiles industry and several mentioned that they were now more aware of the polluting effect of textiles and clothing (*'.... pollution is becoming more in the world'*). However, there were several concerns raised about the useability of the biofilm they had created, once they received the dried biofilms (*'its crumpled and smelly', 'it's weird'*) with some students suggesting this could be a barrier to public adoption of this type of material (*'I think this would cause a lot of controversy with members of the public because they would be disgusted... ... . If we could alter the textures of the fabric the bacteria will make people would be more convinced to buy them'*).

The three posters submitted detailed the students understanding and illustrated their engagement with the biofilm development process. Figure 6 is an example of one of the posters; the student adopted a 'comic strip' approach to detail the process.

4. Discussion

Both the public engagement event and the school science club events showed that there was some awareness of the polluting nature of the textile industry and the impact that fabrics currently used in clothing could have on the environment. Nevertheless, the school students' comments highlighted the need for wider education around recycling of clothing beyond its initial use; there was little appreciation of reusing the textile or componentry of garments for different end uses beyond clothing.

Furthermore, there was little awareness around alternative approaches; none of the participants (across all events) had heard of using bacteria or biofilms to create fabrics and there was reluctance across all the groups to consider this approach when put forward as an idea.

Table 2. BC biofilm pellicles removed from jars (each pellicle 6–7 cm diameter).

	Wet	Dry
Tea culture medium		
H&S culture medium		

Figure 6. Poster produced by student detailing the project and learning journey

This receptiveness to new ideas sits with Kolb's learning cycle which suggests that the learner is more likely to engage with the ideas put forward if this can be grounded in concrete experience, rather than presented as a series of facts or potential solutions to a problem (Kolb, 2022). Discussion showed reluctance to accept BC as an alternative fabric, but the physical activities (experiential learning) gained the trust of the participant, opened pathways to enquiry and ultimately enabled more engagement in consideration of BC as a potential future textile. A further illustration of this is the behaviours observed in the public engagement event: the participants were very happy to use the BC 'fabrics' to create headwear, despite voicing reservations at the beginning of the session when the concept was first presented. In a similar way, the school students used terms such as '*eewwww*' and '*disgusting*' when they first discussed BC as an alternative, and after the first 2 weeks of growing, with students commenting on the vinegar-like smell of the jars. The author explained that this was entirely normal in this type of biofilm development and had been noted many times in other research (Jarrell et al., 2000; Chakravorty et al., 2016; Dufresne, 2012; Mohite and Patil, 2014; Jayabalan et al., 2014). Once engaged with the physical act of growing their own fabric, they started to use words such as '*cool*' and '*innovative*' in their descriptions. There were some reservations voiced at the end of the process, once the dried biofilms were reviewed, but all students could see the benefit of further exploration of this type of material and even came up with some suggestions of what they thought was needed to encourage mainstream adoption (... ... ... *If we could alter the textures of the fabric the bacteria will make people would be more convinced to buy them*'). Despite Kolb's learning theory traditionally being applied to adult learners (Kolb & Kolb, 2022; Reid, 2005), our study illustrates it is also applicable to secondary school students.

Whilst this study illustrated the benefits of the experiential learning model for the use of bacterial cellulose, the activity itself presented some difficulties. The length of time taken to grow the biofilm was approximately five weeks; this could be prohibitive in some educational settings. Additionally, contamination of some of the jars was observed which could also cause issues with cross contamination and subsequent safe disposal. This could be mitigated by the pre-preparation of materials (as per the public engagement event), or by providing the materials and instructions for participants to grow the material at home (and how to dispose of any contaminated jars), documenting the growth cycle in a similar way to the school science club, and bringing the dried material into class for further exploration and discussion. This could also present the possibility of linking across subjects such as design and technical performance to enhance learning opportunities.

Brandenburg and Wilson (2013) suggest that some students hold the belief that learning is something that is done to them; it is a passive activity that is the responsibility of the teacher and not the student. In our study, the actions of all groups suggest that physical activities in which the participants were encouraged to explore the BC, both from a growing and an application viewpoint, elicited better engagement and promoted deeper discussion than the initial didactic presentation of the concept of biofilm fabrics. Brandenburg and Wilson (2013) also suggest that listening to the student voice and adapting to their needs is critical; the findings of this study support this idea; initial rejection of BC turned to acceptance once the approach to learning changed. This is a concept that Brown, Roediger, and McDaniel, and A (2014) support, stating that a mixed approach deepens learning and allows adaptation of knowledge to different scenarios, which was observed in our study.

5. Conclusions

This study observed the perceptions of samples of the public and year seven students on the use of alternative textiles (BC) in the fashion industry using a pedagogical model based on Kolb's experiential learning theory. Whilst the participants across all groups were initially reluctant to consider the use of BC due to its mechanism of manufacture (grown from bacteria), the attendees of the millinery workshop were happy to use the material once they had handled the samples, whilst the year seven students engaged in the process of 'growing your own fabric' during the lunchtime science club, acknowledging the need for alternative textiles.

The study illustrated the difficulties of conveying the concepts of alternative bacteria-based fabrics using didactic methods; for both audiences, the material was rejected as a possibility when described in this way.

Lessons were learned for those planning future events – for example, the H&S medium appeared more susceptible to contamination in this study and could be omitted. In addition, the length of time required to grow the biofilm could exclude this activity from some events, although prefabricated samples could be provided, or, the participants could take the incubation mix home for growth and subsequently feedback observations to the research team (authors). This could also be built into the curriculum and timetabled as part of a structured syllabus.

There is a need to promote radical change in textiles and apparel to address the effects of the industry on climate change. Our study illustrates that alternative textiles such as BC can be used as engaging and illustrative examples by students and the public if they are presented within an experiential learning model.

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References

Abner, M., & Baytar, F. (2019). Apps to increase student engagement: A case of textiles and apparel sustainability education. *International Journal of Fashion Design, Technology and Education, 12*(1), 56–64.

Brandenburg, R., & Wilson, J. Z. (2013). *Pedagogies for the future: Leading quality learning and teaching in higher education*. Rotterdam: SensePublishers.

Brown, P. C., Roediger, H. L., and McDaniel, I. I. I., & A. M. (2014). *Make it stick: The science of successful learning*. Cambridge, MA: The Belknap Press of Harvard University Press.

Burrell, G., & Morgan, G. (1992). *Sociological paradigms and organisational analysis: Elements of the sociology of corporate life*. Aldershot: Ashgate.

Chakravorty, S., Bhattacharya, S., Bhattacharya, D., Chatzinotas, A., Chakraborty, W., & Gachhui, R. (2016). Kombucha tea fermentation: Microbial and biochemical dynamics. *International Journal of Food Microbiology, 220*, 63–72.

Coffield, F. (2005). Kinesthetic nonsense. *Times Educational Supplement*.

Common Objective (2022). What are our clothes made from?: @joinourco. [Online]. Retrieved 18 May, from http://www.commonobjective.co/article/what-are-our-clothes-made-from

Dufresne, A. (2012). *Nanocellulose: From nature to high performance tailored materials*. Walter de Gruyter GmbH.

Fernandes, M., Gama, M., Dourado, F., & Souto, A. P. (2019). Development of novel bacterial cellulose composites for the textile and shoe industry. *Microbial Biotechnology*, *12*(4), 650–661.

García, C., & Prieto, M. A. (2019). Bacterial cellulose as a potential bioleather substitute for the footwear industry. *Microbial Biotechnology*, *12*(4), 582–585.

Ha, J. H., Shehzad, O., Khan, S., Lee, S. Y., Park, J. W., Khan, T., & Park, J. K. (2008). Production of bacterial cellulose by a static cultivation using the waste from beer culture broth. *Korean Journal of Chemical Engineering*, *25*(4), 812–815.

Haq, U. N., & Ite, T. A. (2022). 'Exploratory study of textile undergraduates' knowledge and perception towards eco-friendly clothing in Bangladesh.'. *Tekstilec (Spletna izd)*, *65*(1), 44–57.

Henninger, C. E., Alevizou, P. J., & Oates, C. J. (2016). What is sustainable fashion? *Journal of Fashion Marketing and Management*, *20*(4), 400–416.

Hiller Connell, K. Y., & and Kozar, J. M. (2012). Sustainability knowledge and behaviors of apparel and textile undergraduates. *International Journal of Sustainability in Higher Education*, *13*(4), 394–407.

Jarrell, J., Cal, T., & Bennett, J. W. (2000). The Kombucha consortia of yeasts and bacteria. *Department of Cell and Molecular Biology*, *14*(4), 166. /170.

Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Sathishkumar, M. (2014). A review on kombucha tea – Microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Comprehensive Reviews in Food Science and Food Safety*, *13*(4), 538–550. http://dx.doi.org/10.1111/1541-4337.12073.

Kolb, D. (2022). Home|experience based learning systems, LLC. [Online]. https://learningfromexperience.com/index.html

Kolb, D., & Kolb, A. (2022). Home|experience based learning systems, LLC. [Online]. https://learningfromexperience.com/index.html

Kumbhar, J. V., Rajwade, J. M., & Paknikar, K. M. (2015). Fruit peels support higher yield and superior quality bacterial cellulose production. *Applied Microbiology and Biotechnology*, *99*(16), 6677–6691.

Kurosumi, A., Sasaki, C., Yamashita, Y., & Nakamura, Y. (2009). Utilization of various fruit juices as carbon source for production of bacterial cellulose by Acetobacter xylinum NBRC 13693. *Carbohydrate Polymers*, *76*(2), 333–335.

Landgren, T. M., & Pasricha, A. (2011). Transforming the fashion and apparel curriculum to incorporate sustainability. *International Journal of Fashion Design, Technology and Education*, *4*(3), 187–196.

Mohite, P. V., & Patil, S. V. (2014). A novel biomaterial: Bacterial cellulose and its new era applications. *Biotechnology and Applied Biochemistry*, *61*(2), 101–110.

Mukendi, A., Davies, I., Glozer, S., & McDonagh, P. (2020). Sustainable fashion: Current and future research directions. *European Journal of Marketing*, *54*(11), 2873–2909.

Patagonia. (2022). The cleanest line – Patagonia. [Online]. https://eu.patagonia.com/nl/en/stories/

Reid, G. (2005). *Learning styles and inclusion*. London: Paul Chapman Pub. SAGE Publications.

Schramm, M., Gromet, Z., & Hestrin, S. (1957). Synthesis of cellulose by Acetobacter Xylinum. 3. Substrates and inhibitors. *The Biochemical Journal*, *67*(4), 669–679.

Snider, V. E. (1992). Learning styles and learning to read: A critique. *Remedial and Special Education*, *13*, 6–18.

UN. (2022). *Sustainable development goals; Goal 12: Ensure sustainable consumption and production patterns*. [Online]. Retrieved 19 April, from https://www.un.org/sustainabledevelopment/sustainable-consumption-production/

Wood, J. (2019). Bioinspiration in fashion – A review. *Biomimetics*, *4*(1), 16.1–16.8.

WRAP. (2021). *Textiles 2030*. Banbury.

Yamanaka, S., & Sugiyama, J. (2000). Structural modification of bacterial cellulose. *Cellulose*, *7*(3), 213–225.

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Bacterial cellulose grown from kombucha: Assessment of textile performance properties using fashion apparel tests

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Abstract

Bacterial cellulose (BC) has been suggested as a sustainable alternative textile for apparel. Previous studies have evaluated the production of BC sheets and the suitability of these to form garment shapes. The laboratory measured physical performance characteristics of BC from an apparel perspective remain relatively unexplored.

The aim of this study was to produce reproducible sheets of BC, enabling the evaluation of the performance of the BC in an apparel textile testing context, and comparison to other textile materials. Grown in sterile black tea with glucose, the BC presented as a mesh of non-woven nanofibers, and thus comparison was made with three non-woven fabrics. It has also been suggested that BC could be used as 'vegetable' leather; therefore, performance comparisons were conducted with animal skins.

Utilizing British, European and International standard test methods, the selected fabrics were evaluated for their performance in tensile, elongation, moisture vapor permeability and abrasion tests, relevant for an apparel end-use.

Tensile strength testing revealed that BC is weaker than its animal counterparts but does display similar physical characteristics at the point of failure; however, it displayed a higher tensile strength than the non-woven fabrics chosen for comparison.

BC was the least breathable and most moisture-retentive of all the fabrics tested, raising questions regarding its suitability and comfort for apparel applications in its untreated state.

However, BC displayed superior performance when tested for resistance to abrasion, suggesting it could be best utilized in the form of encapsulated patches in items subjected to this type of damage.

Keywords

Bacterial cellulose, textiles, performance, fashion, apparel, sustainability

By 2030 global apparel consumption is estimated to be 102 million tonnes, a 63% increase from figures collected in 2019.[1] This consumption puts a huge strain on the environment and natural resources, with United Nations' estimates suggesting the equivalent of approximately three planets worth of resources being required by 2050 to sustain the growth in demand.[2] The impact of apparel on the environment is wide reaching; every stage of the manufacturing process is resource-hungry and contributes to environmental pollution from chemicals used in fiber production, processing techniques in fabric creation and manufacturing practices in apparel construction. In addition, environmental pollution is continued during the lifetime of the garment via pollutants due to washing and wear (for example, as detergents and microfibers), with estimates of over 300,000 tonnes of clothing being discarded every year, and approximately 20% of this being sent to landfill.[3]

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There are concerns around both natural and synthetic fibers. Synthetic fibers are currently largely made from virgin plastics and whilst they use less water than natural fibers in their production, they are mostly non-biodegradable and therefore make a higher contribution to greenhouse gas emission than their natural counterparts. However, natural fibers such as cotton and wool require larger amounts of water and synthetic chemicals in their processing to enable them to withstand the demands of the modern consumer. In addition to addressing the issues regarding manufacturing 'traditional' fabric types, the textile industry is seeking alternative sources of raw material to address environmental concerns.

Bacterial cellulose (BC) is one such alternative material. Commonly described in the apparel industry as 'vegetable leather,' it is reported to have numerous 'enhanced' properties in comparison to its plant counterpart, such as high tensile strength, high water retention, extreme insolubility to solvents, the ability to be molded into shape and high degrees of polymerization and high crystallinity. In addition, its production yields cellulose in a pure form, without the presence of impurities and waxes such as lignin, pectin and hemicellulose,[4] which require heavy processing to remove from naturally produced textiles. However, the study of BC as an alternative textile in the fashion apparel industry has been limited, with relatively few studies exploring the application of BC in this field.[5–7]

'Bacterial (or microbial/nano) cellulose is a form of cellulose that is produced by bacteria.'[8] It forms as a product of the microbial metabolic process and can be thought of as a generated protective layer for the bacterial cell, due to the reported protective properties from ultra-violet light and the retention of moisture. BC is generated as micro- (or nano-) fibrils via pores on the surface of the microbial cell, generally at the interface of liquid and air,[9] forming as a mat, or a pellicle. This structure can be considered a biofilm.[8]

BC is produced most vigorously by the bacterium *Komagataeibacter xylinus* (also known as *Gluconacetobacter xylinus* and *Acetobacter xylinus*).[8,10,11] In laboratory conditions, techniques such as the aseptic inoculation of sterile media (e.g., Hestrin and Schram or Yamanaka) are used to develop BC sheets. However, *K. xylinus* is also found in kombucha, known colloquially as Kargasok tea, tea fungus, Haipao and Manchurian mushroom,[12] which is a fermented drink that has been consumed for several thousands of years. This has led to an artisanal approach to the development of BC material with researchers growing sheets in large containers of unsterile tea and sugar, inoculated with kombucha starter cultures, with varied results.

A kombucha pellicle can be considered a consortium of yeasts and bacteria.[10,13] The composition of the pellicle is determined by its geographic and climatic conditions of cultivation,[13] but it is suggested that a kombucha mat consists of a 'core' consortium of bacteria and yeasts[13] that always possesses cellulose-forming properties.[13,14] There have been many studies to evaluate the microbial composition of kombucha cultures and assess the optimum conditions for tea brewing.[10,15–19] Some studies have attempted to replicate the original culture in media other than tea. These have included complex media such as pineapple, watermelon and orange juice,[19–21] coconut water[22] and beer.[23]

Efforts to create a defined medium, essential for reproducibility, are ongoing, but such media have required the addition of complex yeast extract to stimulate pellicle growth.[24,25] Schramm and Hestrin[26] developed the 'standard' (but undefined) complex growth medium (commonly known as H&S) consisting of glucose, bactopeptone and yeast extract.[23,25,27,28] This medium is commonly used in experiments that remove the developed pellicle/biofilm from the surface of the liquid medium, rinsing using distilled water and NaOH to lyse any residual bacterial cells. The biofilm is then dried using various methods (most commonly ambient air-drying) until a constant weight is achieved, from which the overall yield of BC (in terms of weight rather than purity) is calculated.[23,25,27–29]

Studies examining the performance of BC as a textile in apparel have focused on the production of BC sheets,[30,31] the ability of the sheet to be formed into garment shapes[32] or the response of the sheet to textile coloration and finishing techniques from an aesthetic viewpoint.[33,34] Whilst previous studies have postulated the use of BC as an apparel textile, with some suggesting it could be seen as a replacement to animal leather,[7,35] to the authors' knowledge, no studies to date have specifically tested the sheets as an apparel textile to establish their performance characteristics and suitability for fashion garment end-use. If the BC sheet is to be considered as an animal leather alternative in the apparel industry, tests commonly used to assess the performance of leather during garment wear, including tensile strength and abrasion, should be carried out. In addition, wearer comfort in apparel is associated (in part) with the moisture transmission characteristics of a textile, and thus should be evaluated for any textile proposed for apparel purposes.[36–38]

Such work is critical if BC is to be used as a viable alternative textile in fashion apparel. This study aimed to identify the best practice for simple but reproducible small batch fabrication of a BC biofilm so that more reliable data could be generated relating to the textile performance properties of the resultant material, thus allowing evaluation of BC performance against

'comparable' textiles (such as animal leathers, non-wovens and cellulose-based structures) for use in the fashion garment arena.

