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# Visible light photocatalytic bismuth oxide coatings are effective at suppressing aquatic cyanobacteria and degrading free-floating genomic DNA

Running title: Photocatalytic bismuth oxide for water treatment

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## Abstract

Access to safe drinking water free from microbial pollution is an issue of global concern. The use of photocatalytic thin films in water treatment has focused on titanium dioxide, which requires UV-activation, proving a potential barrier to upscaling and implementation in the real world. Visible-light-activated photocatalytic thin films, such as bismuth oxide, have recently been shown to have antimicrobial properties. However, more understanding of the photocatalytic effect on the microbial population in water is required. Glass beads coated with bismuth oxide were incubated with either *Microcystis aeruginosa, Anabaena* sp. or free-

floating genomic DNA. The presence of bismuth oxide-coated glass beads was able to rapidly stop a population of cyanobacteria from increasing. The coated beads were also able to degrade genomic DNA. Leachate from the beads showed no increase in toxicity against human liver cells. This data demonstrates the efficacy of bismuth oxide-coated glass beads for controlling potentially dangerous cyanobacterial populations, whilst potentially reducing the amount of free-floating genomic DNA (an essential issue in the face of antimicrobial resistance) – all of which should be essential considerations in emerging water treatment technologies.

Keywords: Photocatalysis; Bismuth oxide; Cyanobacteria; Water treatment; Antimicrobial

## Introduction

Access to sustainable and safe drinking water is considered a basic human right, recently reinforced by the UN as a designated goal for global sustainable development (United Nations, 2015). However, over two billion people use drinking water contaminated with either human or animal feces, whilst 844 million people lack a basic drinking water service; over 570 million children do not have access to safe drinking water at school (Achtman et al., 2012, World Health Organisation, 2019). Additionally, water scarcity and the lack of access to clean, safe drinking water affects 2.7 billion people for at least one month of the year, with projections of two-thirds of the world's population facing water shortages by 2025 (WWF n.d.).

In drinking water production, contamination from microorganisms and organic pollutants is a problem that must be addressed. Over the past few decades, photocatalysis has gained popularity as a safe, cheap and sustainable method for water decontamination (Fujishima and Zhang 2006, Regmi et al., 2018). Of the practical applications of photocatalysis, water treatment is of particular interest, because photocatalysis provides a relatively simple, yet efficient, technique of mineralizing organic contaminants to carbon dioxide and water without the production of hazardous intermediates. Titanium dioxide (titania, TiO<sub>2</sub>) is the most-studied photocatalytic material for decontamination, depollution and disinfection processes (Fujishima and Zhang, 2006; Chong et al., 2010). It is capable of killing bacteria through the generation of hydroxyl radicals and reactive oxygen species that damage bacterial cell membranes, leading to cell lysis and death (Reddy et al., 2017). Consequently, photocatalysis-mediated disinfection can be favored over the use of other antimicrobial active substances due to its simplicity and long-term antimicrobial action (Markowska-Szczupak et al., 2011). In addition to killing bacteria such as E. coli, photocatalysis has also been shown to break down viruses, and degrade a wide variety of organic contaminants (Lawton et al., 2003; Ryu et al., 2008; Murar and Dhumale, 2015) whilst its potential to damage and break down DNA may help reduce the

sharing of genetic information that can increase the virulence of bacteria (Redfern and Enright, 2020). However, unless doped with another material, TiO<sub>2</sub> is only activated by UV light with a wavelength below 387.5 eV, due to its relatively wide band gap (3.2 eV for the most photocatalytically active anatase phase). This results in the need for UV irradiation sources and causes barriers to upscaling and commercialization. When TiO<sub>2</sub> is doped with certain compounds, it may demonstrate photocatalytic activity in visible light. For example, poly diallyl ammonium chloride-modified TiO<sub>2</sub> has demonstrated the ability to degrade the toxic cyanobacterium Microcystin (MC-LR) under visible light, whilst F-Ce-TiO<sub>2</sub> and N-P co-doped TiO<sub>2</sub> have both demonstrated anti-cyanobacterial activity in degrading MC-LR (Wang et al., 2017a, 2017b, 2018).

However, conventional modification methods such as doping are not always practical for shifting the band gap of titanium dioxide towards the visible, as considerable amounts of dopant are required for significant shifts, owing to the high band gap of TiO<sub>2</sub>. On the other hand, dopant atoms in high amounts typically act as extra charge carrier recombination sites, lowering the photocatalytic activity of the composite material. Therefore, over the past decade the efforts of many researchers have focused on the development and implementation of visible-light-activated photocatalytic materials as alternatives to TiO<sub>2</sub> (Hernandez-Alonso et al., 2009).