Materials and methods

Fabric samples

Bacterial cellulose sheets. A commercially available 200 g Kombucha pellicle in 100 ml of green tea was purchased from 'Happy Kombucha' (https://happykombucha.co.uk/) and stored on a bench top at room temperature in its original green tea broth (as provided) until required for experiments. To produce a standardized inoculum, 100 g of the starter culture was placed in a 500 ml sterile pot containing 250 ml sterile H&S medium. A lid was loosely placed on the pot, which was stored in an incubator (30°C) for 15 days. After 15 days, solids (=pellicles) were removed from the pot by filtration, with the remaining liquid broth used as inoculum.

To determine the most suitable medium to support BC sheet generation, a variety of growth media were used to grow kombucha pellicles (Table 1).

In this study, coconut water was selected for its 'natural' origin.[39] Beer already contains yeast extract and previous studies have suggested that enhanced yeast extract content helps support pellicle development.[41] The exact content of yeast in the supermarket-purchased beer was unknown. Black tea broth was prepared using recipes collected during the literature review as guidance (3 g leaf tea steeped in 1 l boiling water for 15 min).[10,41]

To determine whether sterile conditions were necessary to produce the BC pellicles, each medium was split into two parts: one half was autoclaved and after cooling to ambient temperature, and 10 ml aliquots of media were aseptically dispensed into 30 sterile 25 ml containers. The other half was not autoclaved, and 10 ml aliquots of media were dispensed directly into 30 sterile 25 ml containers.

Each container was then inoculated with 1 ml of original kombucha pellicle tea broth. The lid was placed loosely on top, and the container incubated at 30°C with conditions monitored using a USB datalogger (model: Easylog USB, manufacturer: Lascar Electronics, Wiltshire, UK). Visual observations of pellicle development and sampling took place 27, 34 and 42 days after inoculation (10 pots per sample date).

Pellicles on the surface of the liquid media were removed with sterile forceps and placed on dry filter paper to remove any excess surface moisture. These pellicles were then removed from the paper using forceps and individually placed in petri dishes and weighed twice – (i) in the wet state and (ii) after drying for 24 hours in a fan oven at 60°C (which equaled constant weight, as determined in preliminary work; results not included in this paper).

The weight of BC produced per volume of liquid (% w/v) was calculated.[25,26] Mean and standard deviations of the weights recorded were calculated with outliers removed using the interquartile range statistical method.[42]

The presence of a cellulosic molecular structure in the pellicle samples was confirmed using Fourier transform infrared spectroscopy (FTIR) (model: FTIR UATR Spectrum 2, manufacturer: Perkin Elmer). To determine any differences in the morphology, samples of each of the pellicles were observed under a scanning electron microscope (SEM) (model: Supra 40VP, manufacturer: Carl Zeiss Ltd). The samples were prepared for viewing by storing in a desiccator[42] before being mounted on a metal stub using a sticky carbon disc and sputter coated in gold to 10 nm thickness.[43,44]

Comparison fabrics for assessment. Fabrics were chosen for comparison to the BC according to their physical morphologies (determined by visual assessment, and by

Table 1. Media used for pellicle/biofilm growth

Type of medium	Preparation	Comments
Coconut water	Pre-packaged coconut water[39] was purchased from a local supermarket. The liquid was centrifuged to extract any residual solids.	After preparation/purchase of the media, each liquid was decanted into separate sterile 500 ml Duran bottles and sterilized in an autoclave for 10 min @ 115°C (the lower temperature was used to reduce risk of glucose caramelization).
Bitter	Own brand bitter (2.8%) – Morrisons supermarket[40]	
Black Tea	Tea broth was prepared by steeping 1 tea bag (Yorkshire brand black tea) in 1 l boiling water for 15 min (approx. 3 g tea/l). The bag was removed and 100 g glucose added (10%).[10,41]	
H&S	2% Glucose, 0.5% bactopeptone, 0.5% yeast extract added to 1 l distilled, deionized water.[24]	

reference to BC SEM images), mass per unit areas, thickness and composition.

The purpose of the study was to evaluate BC as an alternative textile for fashion apparel, and therefore common textile performance tests relevant to this field were chosen: tensile strength and elongation, abrasion and moisture permeability. Tests such as thermal insulation/conductivity and air permeability were not considered in this work as these are usually characteristics associated with performance clothing.

Mass per unit area

Three 10 cm × 10 cm square specimens were cut with fabric shears at randomized points from each sample fabric. These samples were weighed individually on a precision laboratory balance (Sartorius, Germany) and the mean of these readings was calculated. The arithmetic mean was multiplied by 100 to determine the mass per unit area of the fabric in g/m^2.

Thickness

Using a thickness gauge (Mercer, UK; model 110), readings were taken at five randomized points for each sample fabric. The arithmetic mean of these readings was calculated to determine the thickness of each fabric sample in mm.

Tensile strength and elongation (BS EN ISO 13934)

In accordance with the specified method, three specimens were taken at randomized points from each sample fabric. The samples were mounted on the tensile tester (Instron, UK; model 33R4465), with a jaw separation width of 100 mm. The machine was set to an extension rate of 100 mm/min and all specimens were extended to breaking point with maximum force at break (N) and breaking elongation (mm) recorded.

Martindale abrasion (BS EN ISO 12947-1)

In accordance with the specified method, three specimens were taken at randomized points from each sample fabric. The samples were mounted on the Martindale Abrasion tester (James Heal, UK: model NU 864) in accordance with test standard BS EN ISO 13934 and the machine set to rub at 5000 revolution intervals against an abrasive cloth (James Heal, UK: original SM25), with a 9 kPa weight per specimen. The specimens were assessed after each 5000 rubs and removed at the point of destruction with the number of rubs recorded.

The test was repeated using a 12 kPa weight for the animal skin and BC specimens only and specimens were assessed after each 5000 rubs and removed at the point of destruction with the number of rubs recorded.

Taber Abraser (BS EN ISO 17076-1:2020)

In accordance with the specified method, three specimens were taken at randomized points from the animal leather, animal suede and BC sample fabrics. The samples were mounted on a Taber Abraser (Taber Industries, USA; model 5135), using Calibrase CS-10 (Taber Industries) abrasive wheels, 1000 g weights and a textile specimen clamp with double-sided tape to ensure no movement of the sample on the mount during the test. The appearance and weight each sample was assessed and noted before the test and at 1000 revolution intervals until the sample broke down or a total of 10,000 revolutions was reached.

Breathability: water vapor transmission (BS 7209:1990)

In accordance with the specified method, three 90 mm diameter circular specimens were taken at randomized points from each sample fabric and individually weighed. Each sample was mounted on an individual pot containing 40 ml distilled water and a gauze cover affixed covering the fabric sample and the pot. Each pot was weighed, mounted on the rotating disc for 24 hours, removed and reweighed, in accordance with test standard BS 7209:1990. The fabric samples were removed from the pots and reweighed immediately.

The water vapor transfer (WVP) of each fabric was calculated by comparing the mass of the pot at the start and end of the test and using the following calculation

$$\mathrm{WVP} = 24M/At$$

where M is the mass lost (g), t is the time (hours), A is the area (m^2), $A = (\pi d^2/4) * 10^{-6}$ and d is the diameter of the dish (mm).

Water vapor absorbed by the fabric and still present at the end of the test was calculated as a percentage mass by weight as follows

((Mass (g) of sample at end of test–Mass (g) of sample at start of test)
/Mass (g) of sample at start of test) * 100

As an additional measure in this test (not specified in the standard test method), each fabric sample was weighed before and after the test was completed, and these weights compared to establish the moisture retention of the material.

Results and discussion

Bacterial cellulose sheet development

Across all the media studied, samples prepared under sterile conditions gave higher yields of BC sheet than their non-sterile counterparts (Figures 1(a)–(c)). It is likely that contaminants might affect the yield, so it is always preferable to maintain an aseptic technique and sterile conditions when appropriate.

Sterile tea always gave the highest yields of BC at each time point and a general trend of increase in yield across time was observed. This contrasts with the other media, which displayed almost constant yields/a slight decrease in yields between 27 and 42 days. However, it should also be noted that the standard deviation of yield measurements was also largest in the sterile tea broth.

H&S broth samples gave the lowest yield of all media across all timepoints observed, and the difference in yield from sterile and non-sterile H&S broths was less than for the other samples measured. It was also noted that H&S broths gave measurements with the lowest standard deviation. It could therefore be considered that whilst the H&S BC yield is low, it is also the most consistent in terms of yield. However, as the tea broth gave the greatest yield of BC sheets, this broth was selected to grow further material for evaluation.

To develop larger sheets of BC in tea, sterile tea liquid was prepared as detailed in the methods section above. Some 300 ml of tea liquid was dispensed into plastic containers (170 (l) × 50 (d) × 110 (w) mm) and inoculated with 30 ml of original Kombucha pellicle tea broth. Lids were loosely placed on the containers and the BC sheets allowed to develop for 40 days. The sheets were removed, rinsed with water and left to dry for 1 week in ambient conditions on a laboratory bench.

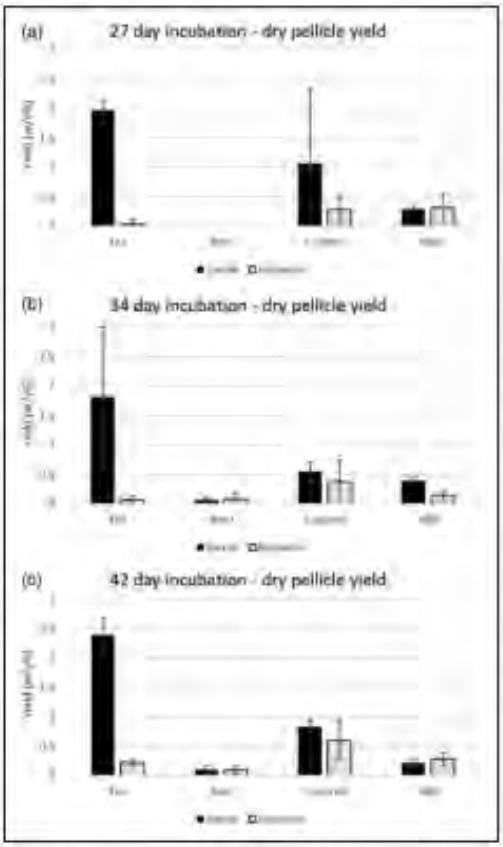

Figure 1. Dry pellicle yield after (a) 27 days, (b) 34 days and (c) 42 days. H&S: Hestrin and Schramm medium.

Physical morphologies and selection of comparison fabrics

FTIR traces performed on the pellicle samples displayed peaks in the regions shown in Table 2. Neera et al.,[43] Dima et al.[45] and Halib et al.[46] all concur that these peaks are indicative of the presence of a cellulosic structure.

There was very little difference in the spectral data in terms of the occurrence of peaks across the growth media permutations, indicating the same molecular structure across the samples.[19]

SEM images (Figure 2) revealed BC sheets to be a random mesh of nanofibers, arranged in a similar structure to a traditional non-woven fabric structure (such as those seen in felt or non-woven interlining fabrics), regardless of the growing liquid.[8,17] The nanofibers all measured a similar diameter (approximately 100 nm).

Non-woven fabrics are defined as textile structures that are created from fibers and formed into webs by bonding or interlocking via mechanical, thermal, chemical or solvent processes.[47–49] Therefore, three non-woven fabrics were selected for comparison, a plant-derived cellulose fiber sheet (mechanically manufactured), a woolen felt (mechanically manufactured)

Table 2. Indicative bacterial cellulose Fourier transform infrared spectroscopy wavelength peaks

Wavelength peak (cm^{-1})	
3350	O-H stretching
2800–2900	C-H stretching
1160	C-O-C stretching
1035–1060	C-O stretching
1300	C-H bending
1400	CH_2 bending

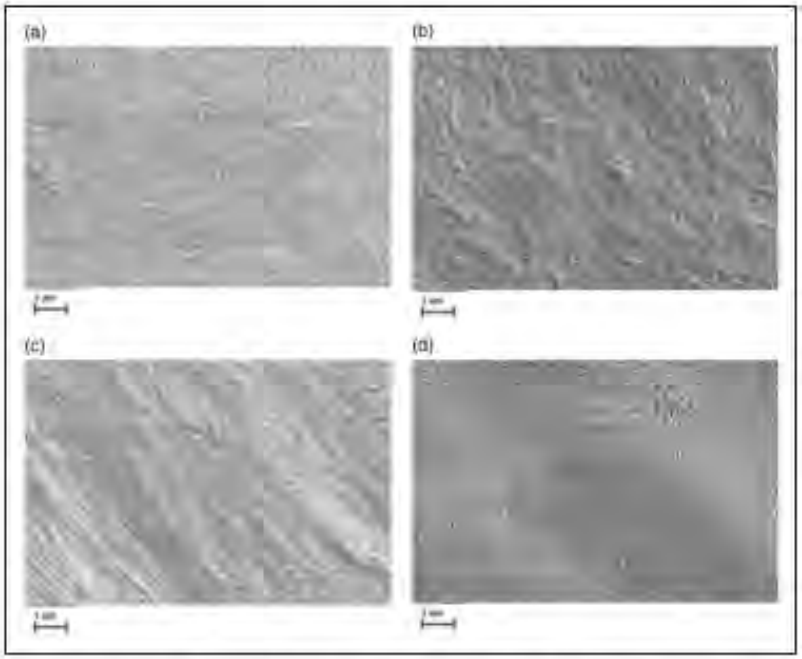

Figure 2. Scanning electron microscope images of bacterial cellulose sheets grown in different liquids (×10 k magnification). H&S: Hestrin and Schramm medium. (a) Tea; (b) beer; (c) coconut and (d) H&S.

Table 3. Fabrics selected for comparison to bacterial cellulose

Sample fabric	Comments
Bacterial cellulose	Vegetable leather sheet.
Animal skin (suede)	Cow hide, napped surface.
Animal skin (leather)	Cow hide, natural grain surface.
Cellulose sheet	Mechanically compressed cellulose fibers.
PA interlining	Mechanically and chemically manufactured sheet
Wool felt	Mechanically engineered wool fibers.

PA: polyamide.

and a polyamide (PA) sheet (thermally and chemically manufactured) (Table 3).

BC is often referred to as vegetable leather, with some studies suggesting it is a viable alternative to animal hide. Therefore, two types of cow hide were selected for comparison: natural grain (leather) and napped surface (suede) (sourced from university fabric stores) (Table 3).

Mass per unit area and thickness

The wool felt, PA, animal suede and cellulose non-woven were of similar weight (169–234 gm^{-2}). BC was slightly heavier (323.67 gm^{-2}), with animal leather displaying the greatest mass per unit area (685.00 g/m^{-2}). However, measurements of the thickness revealed the cellulose non-woven material to be the thickest material (1.88 mm) and animal suede the to be thinnest (0.60 mm). These results can be explained by the physical structure of the materials; the cellulose non-woven is constructed of plant cellulose fibers, mechanically bound together into a loose and open fabric structure (Table 4). The animal suede, leather and BC sheet are much denser in physical structure with fewer visible gaps between their constituent fibers (Table 4). The samples were deemed acceptable for further comparison as they were representative of fabrics used in apparel applications.

It is also worthy of note that the chosen materials had different surface morphologies when examined using the naked eye. The animal leather displayed a relatively smooth surface and the animal suede was napped/raised, whilst the BC had an undulating surface. All the manufactured non-wovens have similar fibrous surface characteristics (Table 3).

Textile testing

To choose the most relevant textile testing methods, it is important to understand textile performance characteristics when designing and manufacturing apparel to

Table 4. Sample mass and thickness

Sample (image ×2 magnification)		Mean mass per unit area (g/m^{-2})	Mean thickness (mm)
Bacterial cellulose		323.67 (SD 2.87)	0.62 (SD 0.14)
Animal skin (suede)		234.64 (SD 0.47)	0.60 (SD 0.02)
Animal skin (leather)		685.00 (SD 0.82)	1.31 (SD 0.06)
Cellulose non-woven		178.67 (SD 2.36)	1.88 (SD 0.14)
Polyamide non-woven		208.33 (SD 0.47)	0.95 (SD 0.03)
Wool felt		169.00 (SD 2.83)	1.09 (SD 0.04)

ensure the garment meets the wearer's expectations in terms of comfort and fitness for purpose. To this end, there are many laboratory-based tests that can establish the physical performance of a textile and therefore its suitability for specific end-uses.[50–53]

Non-woven fabrics are generally used in apparel as a 'support' to give structure and shape definition for the main body fabric used in the garment.[54] There are several textile testing methods that have been modified to cater for the 'unique' structure of non-wovens[49]; however, as this study aimed to establish the suitability of BC as an apparel outer fabric (and not a 'support' fabric), the modified versions of the standard tests were not used.

As BC has been suggested as an animal leather alternative, the most likely apparel end-use is outerwear or apparel that is expected by consumers to have levels of superior durability. Tensile performance and abrasion resistance were therefore chosen as the most relevant indicative tests of these characteristics.

However, as previously mentioned, comfort characteristics cannot be overlooked. It is well documented that moisture management of textiles and clothing is a critical factor in wearer comfort.[37,38,53–59] Moisture vapor transfer and moisture retention capacity were chosen to establish the performance of the sample fabrics.

Tensile strength and elongation. Animal leather, animal suede and BC displayed definite and abrupt points of failure, with 'clean' break points visually observed in the fabric samples (Figure 3(a)). However, the BC withstands lower forces and elongates less before rupture (0.196 kN at 17.85 mm) than its animal leather (0.844 kN at 29.77 mm) and suede (0.515 kN at 59.32 mm) counterparts (Figure 3(b)).

The wool, PA and cellulose non-wovens all displayed low and comparable forces at failure (Figure 3 (b)). Figure 3(a) illustrates the gradual, less abrupt breakdown of the fabric structure in comparison to the abrupt failure observed in the animal skin and BC samples. The wool felt extended most at its failure point and it is suggested this is likely to be due to friction of scales on the wool fiber holding the structure together.[60] By comparison, the cellulose and PA fibers have a smoother surface morphology therefore less mechanical friction between fibers to hold them together, resulting in lower breakdown force and extension.[61]

These results indicate that BC would not be suitable as an animal skin replacement in apparel applications where resistance to stress is critical, but it could still be considered in applications where non-wovens are currently used. Closer fitted garments (such as leather jackets) generally require higher tensile strength performance due to the strain applied to the fabric when the body moves. However, non-woven fabrics can be used in looser fitting garments (such as personal protective equipment (PPE) gowns or outer coats), suggesting the tensile and elongation performance of BC does not completely exclude this from apparel applications.

Moisture performance. Moisture vapor permeability (MVP) measures the rate of transmission of water vapor through a material. In textiles, this is often used to describe the 'breathability' of a fabric; that is, the rate at which the textile allows perspiration moisture to move away from the body, pass through the textile clothing and subsequently evaporate into the atmosphere. The higher the reading from the test, the more transmissible water vapor through the fabric.[62] The breathability of a fabric can be affected by the fiber composition, fabric construction and thickness.[36]

The MVP test suggested the most breathable fabrics were the non-woven plant cellulose, PA and wool structure, with results of 659.65, 685.32 and 690.03 g/m^{-2}.24 h, respectively (Figure 4). These structures have larger gaps between the fibers than their animal skin and BC counterparts, thus allowing moisture vapor to travel more freely through the structure. Conversely, the more densely structured animal skin samples showed less breathability (leather 671.96 and suede 531.28 gm^{-2}.24 h) (Figure 4). BC showed the least degree of breathability (205.39 gm^{-2}.24 h), more than 60% less breathable than animal suede (Figure 4).

Marked differences were also observed when examining residual moisture in the samples. The synthetic PA non-woven retained the least moisture (0.26%) and the 'natural' fabrics (cellulose non-woven, wool felt, animal suede, animal leather) retained between 2.06% and 5.34% (Figure 5). These differences can be explained by previous studies; natural fibers have a greater ability to absorb moisture than synthetics.[36,58,63]

However, BC retained a markedly larger percentage of moisture (14.67%) than the other fabrics tested (Figure 5). Again, this is not surprising, as a characteristic of BC is its enhanced ability to absorb moisture.[8] This also explains the poor moisture vapor transfer result for BC; the material can absorb moisture from the surrounding atmosphere but cannot easily release this. This is an issue for clothing where the textile is in direct contact with the skin; such moisture retention would lead to wearer discomfort.[37,56,57] However, in application such as, for example, wound dressings[64,65] or beauty products (e.g., face masks),[66–68] this degree of water retention could be advantageous and enhance the product performance.

Abrasion durability performance. The Martindale abrasion test is a useful measure of the potential of a fabric to 'wear out' over a lifetime of use. As the test specimens are rubbed over an abrasive cloth, it could be suggested that the thicker specimens should show greater resistance to wear, with thinner specimens potentially failing the test more rapidly. However, various studies have illustrated that it is factors such as fabric/yarn structure, fiber composition[69,70] and molecular structure[71] that are of the most significance when determining resistance to abrasion.

Traditional textile non-woven fabrics are known for their poor abrasion characteristics in comparison to traditional woven and knitted textile structures.[49,72] This is illustrated by the results of the Martindale abrasion test, with the cellulose and PA non-woven fabric

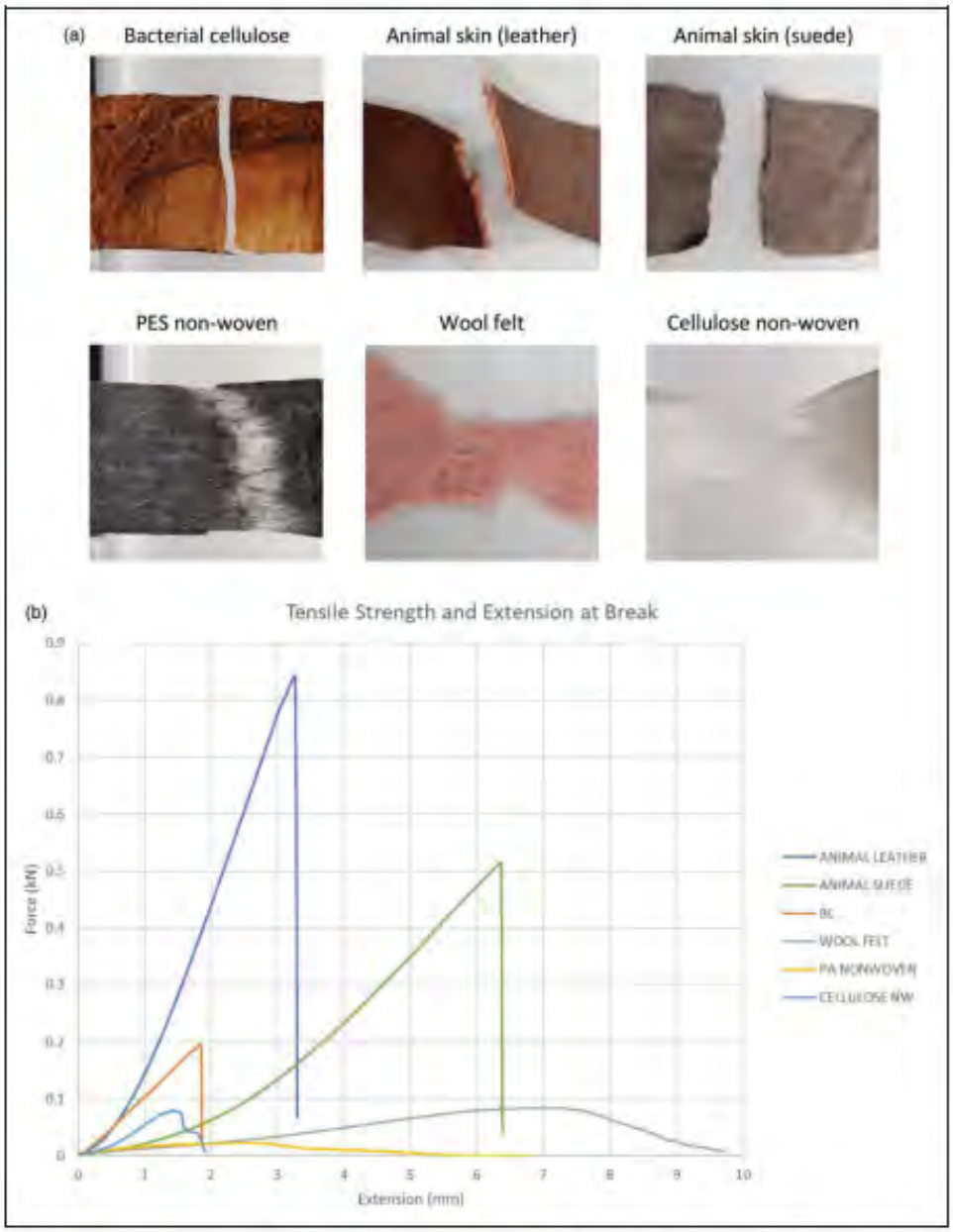

Figure 3. (a) Physical breakdown of samples after the tensile test. (b) Tensile strength and elongation. PES: polyester; BC: bacterial cellulose; PA: polyamide; NW: non-woven.

breaking down at 5000 and 10,000 rubs @ 9 kPa, respectively. Non-woven wool felt performed better, with total breakdown at 15,000 rubs @ 9 kPa (Figure 6). In a similar way to the tensile performance discussed above, this performance could be attributed to the surface morphology of the constituent fibers; scales on the protein wool fiber could cause an interlocking effect and thus enhance the abrasion durability of the fabric in comparison to the smoother surfaces of the cellulose and PA non-woven fabric fibers.

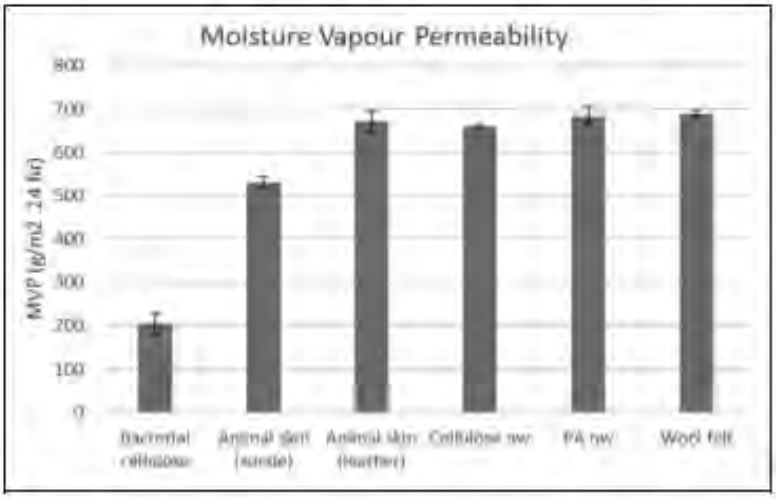

Figure 4. Breathability of the sample fabrics. MVP: moisture vapor permeability; NW: non-woven; PA: polyamide.

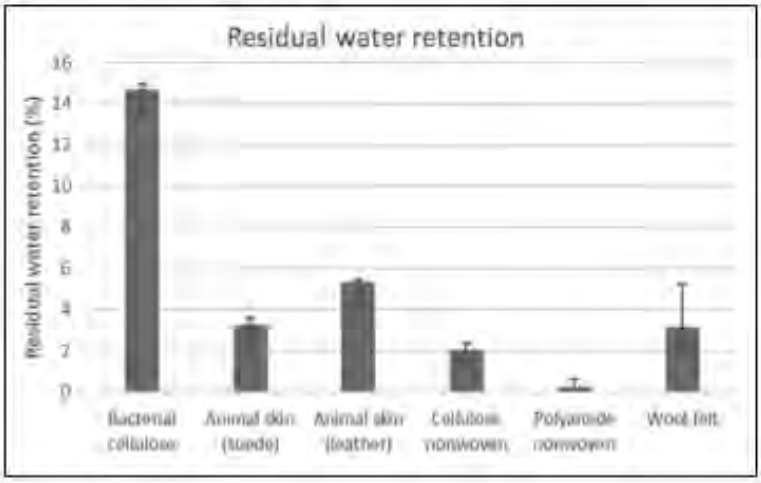

Figure 5. Water retention of the sample fabrics.

The BC, animal suede and leather resisted a much greater number of rubs and were therefore tested using both the 9 and 12 kPa weights (Figure 6). Whilst apparel performance is normally assessed using 9 kPa, superior textile performance (normally attributed to upholstery and other high-performance textile applications) is ascertained using 12 kPa; Martindale abrasion testing for leather is normally conducted using a 12 kPa weight.

BC displayed excellent abrasion resistance, with superior performance to animal skin at 9 kPa. BC showed no signs of breakdown at 150,000 rubs with a 9 kPa weight, whilst animal suede broke down at 90,000 (Figure 6). This level of abrasion is classed as superior performance for everyday apparel applications.[52,72,73]

At 12 kPa BC failed at 105,000 rubs, displaying a similar performance to animal leather (100,000 rubs). Both were superior to animal suede, which failed at 15,000 rubs (Figure 6).

As the BC and both animal leathers displayed superior abrasion characteristics during the Martindale abrasion test, the three materials were subjected to the Taber Abraser test, which is considered a harsher, more destructive test normally reserved for technical textiles where a high degree of abrasion resistance is required.[73]

	BC	Animal skin (leather)	Animal skin (suede)	Cellulose nonwoven	Polyamide nonwoven	Wool felt
Rubs to destruction @ 9kPa	150,000*	90,000	90,000	5,000		15,000
Rubs to destruction @ 12kPa	105,000	92,000	15,000	n/a	n/a	n/a

Figure 6. Martindale abrasion sample appearance at end of the test. *No failure, specimen still intact – test abandoned. BC: bacterial cellulose.

	Before test	1000 revs	2000 revs	3000 revs	4000 revs	6000 revs	8000 revs	10000 revs
Brown leather								
Bacterial Cellulose								
Suede				n/a	n/a	n/a	n/a	n/a

Figure 7. Taber Abraser sample appearance.

Sample	Bacterial Cellulose	Animal Leather	Animal Suede
Sample at 10,000 revs with 1000g weights			

Figure 8. Taber Abraser: sample damage at 10 k revs (suede total breakdown at 2 k).

BC and animal leather were comparable in performance during the Taber Abraser test (Figure 7): animal suede broke down rapidly to failure after 2000 revs (Figure 8). However, BC and animal leather showed a steady and comparable degree of mass loss to 10,000 revs, at which point the test was abandoned as all the surface of the animal leather had been removed (Figure 9). As an indication of the level of performance, European Standard EN388-2016 (protective gloves against mechanical risks) states that at the highest level

Figure 9. Taber Abraser – sample mass loss over time.

of protection a textile should withstand 8000 revs at 1000 g.[74] Upon visual examination, neither of these samples displayed complete physical breakdown, with very little surface damage noted on the BC sample (Figure 8). It could therefore be suggested that the BC is more durable to abrasion than the animal leather sample tested and could be suitable as a high-performance abrasion resistant textile. However, as noted above, the moisture retention properties of BC mean that this material would not be suitable to be worn against the skin, so it is suggested that encapsulation of BC within the structure of the garment would need to be explored. The encapsulant would need to be carefully chosen as to not inhibit the performance of BC.

Conclusion

This study first determined the optimum method for fabrication of reproducible small batch manufacture of BC sheets. To our knowledge, it is the first study to assess BC as a textile for potential fashion apparel applications. Evaluation of BC against standards for textiles used in fashion clothing is important if BC is to ever be considered as a replacement for traditional textile sources in this field.

Whilst laboratory-manufactured liquid growth medium (H&S) gave the most consistent yields, sterile black tea and sugar produced the highest yield of BC sheets. Sterile black tea with glucose was therefore used to grow larger pieces of BC for textile performance evaluation.

SEM examination of the BC sheets revealed a nanofibrillar mat structure, which was directly comparable to the structure of 'traditional' non-woven fabric, thus a selection of non-woven fabrics was used for performance comparison to BC. As many practitioners have suggested BC could be a replacement for animal leather, animal skins were also selected for performance comparison to BC.

There are many test criteria that could be applied when assessing the performance of a textile for an apparel end-use and they are largely dependent on the expectations of the wearer. However, as this study explored the application of BC in fashion apparel, it focused on the aspects of strength and durability in wear (tensile strength and abrasion) and the moisture transfer properties considered important when assessing elements of wearer comfort.

Tensile strength and elongation testing revealed that whilst BC physically behaves as animal skin in terms of abrupt breakdown, the forces and elongation required to elicit this breakdown are considerably lower for BC than its animal skin counterparts. However, BC withstood higher forces at break than the comparable non-woven structures, suggesting that BC could have applications in looser fitting apparel where tensile strength is not a prohibitive factor.

The moisture transfer capabilities of fabrics are critical for the consideration of comfort of apparel. BC was the least breathable of all the samples tested and retained the most moisture at the end of the test. This indicated that BC would not be suitable for use in a garment worn in direct contact with the skin. However, there may be applications for BC in fashion outerwear where moisture absorbance/repellency performance is not a limiting factor in fabric selection (e.g., fashion clothing not designed for wet weather protection).

BC outperformed all other samples in terms of abrasion resistance, showing no signs of breakdown even after points of failure for the animal leathers. This result is of greatest interest from the study, as the BC tested exceeds the most rigorous requirements of European Standards for clothing designed to protect against abrasion.

These findings suggest the BC would not be suitable in close fitting apparel due to its tensile, elongation and vapor transfer/moisture retention characteristics. The application of a specialist finish could go some way to counter this, although this may compromise the sustainable aspect of the BC and require re-evaluation of the performance characteristics to ensure there are no adverse effects. However, the superior abrasion performance of BC could render it useful in clothing for protection; it is suggested from these results that the BC could be used as sealed-in patches in clothing (to prevent moisture absorbance and thus potential negative effects on abrasion performance) rather than the whole garment (for example, in high-impact wear such as motorbike leathers and areas of potential high abrasion (such as knees) in workwear clothing). We would suggest further performance trials of BC in this context. Furthermore, extending the apparel testing regime to include properties such as thermal insulation/conductivity, air permeability and performance to laundering could allow for a wider range of apparel applications to be suggested.

Declaration of conflicting interests

The author(s) declare that there is no conflict of interest regarding the publication of this article.

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References

1. Parliament EAC-U. Fixing fashion: clothing consumption and sustainability – Sixteenth Report of Session 2017–19 In: Committee EA (ed.) London: Environmental Audit Committee, UK Parliament, 2019.
2. UN. Sustainable Development Goals, Goal 12: Ensure sustainable consumption and production patterns, https://www.un.org/sustainabledevelopment/sustainable-consumption-production/ (accessed 19th September 2022).
3. WRAP. *Textiles* 2030. Banbury, 2021, Oxford, UK.
4. Ashjaran A, Yazdanshenas ME, Rashidi A, et al. Overview of bio nanofabric from bacterial cellulose. *J Text Inst* 2013; 104: 121–131.
5. Fernandes M, Gama M, Dourado F, et al. Development of novel bacterial cellulose composites for the textile and shoe industry. *Microb Biotechnol* 2019; 12: 650–661.
6. García C and Prieto MA. Bacterial cellulose as a potential bioleather substitute for the footwear industry. *Microb Biotechnol* 2019; 12: 582–585.
7. Fairs M. Microbes are the "Factories of the Future". Dezeen and mini frontiers exhibition, 2014.
8. Dufresne A. *Nanocellulose: from nature to high performance tailored materials*. Berlin/Boston, MA: Walter de Gruyter GmbH Co.KG, 2012.
9. Brown RM, Willison JHM and Richardson CL. Cellulose biosynthesis in acetobacter xylinum – visualization of site of synthesis and direct measurement of invivo process. *Proc Natl Acad Sci USA* 1976; 73: 4565–4569.
10. Jarrell J, Cal T and Bennett JW. The kombucha consortia of yeasts and bacteria. *Dept Cell Mol Biol* 2000; 14: 166–170.
11. Reva ON, Zaets IE, Ovcharenko LP, et al. Metabarcoding of the kombucha microbial community grown in different microenvironments. *AMB Expr* 2015; 5: 1–8.
12. Kurtzman CP, Robnett CJ and Basehoar-Powers E. Zygosaccharomyces kombuchaensis, a new ascosporogenous yeast from 'Kombucha tea'. *FEMS Yeast Res* 2001; 1: 133–138.
13. Teoh AL, Heard G and Cox J. Yeast ecology of kombucha fermentation. *Int J Food Microbiol* 2004; 95: 119–126.
14. Chakravorty S, Bhattacharya S, Bhattacharya D, et al. Kombucha tea fermentation: microbial and biochemical dynamics. *Int J Food Microbiol* 2016; 220: 63–72.
15. Četojević-Simin DD, Velićanski AS, Cvetković DD, et al. Bioactivity of lemon balm kombucha. *Food Bioproc Technol* 2012; 5: 1756–1765.
16. Gaggia F, Baffoni L, Galiano M, et al. Kombucha beverage from green, black and rooibos teas: a comparative study looking at microbiology, chemistry and antioxidant activity. *Nutrients* 2019; 11: 1.
17. Wood J, van der Gast C, Rivett D, Verran J, et al. Reproducibility of bacterial cellulose nanofibers over sub cultured generations for the development of novel textiles. *Front Bioeng Biotechnol* 2022; 10. DOI: 10.3389/fbioe.2022.876822
18. Marsh AJ, O'Sullivan O, Hill C, et al. Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. *Food microbiol* 2014; 38: 171–178.
19. Castro C, Zuluaga R, Putaux J-L, et al. Structural characterization of bacterial cellulose produced by Gluconacetobacter swingsii sp. from Colombian agroindustrial wastes. *Carbohydr Polym* 2011; 84: 96–102.
20. Jahan F, Kumar V, Rawat G, et al. Production of microbial cellulose by a bacterium isolated from fruit. *Appl Biochem Biotechnol* 2012; 167: 1157–1171.
21. Kurosumi A, Sasaki C, Yamashita Y, et al. Utilization of various fruit juices as carbon source for production of bacterial cellulose by Acetobacter xylinum NBRC 13693. *Carbohydr Polym* 2009; 76: 333–335.
22. Kongruang S. Bacterial cellulose production by acetobacter xylinum strains from agricultural waste products. *Appl Biochem Biotechnol* 2008; 148: 245–256.

23. Shezad O, Khan S, Khan T, et al. Production of bacterial cellulose in static conditions by a simple fed-batch cultivation strategy. *Kor J Chem Eng* 2009; 26: 1689–1692.
24. Schramm M, Gromet Z and Hestrin S. Synthesis of cellulose by Acetobacter xylinum. 3. Substrates and inhibitors. *Biochem J* 1957; 67: 669–679.
25. Yamanaka S and Sugiyama J. Structural modification of bacterial cellulose. *Cellulose* 2000; 7: 213–225.
26. Schramm M and Hestrin S. Factors affecting production of cellulose at the air/liquid interface of a culture of Acetobacter xylinum. *J Gen Microbiol* 1954; 11: 123.
27. Setyawati MI, Chien L-J and Lee C-K. Expressing Vitreoscilla hemoglobin in statically cultured Acetobacter xylinum with reduced O_2 tension maximizes bacterial cellulose pellicle production. *J Biotechnol* 2007; 132: 38.
28. Singh R, Mathur A, Goswami N, et al. Effect of carbon sources on physicochemical properties of bacterial cellulose produced from Gluconacetobacter xylinus MTCC 7795. *E-Polymers* 2016; 16: 331–336.
29. Schramm M and Hestrin S. Synthesis of cellulose by Acetobacter xylinum. I. Micromethod for the determination of celluloses. *Biochem J* 1954; 56: 163.
30. Yim SM, Song JE and Kim HR. Production and characterization of bacterial cellulose fabrics by nitrogen sources of tea and carbon sources of sugar. *Prev Biochem* 2017; 59: 26–36.
31. Ng MCF and Wang W. A study of the receptivity to bacterial cellulosic pellicle for fashion. *Res J Text Apparel* 2015; 19: 65–69.
32. Chan CK, Shin J and Jiang SXK. Development of tailor-shaped bacterial cellulose textile cultivation techniques for zero-waste design. *Clothing Text Res J* 2018; 36: 33–44.
33. Han J, Shim E and Kim HR. Effects of cultivation, washing, and bleaching conditions on bacterial cellulose fabric production. *Text Res J* 2018; 8(6): 1094–1104.
34. Shim E and Kim HR. Coloration of bacterial cellulose using in situ and ex situ methods. *Text Res J* 2019; 89: 1297–1310.
35. Launch. Suzanne Lee: biocouture – launch, https://www.launch.org/innovators/suzanne-lee/ (2019, accessed 20th August 2020).
36. Watkins S. *Clothing the portable environment*. The Iowa State University Press, Ames, Iowa, USA, 1985.
37. Watkins SM and Dunne LE. *Functional clothing design: from sportswear to spacesuits*. New York: Fairchild Books; 2015.
38. Song G. *Improving comfort in clothing*. Oxford; Philadelphia, PA; Woodhead Pub., published in association with the Textile Institute, 2011.
39. Vitacoco. Vita coco, https://www.vitacoco.com/ (2020, 19th October 2020).
40. Morrisons. Morrisons, www.morrisons.com (2020, 10th October 2020).
41. Jayabalan R, Malbaša RV, Lončar ES, et al. A Review on kombucha tea—microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Comprehens Rev Food Sci Food Saf* 2014; 13: 538–550.
42. O'Gorman TW. *Adaptive tests of significance using permutations of residuals with R and SAS*. Hoboken, NJ: Wiley, 2012.
43. Neera RKV and Batta HV. Occurrence of cellulose-producing Gluconacetobacter spp. in fruit samples and kombucha tea, and production of the biopolymer. *Appl Biochem Biotechnol* 2015; 176: 1162–1173.
44. Nguyen VT, Flanagan B, Gidley MJ, et al. Characterization of cellulose production by a Gluconacetobacter xylinus strain from kombucha. *Curr Microbiol* 2008; 57: 449–453.
45. Dima SO, Panaitescu DM, Orban C, et al. Bacterial nanocellulose from side-streams of kombucha beverages production: preparation and physical-chemical properties. *Polymers* 2017; 9: 374.
46. Halib N, Perrone F, Cemazar M, et al. Potential applications of nanocellulose-containing materials in the biomedical field. *Materials (Basel, Switzerland)* 2017; 10: 977.
47. ISO 9092:2011. Textiles - nonwovens - definition.
48. Denton MJ and Daniels PN. *Textile terms and definitions*. 11th ed. Manchester: Textile Institute, 2002.
49. Russell SJ and Textile I. *Handbook of nonwovens*. Boca Raton, FL/Cambridge: CRC Press/Woodhead Pub., 2007.
50. Slater K and Harrison PW, Textile I. *Physical testing and quality control: a critical appreciation of recent developments*. Manchester: Textile Institute, 1993.
51. Booth JE. *Principles of textile testing: an introduction to physical methods of testing textile fibres, yarns and fabrics*. 3rd ed. London: Butterworths, 1983.
52. Hu J. *Fabric testing*. Boca Raton, FL/Cambridge: CRC Press/Woodhead, 2008.
53. Saville BP. Textile I. *Physical testing of textiles*. Cambridge/Boca Raton, FL.: Woodhead Pub., in association with the Textile Institute; in North and South America by CRC Press, 1999.
54. Senthil KP and Punitha V. An overview of nonwoven product development and modelling of their properties. *J Text Sci Eng* 2017; 7. DOI: 10.4172/2165-8064.1000310
55. Li Y and Wong ASW. *Clothing biosensory engineering*. Cambridge: Woodhead Pub., 2006.
56. Fourt L and Hollies NRS. *Clothing: comfort and function*. Ann Arbor, MI: UMI Books on Demand, 2002.
57. Almeida L. *Functional finishes for textiles: improving comfort, performance and protection*. Cambridge: Woodhead Publishing, 2015.
58. Lyle DS. *Performance of textiles*. New York: Wiley, 1977.
59. McLoughlin J and Sabir T. *High-performance apparel: materials, development, and applications*. Oxford: Woodhead Publishing, 2017.
60. Mather RR and Wardman RH. *The chemistry of textile fibres*. Cambridge: Royal Society of Chemistry, 2011.
61. Bunsell AR. Textile I. *Handbook of tensile properties of textile and technical fibres*. Oxford: Woodhead Publishing, 2009.
62. Hu J. Textile I. Fabric testing. Boca Raton, FL/Cambridge: CRC Press/Woodhead; 2008.

63. Horrocks AR and Anand SC. *Handbook of technical textiles. Vol. 2, Technical textile applications*. Duxford: Woodhead Publishing, 2016.
64. Sulaeva I, Henniges U, Rosenau T, et al. Bacterial cellulose as a material for wound treatment: properties and modifications. a review. *Biotechnol Adv* 2015; 33: 1547–1571.
65. Beekmann U, Zahel P, Karl B, et al. Modified bacterial cellulose dressings to treat inflammatory wounds. *Nanomaterials (Basel, Switzerland)* 2020; 10: 2508.
66. Amnuaikit T, Chusuit T, Raknam P, et al. Effects of a cellulose mask synthesized by a bacterium on facial skin characteristics and user satisfaction. *Med Dev (Auckland, NZ)* 2011; 4: 77.
67. Ullah H, Santos HA and Khan T. Applications of bacterial cellulose in food, cosmetics and drug delivery. *Cellulose* 2016; 23: 2291–2314.
68. Bianchet RT, Vieira Cubas AL, Machado MM, et al. Applicability of bacterial cellulose in cosmetics – bibliometric review. *Biotechnol Rep (Amsterdam, Netherlands)* 2020; 27: e00502–e.
69. Miguel R, Lucas J, Carvalho L, et al. Garment abrasion strength evaluation: a comparative methods study. *Int J Clothing Sci Technol* 2007; 19: 194–203.
70. Shaw VP and Mukhopadhyay A. Impact of abrasion on strength, elasticity and elastic recovery properties of stretch-denim fabric. *Int J Clothing Sci Technol* 2022; 34: 241–261.
71. Uzun M, Kanchi Govarthanam K, Rajendran S, et al. Interaction of a non-aqueous solvent system on bamboo, cotton, polyester and their blends: the effect on abrasive wear resistance. *Wear* 2015; 322–323: 10–16.
72. Annis PA. *Understanding and improving the durability of textiles*. Oxford: Woodhead Pub., 2012.
73. Textor T, Derksen L, Bahners T, et al. Abrasion resistance of textiles: Gaining insight into the damaging mechanisms of different test procedures. *J Eng Fibres Fabr* 2019; 14. DOI: 10.1177/1558925019829481
74. BS EN 388:2016. Protective gloves against mechanical risks.

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Reproducibility of Bacterial Cellulose Nanofibers Over Sub-Cultured Generations for the Development of Novel Textiles

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The textile industry is in crisis and under pressure to minimize the environmental impact on its practices. Bacterial cellulose (BC), a naturally occurring form of cellulose, displays properties superior to those of its cotton plant counterpart, such as enhanced purity, crystallinity, tensile strength, and water retention and is thus suitable for an array of textile applications. It is synthesized from a variety of microorganisms but is produced in most abundance by *Komagataeibacter xylinus*. *K. xylinus* is available as a type strain culture and exists in the microbial consortium commonly known as Kombucha. Whilst existing literature studies have described the effectiveness of both *K. xylinus* isolates and Kombucha in the production of BC, this study investigated the change in microbial communities across several generations of sub-culturing and the impact of these communities on BC yield. Using Kombucha and the single isolate strain *K. xylinus* as inocula in Hestrin and Schramm liquid growth media, BC pellicles were propagated. The resulting pellicles and residual liquid media were used to further inoculate fresh liquid media, and this process was repeated over three generations. For each generation, the thickness of the pellicles and their appearance under SEM were recorded. 16S rRNA sequencing was conducted on both pellicles and liquid media samples to assess changes in communities. The results indicated that the genus *Komagataeibacter* was the most abundant species in all samples. Cultures seeded with Kombucha yielded thicker cellulose pellicles than those seeded with *K. xylinus*, but all the pellicles had similar nanofibrillar structures, with a mix of liquid and pellicle inocula producing the best yield of BC after three generations of sub-culturing. Therefore, Kombucha starter cultures produce BC pellicles which are more reproducible across generations than those created from pure isolates of *K. xylinus* and could provide a reproducible sustainable model for generating textile materials.

Keywords: bacterial cellulose, *Komagataeibacter xylinus*, pellicle, textiles, sustainability

INTRODUCTION

The textile industry is a major contributor to greenhouse gas emissions, global water consumption, and contamination (Ellen MacArthur Foundation, 2017). On its current trajectory alongside a heavy reliance on non-renewable resources, the industry will be responsible for 26% of the carbon budget associated with a 2°C global temperature increase by 2050. Under pressure to increase sustainability and minimize the environmental impact on its practices, the textile industry is seeking alternatives to meet global demands (Textiles 2030 project, 2021), with replacements for traditional fiber sources being one area of exploration.

One such traditional fiber cotton accounts for approximately one-third of fibers used worldwide, and the demand is expected to grow due to the versatility of its cellulosic base (Voora et al., 2020). This plant-based cellulosic fiber has a huge environmental impact; it requires a large volume of water to support its growth to maturity, alongside the use of environmentally harmful pesticides and chemicals to achieve high yield and good quality crops (Blackburn, 2009; Stone et al., 2020). Additionally, the cotton fiber structure contains contaminants (for example, non-cellulosic proteins, amino acids, and waxes) which have a negative impact on the end use and need to be removed *via* extensive cleaning processes (Sinclair, 2015).

In contrast to its plant counterpart, bacterial cellulose (BC) is a form of naturally occurring cellulose in a highly pure state (Lin et al., 2013). It possesses properties such as a highly crystalline structure, high tensile strength, and considerable water retention behavior (Dufresne, 2012). As such, this makes it suitable for medical applications (wound dressings, artificial skin, and tissue engineering) (Hoenich, 2006; Bodin et al., 2007; Backdahl et al., 2008; Gatenholm and Klemm, 2010; Huang et al., 2014; Picheth et al., 2017; Torres et al., 2019; Azimi et al., 2021), cosmetics and beauty care (Amnuaikit et al., 2011; Mohite and Patil, 2014), food and packaging (Chawla et al., 2009; Kuswandi et al., 2014; Shi et al., 2014; Ullah et al., 2016; Ashrafi et al., 2017), acoustics (Iguchi et al., 2000), paper (Lee, 2018; Torres et al., 2019), electrical energy storage and sensors (Hu et al., 2011; Jia et al., 2016), filtration, absorbents and adsorbents (Mohite and Patil, 2014; Stoica-Guzun et al., 2016; Zhu et al., 2016), and fabrics for apparel (Fernandes et al., 2019; García and Prieto, 2019; Lee, 2019).

As a biopolymer, BC is reportedly synthesized by a variety of microorganisms (Dufresne, 2012). Studies suggested that it is the *Komagataeibacter* genus (formerly known as *Gluconacetobacter* and *Acetobacter*) that produces BC in the greatest abundance (Yamanaka and Sugiyama, 2000; Jayabalan et al., 2014), while *Komagataeibacter xylinus* is widely accepted as the most productive.

In a liquid medium with a carbon source, *K. xylinus* develops a gelatinous biofilm, or pellicle, on the surface of the liquid, which is reported to consist of BC nanofibrils and extracellular material (Torres et al., 2019). In laboratory work, "standard" complex liquid media are often used to produce BC from a pure isolate of *K. xylinus*. The two most commonly used media consist of yeast extract, glucose and bactopeptone (known as Hestrin and Schramm media) Schramm and Hestrin (1954); (Ovcharenko, 2013) or sucrose, potassium phosphate, magnesium sulfate, and ammonium sulfate (known as Yamanaka media) Yamanaka and Sugiyama (2000). *K. xylinus* can be isolated from rotting fruit, as well as from bacterial and yeast communities such as Kombucha.

Kombucha, also known as Manchurian mushroom, Haipao, or tea fungus, has been used in the fermentation of drinks dating back to several thousand years (Jarrell et al., 2000; Teoh et al., 2004; Četojević-Simin et al., 2012) and is purported to have detoxifying and energizing properties when imbibed (Teoh et al., 2004; Marsh et al., 2014). The Kombucha "tea fungus", or pellicle, can be considered a "core" consortium of bacteria and yeasts, with its exact composition determined by its geographic and climatic conditions of cultivation (Chakravorty et al., 2016). It is thought that the additional "local" bacteria and yeasts have some effect on the growth behavior of the overall community (Jayabalan et al., 2014) and that the microbial community composition can vary with fermentation time. It has been widely documented that the brewing of Kombucha tea for more than 3 days can lead to the "core" consortium producing a BC pellicle on the surface. Similar production is observed when Kombucha is used to inoculate standardized microbiological media such as Hestrin and Schramm (commonly known as H&S) or Yamanaka broth (Schramm and Hestrin 1954; Jarrell et al., 2000; Yamanaka and Sugiyama 2000; Chakravorty et al., 2016).

The impact of different growth liquids and different starter cultures has been demonstrated in the literature with respect to bacterial cellulose yield and changes in bacterial communities. Marsh et al. (2014) used Kombucha pellicles as inoculants from different geographical locations and analyzed the microbial communities present after 3 and 10 days of fermentation in black tea using metagenomic DNA extraction and high-throughput sequencing techniques and found that in all cases, *Komagataeibacter* was the dominant bacterial genus, with the highest diversity of microbial strains found in the cellulosic pellicles. Similar data were identified by Chakravorty et al. (2016) where Kombucha pellicles and liquids were assessed at 3-, 7-, 14-, and 21-day fermentation. *Komagataeibacter* was the dominant bacterial genus in both the liquid and pellicle at all time-points with microbial diversity declining throughout the study.

When examining microbial communities in Kombucha tea, Reva et al. (2015) also discovered diversity was found to be less in the pellicles and more in the liquid phase. Reva et al. (2015) postulated more work is required to evaluate the core species in Kombucha that are responsible for pellicle formation and that this could be used as a platform to assess potential end uses of the formed pellicles.

However, to date, there is little research to examine the changes in the bacterial communities in both the liquid and pellicle over several generations of sub-culturing, where previous studies have instead focused on the age of a single culture. If BC is going to provide the textile industry with a more sustainable model for generating materials, it is essential that the effect of sub-culturing over numerous generations is understood as reproducibility would be an essential requirement for any

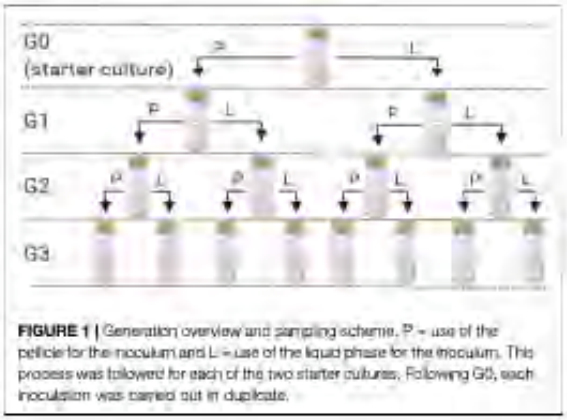

FIGURE 1 | Generation overview and sampling scheme. P = use of the pellicle for the inoculum and L = use of the liquid phase for the inoculum. This process was followed for each of the two starter cultures. Following G0, each inoculation was carried out in duplicate.

industrial use. Therefore, the aim of this study was to analyze the effect of multi-generational sub-cultures of BC pellicles and the liquid environment it grows in, with respect to physical properties and microbial community composition and changes.

MATERIALS AND METHODS

Starter Cultures

This study used either *Gluconacetobacter xylinus* (ATCC 23767) (now named *Komagataeibacter xylinus*) as a single isolate strain or a Kombucha starter culture (KSC—www.happykombucha.co.uk) to propagate bacterial cellulose over three generations. *K. xylinus* was stored at −80°C in a cryopreservative solution [3.6% KH_2PO_4, 12.6% K_2HPO_4, 0.9% $Na_3C_6H_5O_7$, and 1.8% $(NH_4)_2SO_4$]. When required, it was grown on H&S agar overnight at 30°C and was then used to inoculate a liquid medium for experiments detailed as follows. KSC, as provided by suppliers, comprises a solid surface (hereafter termed "pellicle") and liquid phase. Storage of KSC was at room temperature in the absence of light in a sealed container, following manufacturer's instructions.

Growing Bacterial Communities Across Generations

Sterile "universal" tubes (25 ml) containing 10 ml of sterile H&S broth were inoculated with either 1 ml of *K. xylinus* or 1 g of KSC pellicles. The tubes were covered with loose-fitting lids and left at 30°C to incubate for 10 days—producing generation zero (G0). Following the 10-day incubation, pellicles had formed at the liquid–air interface in both starter cultures. Each pellicle was removed aseptically and divided using a sterile scalpel. Half of each pellicle was frozen at −80°C for DNA extraction and analysis (see below). The other half of the pellicle was further divided into two and used as the inoculum for generation 1 (in duplicate) 10 ml sterile H&S broth (G1P; **Figure 1**). Further duplicate G1 cultures were established using 10 ml of H&S broth that had been inoculated with 1 ml of the G0 liquid phase (G1L; **Figure 1**). This resulted in eight new starter cultures, inoculated from either the pellicle or liquid phase for both the community started by *G. xylinus* or the KSC. These cultures were incubated at 30°C for 30 days. After 30 days, the pellicle and broth were removed and sampled as mentioned earlier. The process was repeated to create generation 2 (G2) and generation 3 (G3), where each of the previous generation bacterial communities resulted in two new inoculations (one from the pellicle and one from the liquid phase) (**Figure 1**).

Physical Quantification of Samples

At the end of each generation, before the pellicle was removed from the pot, the thickness was measured, using a ruler on the outside of the pot, and recorded. Pellicles were photographed both before and after removal from the pot, and visual appearance observations (color and opacity/transparency) were noted.

Samples of all pellicles were prepared for scanning electron microscopy (SEM) analysis by immersing each sample in 0.1 M glutaraldehyde overnight (to fix and preserve the cell structure), removing, and then dehydrating by passing through sequential ethanol baths (10 min per bath) of increasing concentrations (50%/70%/80%/90%/99.9%). The samples were kept in a desiccator until ready to be viewed on the SEM (Carl Zeiss Ltd., model Supra 40VP).

Molecular Analysis of Microbial Communities in Samples

Nucleic acid extractions were carried out using the DNeasy PowerSoil kit (QIAGEN Ltd., Manchester), cleaned using the ZR-96 DNA Sequencing Clean-Up kit (Zymo Research, United States), and quantified using the Qubit dsDNA high sensitivity assay kit (Invitrogen, Paisley).

Amplicon sequencing of bacterial ribosomal rRNA genes (16S rRNA) was undertaken, as previously described (Thompson et al., 2017). Briefly, PCR reactions for initial amplifications consisted of 5 µl NEB Q5 reaction buffer, 0.25 µl NEB Q5 High-Fidelity DNA Polymerase, 0.5 µl NEB X nM dNTPs, 15 µl DNA template (c. X ngµl^{-1}), and 0.5 µl X pM primers 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806_R (5′-GGACTACNVGGGTWTCTAAT-3′) or EUK1391_F (5′-GTACACACCGCCCGTC-3′) and 1510_R (5′-CCTTCYGCAGGTTCACCTAC -3′) for 16S and 18S rRNA genes, respectively, and made up to a final reaction volume of 25 µl with nuclease-free water. Cycling conditions comprised an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 60 s, and extension at 72°C for 90 s, with a final extension at 72°C for 10 min. Amplification was confirmed visually by 1.5% (w/v) Tris-acetate-EDTA (TAE)-agarose gel electrophoresis. A second-stage PCR was carried out to attach barcodes for Illumina sequencing. The constituents of the second-stage PCR reaction are as follows: 10 µl NEB Q5 reaction buffer, 0.5 µl NEB Q5 High-Fidelity DNA Polymerase, 1 µl NEB (X nM) dNTPs, 0.5 µl (X pM) forward primer, 0.5 µl (X pM) reverse primer, and 20 µl cleaned amplicon template, and they were

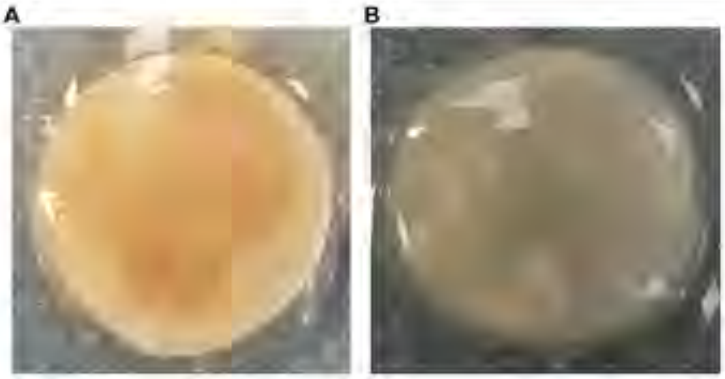

FIGURE 2 | Examples of pellicles (approx. 2 cm across) produced in generation 2 from KSC **(A)** and the *K. xylinus* isolate **(B)**.

made up to a final reaction volume of 50 µl with nuclease-free water. Cycling parameters comprised an initial denaturation at 98°C for 30 s, followed by 10 cycles of denaturation at 98°C for 10 s, annealing at 62°C for 20 s, and extension at 72°C for 30 s, with a final extension at 72°C for 2 min. Amplification and attachment were confirmed by 1.5% (w/v) TAE-agarose gel electrophoresis.

Following second-stage PCR, all amplified DNA strands were normalized using the SequalPrep Normalization kit (Fischer Scientific, Loughborough) and pooled into libraries for sequencing on a MiSeq Illumina platform with a MiSeq Reagent Kit v2 (300-cycle) flow cell.

Data Analysis

Amplicon sequence variants (ASVs) were extracted from the raw sequence data using the dada2 pipeline (Callahan et al., 2016) using the default parameters. Taxa were assigned using the SILVA database and sequencing information files deposited in the NCBI BioProject database (BioProject PRJNA787576; accession numbers SAMN23827663–SAMN23827813). Statistical analysis is described in the results section, with α set at 0.05. All assumptions for parametric statistics were assessed visually prior to analysis. Multivariate ordination plots were calculated using the Bray–Curtis dissimilarity measure and non-metric multidimensional scaling (NMDS). Analysis of similarity (ANOSIM) was used to compare compositional differences in communities between two groups, with permutational ANOVAs (PERMANOVAs) used to analyze differences between communities as a factor of multiple factors using Bray–Curtis dissimilarity measures in the vegan package (v.3.5-0) (Oksanen et al., 2016).

Comparisons of community shifts were achieved using multivariate ANOVAs (MANOVAs). Univariate analyses were performed using repeated measures ANOVA (linear mixed-effects modeling fit by REML) in the lme4 (Bates et al., 2015) package, with significance assigned from the lmerTest (Kuznetsova et al., 2017) package, where generation was both a random effect and a fixed effect along with the original seed culture and phase measured as fixed effects.

RESULTS AND DISCUSSION

Physical Characterization

Across all generations, the Kombucha (KSC) pellicles had a consistent pale brown gel-like appearance, as noted from previous studies (Jarrell et al., 2000; Dumsakarne et al., 2019). However, previous work has not highlighted the visual variations our study found in the *K. xylinus* pellicles. In contrast to the consistent KSC pellicles, white, opaque spots were observed within the *K. xylinus* pellicle structure, as indicated in **Figure 2**.

In contrast to the KSC pellicles, *K. xylinus* pellicles, from both inoculum types, were initially (generation 1) translucent with a gelatinous appearance and were difficult to remove from the vessel due to their gel-like adherent consistency. In both second and third generations, however, the pellicles developed regions of opaqueness and were more robust.

Pellicle thickness increased as the generations progressed (**Table 1**), from 1 mm in all samples observed in generation 1 to between 2.4 mm *K. xylinus* pellicles (KXP) and 3.9 mm Kombucha pellicles (KP) in generation 3, despite all generations having the same incubation time. This trend was observed regardless of the starter culture. However, the inoculum type had an effect on the pellicle yield in generation 3; the highest yields by the thickness of pellicle were achieved when a liquid phase inoculum had been used at some stage of the process, with a lower G3 yield when only pellicle phase inocula had been used throughout the preceding generations (**Table 1**). Dima et al. (2017) and Blanco Parra et al. (2020) noted that the bacterial communities responsible for BC production are more mobile in the liquid phase and have greater access to nutrients, thereby improving their BC production potential and suggesting a reason for the observed differences in pellicle thickness. This leads to the hypothesis that liquid inocula achieve higher yields of pellicles,

TABLE 1 | Observed mean thickness of pellicles.

Starter culture	Generation 1			Generation 2			Generation 3		
KSC	KP		0.5 mm (0)	KP		2.0 mm (0)	KP		2.5 mm (1.6)
							KL		3.0 mm (1.8)
				KL		4.0 mm (0)	KP		3.9 mm (1.3)
							KL		4.0 mm (1.6)
	KL		0.5 mm (0)	KP		2.0 mm (0)	KP		2.6 mm (0.4)
							KL		3.9 mm (0.9)
				KL		2.0 mm (0)	KP		3.8 mm (1.8)
							KL		4.1 mm (1.4)
KXSC	KXP		0.5 mm (0)	KXP		3.0 mm (1.0)	KXP		2.6 mm (1.5)
							KXL		1.8 mm (0.4)
				KXL		2.0 mm (0)	KXP		2.8 mm (1.0)
							KXL		2.3 mm (0.4)
	KXL		0.5 mm (0)	KXP		2.0 mm (1.0)	KXP		2.8 mm (0.8)
							KXL		2.3 mm (0.4)
				KXL		2.0 mm (0)	KXP		2.4 mm (0.4)
							KXL		2.5 mm (0.5)

KSC, Kombucha starter culture; KXSC, K. xylinus starter culture; KP, Kombucha pellicle; KB, Kombucha liquid; KXP, K. xylinus pellicle; KXL, K. xylinus liquid. Generation 1, n = 1; Generation 2, n = 2; Generation 3, n = 8.

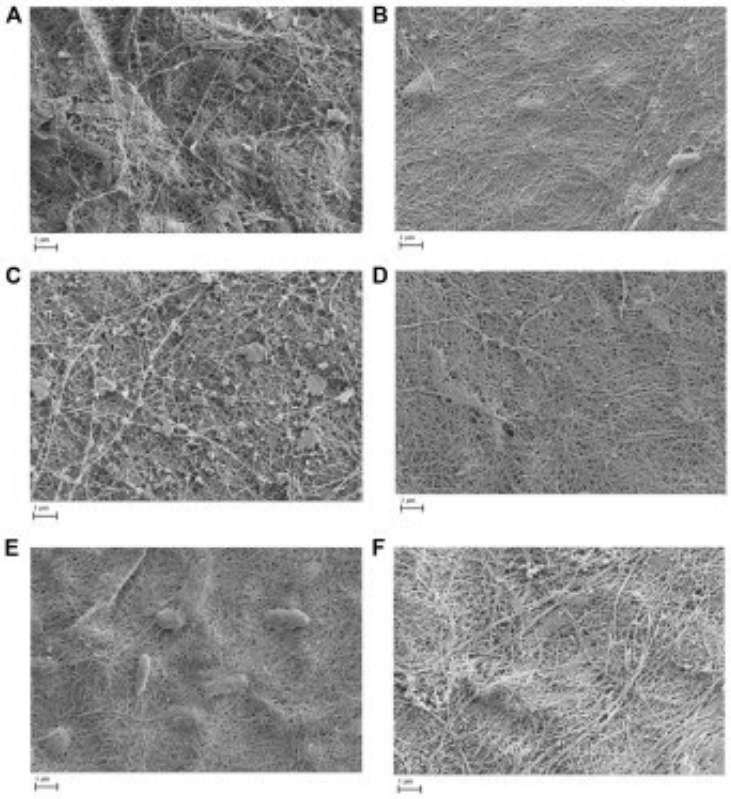

FIGURE 3 | Scanning electron microscope images (x 20k) of generation 3 pellicles showing microfibrillar mat formation from various inoculants (**A** = Kombucha pellicle, **B** = *K. xylinus* pellicle, **C** = Kombucha broth, **D** = *K. xylinus* broth, **E** = Kombucha pellicle and broth, **F** = *K. xylinus* pellicle and broth).

TABLE 2 | Median ASV relative abundancies of *Komagataeibacter* in pellicles across generations.

Genus	G0		G1		G2		G3	
	GX[a]	K[a]	GX[a]	K[a]	GX[b]	K[b]	GX[c]	K[c]
Komagataeibacter	99.31 (99.22–99.39)	92.93 (91–72–94.13)	97.54 (95.74–99.17)	98.81 (98.07–99.56)	99.69 (93.81–99.96)	99.77 (70.40–99.99)	94.81 (49.13–100.00)	99.57 (44.09–99.97)

[a]n = 2.
[b]n = 16.
[c]n = 32.

TABLE 3 | Median ASV relative abundancies of *Komagataeibacter* in liquids across generations.

N	G0		G1		G2	
	GX[a]	K[a]	GX[b]	K[b]	GX[c]	K[c]
Komagataeibacter	100 (67.51–100)	79.53 (68.72–98.30)	68.07 (61.11–99.17)	93.87 (91.30–94.27)	94.59 (64.78–100)	85.60 (50.13–96.00)

[a]n = 2.
[b]n = 4.
[c]n = 16.

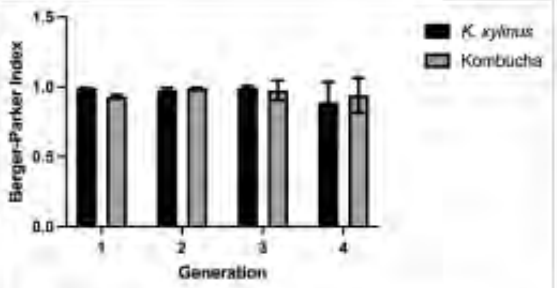

FIGURE 4 | Relative abundance of dominant species (*Komagataeibacter*) across generations using the Berger-Parker Index.

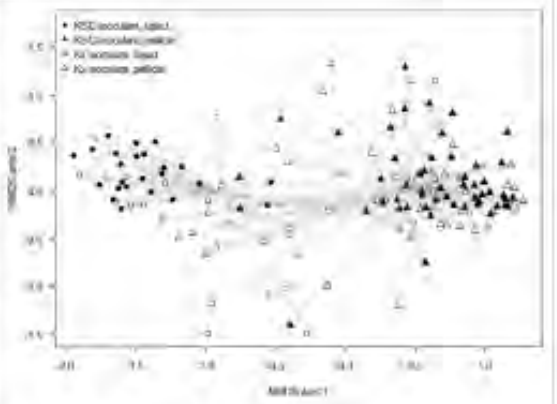

FIGURE 5 | Non-metric multidimensional scaling ordination plot of composition differences of samples (inoculated with either the liquid or pellicle *K. xylinus*/Kombucha) using the Bray–Curtis dissimilarity index.

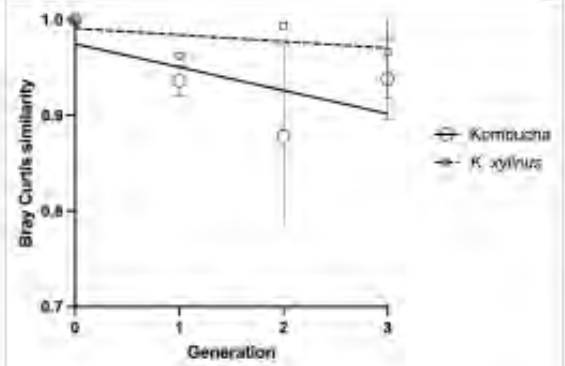

FIGURE 6 | Trend of changes in the pellicle bacterial community relative to the original inoculant using the Bray–Curtis index.

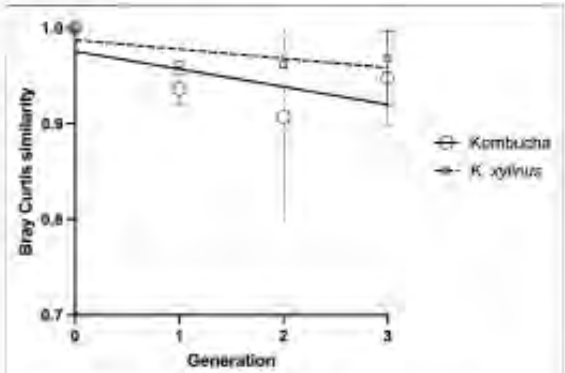

FIGURE 7 | Trend of changes in pellicle bacterial community relative to previous generation using the Bray–Curtis index.

with Kombucha-derived liquid inocula producing higher yields than pure strain *K. xylinus*.

Additionally, previous studies of the fermentation of the Kombucha tea state that the yeast species present in the starter culture are instrumental in making carbon sources freely available (by breaking down complex sugars into more simple molecules) and in increasing the acidity of the broth as a by-product of the metabolic process (Teoh et al., 2004; Marsh et al., 2014). Both are favored conditions for BC-producing bacteria. Thus, the presence of yeast in the Kombucha created an advantage over the single isolate in the development of BC pellicles, particularly in broths such as H&S used in our study where there is no adjustment of pH. Furthermore, Jefferson, (2004) suggested there may be an ability to "store" carbon sources as extracellular polymeric substances (EPS) within the bacterial cellulose fibrillar matrix, thus creating a supply of nutrients which can be utilized when the source in the growth broth is depleted. This could explain the more rapid and consistent development of pellicles from Kombucha starter cultures. Here, a sequestered carbon supply is present when Kombucha is cultured compared to a single isolate where the nutrient source declines.

Characterization by Scanning Electron Microscopy

All pellicles were examined by scanning electron microscopy. **Figure 3** shows the comparisons of generation 3 pellicles as examples, illustrating similarities in the microfibrillar structure. However, SEM revealed pellicles developed from *K. xylinus* tended to develop more consistent and smoother microfibrillar mats. The pellicles developed from the Kombucha culture, whilst exhibiting a microfibrillar structure, also displayed a degree of contamination, which were suggested to be extracellular polymeric substances and a documented part of the Kombucha microbial consortium (Jefferson, 2004). Additionally, all pellicles showed similar size and distribution of nanofibrils, ranging from a (all broth inoculant) mean diameter of 81 (+/− 7.8) nm in *K. xylinus* to (mixed inoculant) a mean

diameter of 92.5 (+/− 6.6) nm in Kombucha. The measured nanofibril size is in accordance with the findings of previous studies (Castro et al., 2014; Kumbhar et al., 2015; Chen et al., 2017). Furthermore, there were no other observed differences between the pellicle structures of those formed from liquid, solid, or mixed inocula.

Community Abundance

The analysis of the 16S rRNA gene sequence data indicated that *Komagataeibacter* was the dominant genus in all samples, agreeing with findings of the previous work (Jayabalan et al., 2009; Marsh et al., 2014; Gaggia et al., 2019). The results indicate that pellicles inoculated with Kombucha (98.31% range: 44.09–99.99%) demonstrated a consistent (Wilcoxon rank sum test: W = 2,986 and $p = 0.614$) relative abundance of *Komagataeibacter* compared to those from a *K. xylinus* starter (97.70% range: 49.12–100%) (**Tables 2, 3**).

Additionally, the proportional abundance of *Komagataeibacter* in the pellicles was analyzed using the Berger–Parker index to establish the numerical importance of this as the most abundant species. **Figure 4** illustrates the relative abundance of *Komagataeibacter* across generations of pellicles inoculated with either *K. xylinus* or Kombucha. Whilst *Komagataeibacter* is abundant in early generations of pellicles inoculated with *K. xylinus*, the trend of abundance slightly decreases over subsequent generations. Conversely, in pellicles inoculated with Kombucha, the trend of abundance of *K. xylinus* increases in later generations.

Community Composition

When considering the non-dominant composition data, there were significant differences between communities due to the inoculation type (ANOSIM: R = 0.28 and $p < 0.001$), during generations 0–2, and significant differences between cultures seeded from Kombucha or *K. xylinus* (ANOSIM: R = 0.14 and $p < 0.001$) across all generations. **Figure 5** provides a visual illustration of these differences. The points represent the mean ordinations of the communities grown using liquid (circular points) or pellicle (triangular points) inocula, which were found to be significantly different ($p < 0.001$). Communities derived from a KSC inoculum (filled points) were found to be significantly different ($p < 0.001$) from those derived from a *K. xylinus* (KX) starter inoculum.

Using the Bray–Curtis similarity index to further explore and quantify the difference in the communities, a more marked drift away from the original pellicle community is noted in pellicles developed from a Kombucha inoculant (**Figure 6**) than those inoculated with *K. xylinus*. However, the rate of pellicle community composition change when compared to the previous generation is similar in trend for both Kombucha and *K. xylinus* (**Figure 7**).

Previous work studying the microbial communities in Kombucha tea liquid observed microbial community stabilization over time. In studies sampling tea broth, it was suggested that stabilization occurs after approximately 21 days (Chakravorty et al., 2016). This implies that stabilization of the community is an important factor when studying the BC pellicle-forming effects of both Kombucha and single isolates. Previous studies investigating the properties of the liquid culture consortium have suggested that there are critical times in fermentation at which the microbial community is most stable (Marsh et al., 2014; Chakravorty et al., 2016). However, it is important to note that some previous studies have used non-sterile sweetened black tea liquid as it is suggested the autoclave process can cause a build-up of toxins which inhibit the fermentation of Kombucha pellicles (Marsh et al., 2014). Further work will be required to tailor the methodology to ensure reproducibility of all aspects of BC production, for example, thickness of BC, and any specific properties required for commercialization. Therefore, it could be argued that the microbial diversity found in these studies was not adequately controlled. Nevertheless, the results of our study lead to the hypothesis that a community stabilization period (in the region of 30 days) is required to observe consistent, reproducible BC pellicle production.

CONCLUSION

This study examined the changes of bacterial communities over several generations of sub-culturing (using either a Kombucha consortium or *Komagataeibacter xylinus* single isolate as a starter inoculum) to establish the reproducibility of the BC pellicle as a potential alternative textile for industrial use. *Komagataeibacter* (the genus responsible for the most prolific production of BC) was found to be the most abundant species in all samples tested. *Komagataeibacter xylinus* starter culture BC pellicle yield improved over subsequent generations; however, Kombucha starter cultures produced the highest yield of BC pellicles from generation 1. This leads to the hypothesis that it is the microbial and fungal community and extracellular polymeric substances in the Kombucha consortium that support more vigorous BC production, giving more stability to the BC-forming bacterial strains. It is also suggested that an increase of diversity negatively impacts the ability of the *Komagataeibacter* genus to produce BC in the case of single isolate inocula.

Additionally, this study has shown that it is a mix of pellicle and liquid inocula that gives the best yield of BC. As the microbial community is more mobile in a liquid than a solid pellicle, it is proposed that the BC-forming microbes can more easily access carbon sources and thus more quickly produce BC.

Reproducibility of the BC pellicle across generations is essential for effective applications across the fashion and biotechnology industries. We conclude that Kombucha starter cultures produce BC pellicles which are more reproducible across generations than those created from pure isolates of *K. xylinus*. However, the Kombucha community needs to reach a critical point to maximize the yield of BC production. The study suggests this may be after a minimum of two generations, but this could be confirmed by examination of further generations of sub-culturing.

The findings of this study suggest that BC pellicles produced from Kombucha starter cultures could provide a reproducible sustainable model for generating textile materials. Further works should now examine the effects of sub-culturing on performance

properties of the BC pellicles to establish potential end uses for the material.

DATA AVAILABILITY STATEMENT

Sequence data that support the findings of this study have been deposited in NCBI BioProject database with accession number SAMN23827663 to SAMN23827813.

AUTHOR CONTRIBUTIONS

JW: conceptualization, data curation, formal analysis, investigation, methodology, visualization, and writing—original draft preparation. DR: data curation, formal analysis, methodology, visualization, and writing—review and editing. CVDG: conceptualization, data curation, formal analysis, methodology, visualization, and writing—review and editing. JV: conceptualization, methodology, supervision, and writing—review and editing. JR: conceptualization, formal analysis, investigation, methodology, supervision, visualization, and writing—review and editing.

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REFERENCES

Amnuaikit, T., Chusuit, T., Raknam, P., and Boonme, P. (2011). Effects of a Cellulose Mask Synthesized by a Bacterium on Facial Skin Characteristics and User Satisfaction. *Med. Devices (Auckl.)* 4, 77–81. doi:10.2147/MDER.S20935

Ashrafi, A., Jokar, M., and Mohammadi Nafchi, A. (2017). Preparation and Characterization of Biocomposite Film Based on Chitosan and Kombucha tea as Active Food Packaging. *Int. J. Biol. Macromol.* 108, 444–454. doi:10.1016/j.ijbiomac.2017.12.028

Azimi, B., Milazzo, M., and Danti, S. (2021). Cellulose-Based Fibrous Materials from Bacteria to Repair Tympanic Membrane Perforations. *Front. Bioeng. Biotechnol.* 7, 669863. doi:10.3389/fbioe.2021.669863

Backdahl, H., Esguerra, M., Delbro, D., Risberg, B., and Gatenholm, P. (2008). Engineering Microporosity in Bacterial Cellulose Scaffolds. *J. Tissue Eng. Regen. Med.* 2, 320–330. doi:10.1002/term.97

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* 67, 48. doi:10.18637/jss.v067.i01

Blackburn, R. S. (2009). *Sustainable Textiles: Life Cycle and Environmental Impact*. Cambridge: Woodhead.

Blanco Parte, F. G., Santoso, S. P., Chou, C.-C., Verma, V., Wang, H.-T., Ismadji, S., et al. (2020). Current Progress on the Production, Modification, and Applications of Bacterial Cellulose. *Crit. Rev. Biotechnol.* 40, 397–414. doi:10.1080/07388551.2020.1713721

Bodin, A., Bäckdahl, H., Fink, H., Gustafsson, L., Risberg, B., and Gatenholm, P. (2007). Influence of Cultivation Conditions on Mechanical and Morphological Properties of Bacterial Cellulose Tubes. *Biotechnol. Bioeng.* 97, 425–434. doi:10.1002/bit.21314

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: High-Resolution Sample Inference from Illumina Amplicon Data. *Nat. Methods* 13, 581–583. doi:10.1038/nmeth.3869

Castro, C., Zuluaga, R., Putaux, J.-L., Caro, G., Mondragon, I., and Gañán, P. (2011). Structural Characterization of Bacterial Cellulose Produced by Gluconacetobacter Swingsii Sp. From Colombian Agroindustrial Wastes. *Carbohydr. Polym.* 84, 96–102. doi:10.1016/j.carbpol.2010.10.072

Četojević-Simin, D. D., Velićanski, A. S., Cvetković, D. D., Markov, S. L., Mrdanović, J. Ž., Bogdanović, V. V., et al. (2012). Bioactivity of Lemon Balm Kombucha. *Food Bioproc. Tech.* 5, 1756–1765. doi:10.1007/s11947-010-0458-6

Chakravorty, S., Bhattacharya, S., Chatzinotas, A., Chakraborty, W., Bhattacharya, D., and Gachhui, R. (2016). Kombucha tea Fermentation: Microbial and Biochemical Dynamics. *Int. J. Food Microbiol.* 220, 63–72. doi:10.1016/j.ijfoodmicro.2015.12.015

Chawla, P. R., Bajaj, I. B., Survase, S. A., and Singhal, R. S. (2009). Microbial Cellulose: Fermentative Production and Applications. *Food Tech. Biotechnol.* 47, 107–124.

Chen, S.-Q., Mikkelsen, D., Lopez-Sanchez, P., Wang, D., Martinez-Sanz, M., Gilbert, E. P., et al. (2017). Characterisation of Bacterial Cellulose from Diverse Komagataeibacter Strains and Their Application to Construct Plant Cell wall Analogues. *Cellulose* 24, 1211–1226. doi:10.1007/s10570-017-1203-3

Dima, S.-O., Panaitescu, D.-M., Orban, C., Ghiurea, M., Doncea, S.-M., Fierascu, R., et al. (2017). Bacterial Nanocellulose from Side-Streams of Kombucha Beverages Production: Preparation and Physical-Chemical Properties. *Polymers* 9, 374. doi:10.3390/polym9080374

Domskiene, J., Saleraviciute, P., and Simonaityte, J. (2019). Kombucha Bacterial Cellulose for Sustainable Fashion. *Ijcst* 31, 644–652. doi:10.1108/ijcst-02-2019-1010

Dufresne, A. (2012). *Nanocellulose: From Nature to High Performance Tailored Materials*. Berlin/Boston: Walter de Gruyter GmbH Co.KG.

Ellen MacArthur Foundation (2017). *A New Textiles Economy: Redesigning Fashion's Future*. Cowes, Isle of Wight: Ellen MacArthur Foundation

Fernandes, M., Gama, M., Dourado, F., and Souto, A. P. (2019). Development of Novel Bacterial Cellulose Composites for the Textile and Shoe Industry. *Microb. Biotechnol.* 12, 650–661. doi:10.1111/1751-7915.13387

Gaggia, F., Baffoni, L., Galiano, M., Nielsen, D. S., Jakobsen, R. R., Castro-Mejía, J. L., et al. (2019). Kombucha Beverage from green, Black and Rooibos Teas: A Comparative Study Looking at Microbiology, Chemistry and Antioxidant Activity. *Nutrients* 11, 1. doi:10.3390/nu11010001

García, C., and Prieto, M. A. (2019). Bacterial Cellulose as a Potential Bioleather Substitute for the Footwear Industry. *Microb. Biotechnol.* 12, 582–585. doi:10.1111/1751-7915.13306

Gatenholm, P., and Klemm, D. (2010). Bacterial Nanocellulose as a Renewable Material for Biomedical Applications. *MRS Bull.* 35, 208–213. doi:10.1557/mrs2010.653

Hoenich, N. (2006). CELLULOSE FOR MEDICAL APPLICATIONS: PAST, PRESENT, AND FUTURE. *BioRes* 1, 270–280. doi:10.15376/biores.1.2.270-280

Hu, W., Chen, S., Liu, L., Ding, B., and Wang, H. (2011). Formaldehyde Sensors Based on Nanofibrous Polyethyleneimine/bacterial Cellulose Membranes Coated Quartz crystal Microbalance. *Sensors Actuators B: Chem.* 157, 554–559. doi:10.1016/j.snb.2011.05.021

Huang, Y., Zhu, C., Yang, J., Nie, Y., Chen, C., and Sun, D. (2014). Recent Advances in Bacterial Cellulose. *Cellulose* 21, 1–30. doi:10.1007/s10570-013-0088-z

Iguchi, M., Yamanaka, S., and Budhiono, A. (2000). Bacterial Cellulose—A Masterpiece of Nature's Arts. *J. Mater. Sci.* 35, 261–270. doi:10.1023/a:1004775229149

Jarrell, J., Cal, T., and Bennett, J. W. (2000). The Kombucha Consortia of Yeasts and Bacteria. *Mycologist* 14, 166–170. doi:10.1016/s0269-915x(00)80034-8

Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S., and Sathishkumar, M. (2014). A Review on Kombucha Tea-Microbiology, Composition, Fermentation, Beneficial Effects, Toxicity, and Tea Fungus. *Compr. Rev. Food Sci. Food Saf.* 13, 538–550. doi:10.1111/1541-4337.12073.

Jefferson, K. K. (2004). What Drives Bacteria to Produce a Biofilm? *Front. Microbiol.* 236, 163–173. doi:10.1111/j.1574-6968.2004.tb09643.x

Jia, Y., Yu, H., Zhang, Y., Dong, F., and Li, Z. (2016). Cellulose Acetate Nanofibers Coated Layer-By-Layer with Polyethyleneimine and Graphene Oxide on a Quartz crystal Microbalance for Use as a Highly Sensitive Ammonia Sensor. *Colloids Surf. B. Biointerfaces* 148, 263–269. doi:10.1016/j.colsurfb.2016.09.007

Kumbhar, J. V., Rajwade, J. M., and Paknikar, K. M. (2015). Fruit Peels Support Higher Yield and superior Quality Bacterial Cellulose Production. *Appl. Microbiol. Biotechnol.* 99, 6677–6691. doi:10.1007/s00253-015-6644-8

Kuswandi, B., Jayus, Oktaviana, R., Abdullah, A., and Heng, L. Y. (2014). A Novel On-Package Sticker Sensor Based on Methyl Red for Real-Time Monitoring of Broiler Chicken Cut Freshness. *Packag. Technol. Sci.* 27, 69–81. doi:10.1002/pts.2016

Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *J. Statistical Softw.* 82, 26. doi:10.18637/jss.v082.i13

Lee, K.-Y. (2018). *Nanocellulose and Sustainability: Production, Properties, Applications, and Case Studies*. UK: CRC.

Lee, S. (2019). *Suzanne Lee: Biocouture Launch*. Available from https://www.launch.org/innovators/suzanne-lee/ Accessed 11/04/22.

Lin, S.-P., Loira Calvar, I., Catchmark, J. M., Liu, J.-R., Demirci, A., and Cheng, K.-C. (2013). Biosynthesis, Production and Applications of Bacterial Cellulose. *Cellulose* 20, 2191–2219. doi:10.1007/s10570-013-9994-3

Marsh, A. J., O'Sullivan, O., Hill, C., Ross, R. P., and Cotter, P. D. (2014). Sequence-based Analysis of the Bacterial and Fungal Compositions of Multiple Kombucha (tea Fungus) Samples. *Food Microbiol.* 38, 171–178. doi:10.1016/j.fm.2013.09.003

Mohite, B. V., and Patil, S. V. (2014). A Novel Biomaterial: Bacterial Cellulose and its new era Applications. *Biotechnol. Appl. Biochem.* 61, 101–110. doi:10.1002/bab.1148

Oksanen, J. B., Guillaume, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2016). Community Ecology Package. Available At: http://CRAN.R-project.org/package=vegan.

Ovcharenko, L. P. (2013). Metagenomic Analysis of Domesticated Kombucha Multi-Microbial Culture. *Biopolymers and Cell* 29, 16.

Picheth, G. F., Pirich, C. L., Sierakowski, M. R., Woehl, M. A., Sakakibara, C. N., de Souza, C. F., et al. (2017). Bacterial Cellulose in Biomedical Applications: A Review. *Int. J. Biol. Macromolecules* 104, 97–106. doi:10.1016/j.ijbiomac.2017.05.171

Reva, O. N., Zaets, I. E., Ovcharenko, L. P., Kukharenko, O. E., Shpylova, S. P., Podolich, O. V., *et al.* (2015). Metabarcoding of the Kombucha Microbial Community Grown in Different Microenvironments. *AMB Express* 5, 124–128. doi:10.1186/s13568-015-0124-5

Schramm, M., and Hestrin, S. (1954). Factors Affecting Production of Cellulose at the Air/Liquid Interface of a Culture of Acetobacter Xylinum. *J. Gen. Microbiol.* 11, 123–129. doi:10.1099/00221287-11-1-123

Shi, Z., Zhang, Y., Phillips, G. O., and Yang, G. (2014). Utilization of Bacterial Cellulose in Food. *Food Hydrocolloids* 35, 539–545. doi:10.1016/j.foodhyd.2013.07.012

Sinclair, R. (2015). *Textiles and Fashion: Materials, Design and Technology*. Cambridge: Elsevier/Woodhead Publishing.

Stoica-Guzun, A., Stroescu, M., Jinga, S. I., Mihalache, N., Botez, A., Matei, C., et al. (2016). Box-Behnken Experimental Design for Chromium(VI) Ions Removal by Bacterial Cellulose-Magnetite Composites. *Int. J. Biol. Macromolecules* 91, 1062–1072. doi:10.1016/j.ijbiomac.2016.06.070

Stone, C., Windsor, F. M., Munday, M., and Durance, I. (2020). Natural or Synthetic – How Global Trends in Textile Usage Threaten Freshwater Environments. *Sci. Total Environ.* 718, 134689. doi:10.1016/j.scitotenv.2019.134689

Teoh, A. L., Heard, G., and Cox, J. (2004). Yeast Ecology of Kombucha Fermentation. *Int. J. Food Microbiol.* 95, 119–126. doi:10.1016/j.ijfoodmicro.2004.02.020

Textiles 2030 project (2021). *WRAP*. Banbury, UK: Textiles 2030.

Thompson, L. R., Sanders, J. G., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., et al. (2017). A Communal Catalogue Reveals Earth's Multiscale Microbial Diversity. *Nature* 551, 457–463. doi:10.1038/nature24621

Torres, F. G., Arroyo, J. J., and Troncoso, O. P. (2019). Bacterial Cellulose Nanocomposites: An All-Nano Type of Material. *Mater. Sci. Eng. C* 98, 1277–1293. doi:10.1016/j.msec.2019.01.064

Ullah, H., Santos, H. A., and Khan, T. (2016). Applications of Bacterial Cellulose in Food, Cosmetics and Drug Delivery. *Cellulose* 23, 2291–2314. doi:10.1007/s10570-016-0986-y

Veera, V., Larrea, C., and Bermudez, S. (2020). "Global Market Report: Cotton," in *Sustainable Commodities Marketplace Series 2019*. Editor S. Balint (Ottawa: International Institute for Sustainable Development).

Yamanaka, S., and Sugiyama, J. (2000). Structural Modification of Bacterial Cellulose. *Cellulose* 7, 213–225. doi:10.1023/a:1009208022957

Zhu, W., Liu, L., Luan, Q., Chen, X., Qian, Z., Shen, J., et al. (2016). Functionalization of Cellulose with Hyperbranched Polyethyleneimine for Selective Dye Adsorption and Separation. *Cellulose* 23, 3785–3797. doi:10.1007/s10570-016-1045-4

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

biomimetics

MDPI

Review

Bioinspiration in Fashion—A Review

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Abstract: This paper provides an overview of the main technologies currently being investigated in the textile industry as alternatives to contemporary fashion fabrics. The present status of the textile industry and its impact on the environment is discussed, and the key drivers for change are highlighted. Historical use of bioinspiration in synthetic textiles is evaluated, with the impact of these developments on the fashion and apparel industries described. The review then discusses the move to nature as a supplier of new fabric sources with several alternatives explored, drawing special attention to the sustainability and performance aspects of these new sources.

Keywords: sustainability; biomimicry; fashion; apparel; bacterial cellulose; mycelium; textiles

1. Introduction

The unsustainable rate of consumption attributed to the fashion industry has been well documented [1]. It is estimated that £140 million worth of clothing goes into landfills each year, with 24% of consumers surveyed stating they had disposed of clothing after only one wear [2]. The Waste and Resources Action Plan (WRAP), Valuing Our Clothes, report suggests that the annual footprint of a household's newly bought clothing, along with the washing and cleaning of its clothes, is estimated to be equivalent to the carbon emissions from driving an average modern car for 6000 miles and the water needed to fill over 1000 bathtubs [2]. In addition to this, there is waste hidden in the supply chain with estimates that 15% of fabric is rejected and discarded before it leaves the factory [3]. Whilst large volumes of synthetic fabrics and garments find their way to landfill, the environmental impact of natural fiber production cannot be ignored. Cotton, considered by many to be eco-friendly, requires large volumes of chemicals to ensure production volumes meet demand; this has devastated some regions, such as the Aral Sea in Central Asia. Once a rich and fertile land, this region of the world has become near desert-like due to the overfarming of cotton.

These facts paint a bleak picture and suggest the textile industry is in crisis. With added consumer awareness around sustainability issues, the need for change is high on the agenda.

This review will discuss how the textile industry has historically used inspiration from nature to manipulate man-made products and how it is now moving into a new era, looking at nature as a source of new materials.

2. Inspiration from Nature

2.1. Velcro

Velcro® is possibly the most well-known example of biomimicry in textiles [4,5]. In 1941, the Swiss inventor George de Mestral noticed that his dog's fur was covered in small burdock plant burrs after walking in the fields. Upon closer inspection, Mestral discovered that the burrs were covered in small hooked spines, allowing them to attach to other materials [6]. Further investigation led to the development of the hook and loop tape that continues to have numerous uses across the whole textile

industry. This type of development is the perfect example of true inspiration from nature and how a simple investigation of the natural world can be translated into product, which continues to have a huge impact in a variety of end-uses.

2.2. *Pinecones*

The current trend in technical textiles is smart fabrics, those that can sense, adapt, and react to their surroundings [7]. While most of this effect is created on a polymeric scale, some of the principles are based on those found in nature.

The structure of a pinecone is such that seeds are only released once optimal conditions for germination are sensed. In nonoptimal conditions, the pinecone structure remains tightly closed, protecting the seeds. However, at appropriate temperature and humidity levels, the bract scales of the cone bend. This bending is caused by differential swelling of the two types of cellulose found in the bract scale [4,5,8]. The opening of the cone allows seed dispersion and the potential for germination.

This principle has been adopted in both fiber/fabric structures and chemical finishes. Schoeller Textil AG (Sevelen, Switzerland), a Swiss technical textile manufacturer, states that the opening and closing of the pine cone structure was used as inspiration for the development of their c_change fabric [9]. This fabric is already manufactured in bulk and can be found in premium range sports garments [10]. It consists of a polymer membrane sandwiched between two textile layers. When the body is warm, the molecular structure of the polymeric membrane opens to allow the escape of excess heat and moisture. As the body cools, the structure contracts, increasing the insulating properties of the fabric, thus trapping heat against the skin [9]. The company claims that this fabric ensures an optimal body climate [11] due to the adaptive nature of the textile.

MMT Textiles Ltd. (London, UK) has used the reaction to atmospheric conditions of the bract scales found in pine cones as inspiration for the development of Inotek^{TM} fibers [12]. Using the differential swelling principle of pine cone cellulose as a starting point, Inotek^{TM} fibers are engineered to curl and become shorter in length as atmospheric humidity increases. Due to the placement of the fibers in relation to the yarn surface, the yarn becomes thinner as the humidity increases, effectively "opening" the structure. Therefore, the fiber becomes more permeable to air, enabling the flow of moist warm air away from the body and allowing a comfortable temperature to be maintained [5]. As humidity decreases, the fibers return to their original state, thus closing the structure and imparting a degree of insulation to the body. Yet to be found in bulk production, MMT Textiles Ltd. states that they are working with a number of global industry partners to bring this product to the wider market, and that the fiber can be easily spun into yarns to suit both knitted and woven fabric structures [12].

2.3. *Sharkskin*

While many innovations have focused on the surface structure of the textile, the development of the Fastskin suit (Speedo International Ltd., Nottingham, UK) considered the whole garment and how the shape of the animal itself could influence this.

Initially, the skin of the shark was studied with special attention given to the dermal denticles (scale-like structures) on the surface [13]. The denticles are arranged longitudinally on the body axis and in line with the flow of water, but their spacing varies depending on their location on the body [5,14]. It is understood that this arrangement minimizes friction when moving in the water [15]. Developers mimicked this denticle arrangement on the surface of the fabric using a knitted, ribbed structure [16], with tests showing that water friction was reduced by 7.5% [17]. However, it is not only the dermal denticles that contribute to the hydrodynamics of a shark's movement, the shape of the body itself is also a key contributor. Therefore, the swimsuit was engineered to give compression in certain areas, effectively changing the shape of the swimmer's body to further decrease water drag. The seams of the garment were bonded (rather than stitched) to enhance the fit of the garment, allowing the seams to lie flatter against the body [18]. It was rumored that the suit took around 25 min to put on, and swimmers needed to be cut out of the garments due to the closeness of the fit.

Such was the improvement in speed of the swimmers through the water, over 300 records were set in the 2008–2009 season. This led to questions being raised by the International Swimming Federation (FINA), the governing body for competitive swimming, and ultimately a ban on these suits being used in competitions [5].

2.4. Stomatex

The mechanism of respiration in plants requires gaseous exchange of oxygen, carbon dioxide, and water vapor. Stomata (tiny pores) on the surface of plant leaves allow this process to occur. The stomata are thought to open in daylight and close during the hours of darkness, with the motion being controlled by guard cells that react to internal pressure within the plant structure [5].

Stomatex® is a composite technical textile [19,20] that is based on these principles. The base textile is neoprene (a synthetic polymer foam rubber) encapsulated within a synthetic knitted outer layer, into which small dome-shaped structures have been embossed. Each dome has a small hole (pore) at its apex, as illustrated in Figure 1. The textile is designed to be close-fitting, thus able to react to the body's movements. At rest, any excess heat and moisture rises into the domes and is released via the pore. When the body is moving, the domes (and pores) flex and move, allowing heat and moisture out, and cooler air in, thus maintaining a comfortable microclimate [11]. Stomatex® has found specific applications in garments for athletes, particularly those using compression garments to enhance performance and recovery, and in medical support appliances [20].

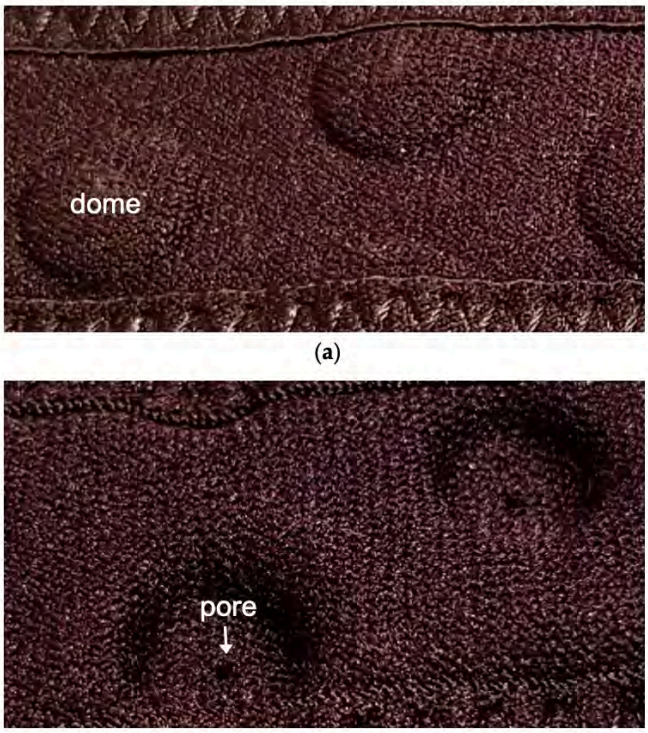

Figure 1. (**a**) Front view and (**b**) reverse view of Stomatex® fabric showing the fabric domes and the pore at the dome apex.

3. New Materials from Nature

3.1. Textiles from Food Waste

Many researchers have investigated the potential of using the waste from the food industry as raw material for textile production and the creation of more sustainable products. Using the unwanted skins and pulps of fruit from juice manufacturing has revealed a rich supply of raw material.

Companies such as Orange Fiber S.R.L. (Catania, Italy), who are utilizing some of the 700 million tonnes of waste from the Sicilian orange juice industry, are creating what is claimed to be a sustainable fibre. Cellulose is extracted from the waste, and is then processed and spun into useable yarn for both knitted and woven fabric production. The company also claims that additional benefits can be gained from the product. It has been well documented that extracts of citrus fruit peel can contain compounds such as essential oils, natural colors, and phenols, which all have associated biological activities such as antioxidant, antimicrobial and anti-inflammatory effects [21]. Orange Fiber is particularly marketing the additional skin moisturizing benefits of their textile, claiming this is due to embedded essential oils [22]. The process is currently filed for patent [23].

However, the true sustainable nature of the fiber is to be questioned. While the raw material would otherwise be waste, the extraction of the cellulose and its manufacture into useable textiles requires several stages of processing, some of which involve chemicals such as hydrogen peroxide, that could be deemed harmful to the environment [24]. Additionally, the performance characteristics of the end textile are not clearly documented and may need some development. The process, detailed in the patent application, suggests that the orange extract is blended with cellulose fibers extracted from wood pulp [23]. The extraction of cellulose from wood pulp is an established route for textile production and is available in the mass market under trade names such as Tencel™ [25].

A different approach to waste utilization is to take the leaves of the plant and use this as fiber to create textiles. Pinatex® uses this principle, taking waste pineapple leaves from food production in the Philippines and processing these into fiber via a manufacturing unit in Spain. The leaves are stripped of excess surface biomatter (decortified) leaving the fibrous inner [26], with any waste from this process being used as biofuel. The fibers then undergo standard textile bulk manufacturing techniques to create rolls of fabric in a nonwoven textile structure, similar to felt. The nonwoven fabric is then chemically treated using established textile finishing processes to give the resulting material a leather-/canvas-like appearance and feel. The resultant textile can be made thicker or thinner to suit the end requirement. It can be coated to enhance durability and performance characteristics such as waterproofness or strength. However, while the fiber itself is 100% biodegradable, the finishing and coating chemicals that are used to make the resultant textile useable are not biodegradable, they are petroleum-based [27]. This is an area that needs to be resolved to meet the demands of a completely sustainable product. Nevertheless, the product is extremely versatile and is being used for a range of commercially available high-end fashion applications where sustainability is used as a marketing focus, such as shoes (Hugo Boss, Metzingen, Germany) and handmade bags (Artesano, Miami, FL, USA) [28]. The product has also been used for clothing, however, these are predominantly signature pieces from independent designers [29,30]; therefore, the ability of the garments to be subjected to standard consumer wash and wear demands should be questioned. Additionally, Pinatex® fabrics have been used in automotive upholstery [27], albeit a concept product only.

One of the largest sources of waste in the food industry is that of seafood shells. Once the edible part of the animal has been prepared and eaten, the shell is discarded. While the shells do biodegrade, and are not contributing to ever increasing landfill, the exoskeleton structure is now seen as an untapped resource. Chitin is the long-chain polysaccharide that is found in the shells; chitosan is created by the deacetylation of chitin. Chitosan has previously found applications in the biomedical and agricultural industries. It can also be spun into fiber using solution spinning techniques, and has been explored as a textile for garment use. While not suitable in its pure form, it has been blended with viscose by Swicofil AG (Emmen, Switzerland), a commercial specialty fiber

and yarn manufacturer, to create Crabyon© [31]. The resultant yarn can be processed into knitted or woven fabric structures, is extremely soft, and absorbs dyes readily. Additionally, the manufacturing process has been developed such that harmful organic solvents are not required, thus improving its eco-friendly status and preserving the natural biodegradability of the chitosan. The performance of the fabric allows it to be used in a range of commercially available garment applications from underwear and lingerie, to sportswear and school uniforms. The range of garment end-uses highlights the durability of the resultant product to domestic wash and wear processes.

3.2. Mushrooms

Mycelium has been explored for some time in the packaging industry, with companies such as Ecovative Design LLC (Green Island, NY, USA) leading the market in this innovation [32]. The technology uses the mycelium of the mushroom and grows these under controlled conditions to produce three-dimensional (3D) packaging structures. This principle has been applied to the more traditional textile fabric structure, with companies such as MycoWorks (San Francisco, CA, USA) leading in the field [33]. The company uses the growing process of mycelium to bind with organic matter, thus creating a "solid" textile, which is more akin to leather in appearance, rather than a traditional knit or woven fabric. The resulting material is flexible, durable, can be dyed easily and with natural dyestuffs, and has a degree of water repellency. It can be grown to shape, thus minimizing any waste. The mycelium structure is biodegradable, but the process is only initiated when the material comes into contact with soil-based bacteria. Therefore, it is suitable for various apparel and wider indoor textile applications [33]. Due to the ability to grow the mycelium-based material into 3D forms, this material has also found applications in furniture and architectural structures [34].

3.3. Microbes

Bacterial cellulose, a relation to plant cellulose or cotton, is one of the most abundant, naturally occurring polymers on the planet. It is most prolifically produced by the *Gluconacetobacter* bacteria species, which can be most commonly found in rotting fruit [35–37]. In contrast to plant cellulose, bacterial production yields cellulose in a highly crystalline state, imparting properties such as improved tensile strength and enhanced water absorbency [35,38]. Additionally, bacterial cellulose occurs in a highly pure form of the polymer, eliminating the need to heavily process the material before it can be subjected to further treatments, such as dyeing, which is the case with plant cellulose. Bacterial cellulose has found applications in the food, paper, and medical industries [39]. However, it has yet to break through into the mainstream fashion arena, despite applications being explored in synthetic fiber coatings and nonwoven cloths [40,41].

Fashion design researchers, such as Suzanne Lee, have experimented with kombucha [42], a symbiotic culture of bacteria and yeast (SCOBY), which is used to ferment tea and is purported to have health-giving properties. When brewed for extended periods, a biofilm forms on the surface of the tea liquor (Figure 2) made up of bacterial cellulose nanofibrils. This biofilm (or mat), when removed from the liquor, and then rinsed and dried, has aesthetic and physical properties like those of fine animal leather. It is commonly referred to as "vegetable leather", however, under high magnification, the structure resembles that of a nonwoven fabric (Figure 3). Furthermore, the biofilm takes the shape of the container in which it is grown, so while whole mats of textile are produced, there is the potential to grow the mats to the shape required for the end product, thus addressing the issue of excess waste fabric in traditional textile manufacturing processes [3]. Conceptual garments have been produced to illustrate the potential of the vegetable leather to be subjected to garment construction techniques such as stitching, bonding and forming into 3D shapes. However, the extremely hydrophilic nature of the bacterial cellulose means it is not suitable for wear in conditions where there may be increased humidity, such as next to the human skin, and therefore, cannot be subjected to domestic washing. Despite this, research continues in this field as the "vegetable leather" can be composted at the end of its useful life and is seen as a viable proposition to alleviate the issue of fabric going to landfill sites.

Figure 2. A bacterial cellulose "biofilm" being formed on the surface of a liquid medium.

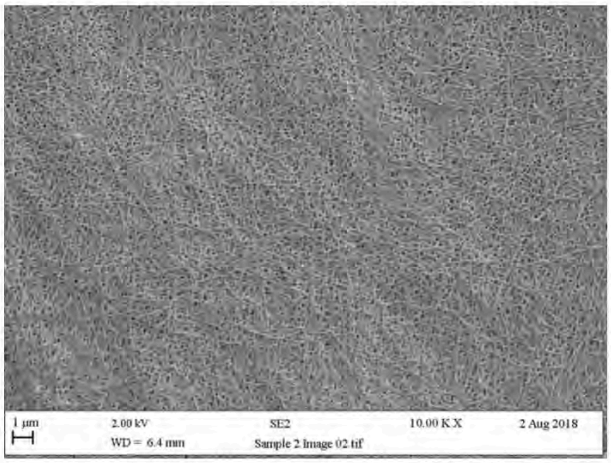

Figure 3. Scanning electron microscopy image of a dried bacterial cellulose biofilm (10,000× magnification).

4. Conclusions

There is no doubt that the textile industry is in crisis and the need for radical change is required. Textiles from natural sources are often assumed as eco-friendly or sustainable, but the evolution of both crop and animal farming has led to extreme demands on land, often with devastating effects. The advancement of synthetic textiles has also impacted the environment, with many of the textiles being nonbiodegradable and adding to landfill growth.

While the textile industry has historically been influenced by nature (with developments such as Velcro®), these have generally been the adaptation of structural principles rather than using alternative natural resources. With consumer focus on minimizing waste, the use of food industry byproducts to create textiles is pertinent and shows promise. True alternative sources, such as mycelium and bacterial cellulose, are still in the early stages of development, but show potential in terms of textile performance properties.

However, textile manufacturers must create profit in order to be viable businesses. There is no doubt that product development costs money, and large investments are required for the investigation of new materials, particularly in such an innovative field as biomimetics. As is the case with Velcro ®, first introduced to the market over seventy years ago, these developments can be highly successful and profitable. Large, well-established, global businesses such as Swicofil AG and Schoeller Textil AG

generally already have a steady product offering that generates income to support such developments and can explore relatively unknown fields. In the case of Schoeller Textil AG specifically, this has proved to be extremely successful as they are constantly broadening their offer of bulk-produced, bioinspired textiles. However, for smaller business where the innovative material is their only product, the future is more uncertain. These companies often rely on business and innovation awards to first become established, and are then often reliant on news or social media to promote their product and fuel further interest. They can also lack ease of access to established manufacturing equipment and expertise, which can prove prohibitive to their growth. Often, the best route to larger scale production for these innovations (such as MMT Textiles Inotek™) is to patent the idea, then look for a more established manufacturer to develop a market and establish a viable bulk manufacturing process.

One must be also mindful that if any of these new sources are to become commercially viable, they must be able to perform to the standard required by the consumer. In apparel and fashion, comfort, flexibility, durability, and the potential to be washed are all key drivers. It is well established in the textile industry that many of these properties can be imparted and enhanced by synthetic chemical finishes. However, this impacts the environment and diminishes the validity of the novel material. True natural alternatives must be engineered to ensure they meet this criterion if they are to present themselves as viable substitutes.

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References

1. Siegle, L. To Die For: Is Fashion Wearing out the World? Fourth Estate: London, UK, 2011.
2. Sustainable Textiles—WRAP UK. Available online: http://www.wrap.org.uk/sustainable-textiles (accessed on 6 December 2018).
3. Fashion Industry Waste Statistics—EDGE. Available online: https://edgexpo.com/fashion-industry-waste-statistics/ (accessed on 22 January 2019).
4. Eadie, L.; Ghosh, T.K. Biomimicry in textiles: Past, present and potential. An overview. *J. R. Soc. Interface* **2011**, *8*, 761–765. [CrossRef] [PubMed]
5. Kapsali, V. *Biomimetics for Designers*; Thames and Hudson: London, UK, 2016.
6. VELCRO® Brand History. Available online: https://www.velcro.co.uk/about-us/history/ (accessed on 22 January 2019).
7. Tao, X. *Smart Fibres, Fabrics and Clothing*; Woodhead: London, UK, 2001.
8. Paris, O.; Burgert, I.; Fratzl, P. Biomimetics and biotemplating of natural materials. *MRS Bull.* **2010**, *35*, 219–225. [CrossRef]
9. c_change–Technologien, Schoeller Textil AG. Available online: https://www.schoeller-textiles.com/de/technologien/c_change (accessed on 4 December 2018).
10. KATUSHA Sports—Premium Cycling Clothing—Apparel Technologies. Available online: https://katusha-sports.com/pages/technologies (accessed on 21 January 2019).
11. Ledbury, J.; Jenkins, E. Composite fabrics for functional clothing. In *Materials and Technology for Sportswear and Performance Apparel*; Hayes, S., Venkatraman, P., Eds.; Woodhead: London, UK, 2015; pp. 104–152.
12. Inotek Textiles. Available online: http://www.inotektextiles.com/ (accessed on 6 December 2018).
13. Teodorescu, M. Applied Biomimetics: A new fresh look of textiles. *J. Text.* **2014**. [CrossRef]
14. Bushnell, D.M.; Moore, K.J. Drag reduction in nature. *Ann. Rev. Fluid Mech.* **1991**, *3*, 65–79. [CrossRef]
15. Koeltzsch, K.; Dinkelacker, A.; Grundmann, R. Flow over convergent and divergent wall riblets. *Exp. Fluids* **2002**, *2*, 346–350. [CrossRef]
16. Wood, J.; Sabir, S. Fabrics for performance clothing. In *Materials and Technology for Sportswear and Performance Apparel*; Hayes, S., Venkatraman, P., Eds.; Woodhead: London, UK, 2015; pp. 87–103.
17. Toussaint, H.M.; Truijens, M.; Elzinga, M. Effect of a Fast-skin 'body' suit on drag during front crawl swimming. *Sport Biomech.* **2002**, *1*, 1–10. [CrossRef] [PubMed]

18. Petrie, E.M. Alternative fabric joining technologies. In *Garment Manufacturing Technology*; Nayak, R., Padhye, R., Eds.; Woodhead: London, UK, 2015; pp. 337–372.
19. Lawler, B.; Wilson, H. *Textiles Technology*; Heinemann Educational Publishers: Oxford, UK, 2002.
20. Floprene, Neoprene Supplier for Neoprene Protective Clothing. Available online: http://www.stomatex.com/index.html (accessed on 4 December 2018).
21. Rafiq, S.; Kaul, R.; Sofi, S.; Bashir, N.; Nazir, F.; Nayik, G.A. Citrus peel as a source of functional ingredient: A review. *J. Saudi Soc. Agric. Sci.* **2016**. [CrossRef]
22. Orange Fiber is the New Green—Orange Fiber. Available online: http://orangefiber.it/en/orange-fiber-is-the-new-green/ (accessed on 4 December 2018).
23. Vismara, E.; Santanocito, A.M. Production of textile from citrus fruit. International Application No. PCT/EP2014/066377, 15 February 2015.
24. Rehan, M.; Nayera, A.M.; Farouk, A.; El-Zawahry, M. Extraction of valuable compounds from orange peel waste for advanced functionalization of cellulosic surfaces. *ACS Sustain. Chem. Eng.* **2018**, *5*, 5911–5928. [CrossRef]
25. TENCEL™ Official Site: Lyocell fibers, Modal fibers, Soft Fabric Textile. Available online: https://www.tencel.com/ (accessed on 4 December 2018).
26. Lo, J.; Hoa, S.V. Design, Manufacturing and Applications of Composites. In Proceedings of the 6th Joint Canada-Japan Workshop on Composites, Toronto, ON, Canada, 23–26 August 2006; DEStech Publications, Inc.: Toronto, ON, Canada, 2006.
27. Ananas Anam. Available online: https://www.ananas-anam.com/ (accessed on 4 December 2018).
28. Artesano—Sustainable Style—Pinatex Bags. Available online: https://artesano.net/collections/sustainability-meets-imagination (accessed on 21 January 2019).
29. Laura Strambi—Eco Friendly Fashion—Milano—Made in Italy. Available online: https://www.laurastrambiyoj.com/ (accessed on 21 January 2019).
30. Studio Frowijn: Home. Available online: https://studiofrowijn.com/ (accessed on 21 January 2019).
31. Crabyon Fibers for Anti-Bacteria Textile Products—Swicofil. Available online: https://www.swicofil.com/commerce/brands/various/crabyon (accessed on 6 December 2018).
32. Grow-It-Yourself—Ecovative Shop. Available online: https://shop.ecovativedesign.com/collections/packaging (accessed on 4 December 2018).
33. MycoWorks. Available online: http://www.mycoworks.com/ (accessed on 4 December 2018).
34. Mushroom mycelium design—Dezeen. Available online: https://www.dezeen.com/tag/mycelium-design/ (accessed on 4 December 2018).
35. Dufresne, A. *Nanocellulose: From Nature to High Performance Tailored Materials*, 1st ed.; Walter de Gruyter: Berlin, Germany, 2012.
36. Jarrell, J.; Cal, T.; Bennett, J.W. The Kombucha consortia of yeasts and bacteria. *Mycologist* **2000**, *14*, 166–170. [CrossRef]
37. Reva, O.N.; Zaets, I.E.; Ovcharenko, L.P.; Kukharenko, O.E.; Shpylova, S.P.; Podolich, O.V.; de Vera, J.-P.; Kozyrovska, N.O. Metabarcoding of the Kombucha microbial community grown in different microenvironments. *AMB Express* **2015**, *5*, 1–8. [CrossRef] [PubMed]
38. Sun, D.; Zhou, L.; Wu, Q.J. Preliminary research on structure and properties of nano-cellulose. *J. Wuhan Univ. Tech.–Mat. Sci.* **2007**, *22*, 677. [CrossRef]
39. Lin, S.P.; Loira Calvar, I.; Catchmark, J.M.; Liu, J.R.; Demirci, A.; Cheng, K.C. Biosynthesis, production and applications of bacterial cellulose. *Cellulose* **2013**, *20*, 2191–2219. [CrossRef]
40. Shoda, M.; Sugano, Y. Recent advances in bacterial cellulose production. *Biotech. Bioprocess. Eng.* **2005**, *10*, 1–8. [CrossRef]
41. Ashjaran, A.; Yazdanshenas, M.E.; Rashidi, A.; Khajavi, R.; Rezaee, A. Overview of bio nanofabric from bacterial cellulose. *J. Text. Inst.* **2013**, *104*, 121–131. [CrossRef]
42. Suzanne Lee: Biocouture—Launch. Available online: https://www.launch.org/innovators/suzanne-lee/ (accessed on 17 December 2018).