Photocatalytic coatings with narrower band gaps than  $TiO_2$  can absorb light with wavelengths in the visible range and are not solely reliant on UV exposure for activation. Bismuth oxide is a narrow band-gap visible-light-activated photocatalyst (Ratova et al., 2017), with a number of studies demonstrating antimicrobial properties through its ability to kill *E. coli* in water (Xu et al., 2013; Helali et al., 2014; Sharma et al., 2016). However, these studies employed freely dispersed particles of bismuth oxide, the use of which may prove difficult in water treatment due to the requirement to recover/remove them from the water before release into the environment or dispersal into the water supply network.

Immobilization of bismuth oxide in the form of thin films on a solid substrate may have greater applicability to water treatment. One such immobilization method is deposition onto a solid substrate via magnetron sputtering, a method that has the advantages of precise composition control, high film uniformity, and scalability (Kelly and Arnell, 2000; Kelly et al., 2014). From this perspective, coated spherical glass beads can be a solution of choice, as they can provide higher area of contact with polluted media compared to flat counterparts, yet can be easily recovered from treated media, unlike nanoparticles. Hence, coated glass beads are frequently used for photocatalytic water treatment processes (Karches et al., 2002; Ratova et al., 2017; Cunha et al., 2018). However, to date there has only been one published paper (Ratova

et al., 2018) detailing the antimicrobial properties of these bismuth oxide coatings, where 2 mm bismuth oxide-coated glass beads reduced the number of *E. coli* from  $10^7$  CFU/mL to below the limit of detection within 24 hr. In addition, the bismuth oxide-coated beads reduced the number of living microbial cells adhered to a surface in a biofilm compared to uncoated controls, therefore demonstrating the presence of antifouling properties. The antimicrobial activity of bismuth oxide coatings against *E. coli* suggests that it has potential as a photocatalytic material for water treatment. However, a real-world water system has a highly diverse microbial community, and it is not possible to assume that an antimicrobial effect demonstrated on one species of microorganism would be the same for all. Therefore, the applicability of bismuth oxide as an efficient material for solar water treatment needs further study, including its ability to inactivate other microorganisms such as cyanobacteria, and degrade organic pollutants.

Cyanobacteria are photosynthetic bacteria, the presence of which can be highly detrimental to the palatability and safety of drinking water. Levels of cyanobacteria in waterbodies are increasing worldwide due to increasing nutrient (N and P) pollution. The additional nutrient input allows cyanobacteria to rapidly increase, leading to a phenomenon known as a harmful algal bloom (HAB). Cyanobacteria can produce some of the most potent natural toxins known (Backer et al., 2015), potentially causing illness and mortality to humans and other members of the ecosystem (Codd, 1995). For example, the cyanobacterial species *Microcystis aeruginosa* produces the hepatotoxic substance microcystin, which can cause serious damage to the liver in humans, as well as damage to other members of the aquatic ecosystem (Jones and Orr, 1994; Oh et al., 2000). In addition to toxins, cyanobacteria can also produce water-tainting compounds such as geosmin (van der Ploeg and Boyd, 1991), the presence of which can adversely affect the taste and odor of drinking water (Srinivasan and Sorial, 2011). Economic loss due to HAB was estimated to be \$2.2 billion in the US alone (Dodds et al., 2009), with blooms likely to increase as a result of the continued effects of climate change (O'Neil et al., 2012).

In addition to the ability to inactivate microorganisms, the safety of the novel photocatalytic coatings in regard to human health also needs to be demonstrated.  $TiO_2$  has long been considered a safe material and categorized as 'biologically inert', leading to wide acceptance in public use (Skocaj et al., 2011). However, little work has been done to evaluate the potential toxicity of bismuth oxide coatings, which must be addressed for use in a real-world application.

Therefore, to be considered as a potential water-treatment technology, bismuth oxide photocatalytic coatings require further study to investigate their various biological effects. This paper describes how bismuth oxide-coated beads may affect the growth of cyanobacterial species important to the production of toxins/organic compounds; the effects on the presence of extracellular genomic DNA; and the safety of the coatings for human cells.

## **1** Materials and methods

#### 1.1 Deposition and characterization of coatings

The deposition and characterization of the coatings has been previously described in detail (Ratova et al., 2018). In brief, 15 g batches of 2 mm diameter glass beads (Sigma Aldrich, UK) were placed in a bowl positioned directly underneath a magnetron mounted in a vacuum chamber. To produce bismuth oxide coatings on the beads the magnetron was fitted with a 99.5% pure bismuth target (300 mm  $\times$  100 mm, Teer Coatings, UK), which was reactively sputtered in an argon and oxygen atmosphere in pulsed DC mode (applied power 600 W, pulse frequency 100 kHz and duty cycle of 50%). The bowl was connected to a vibratory system, which caused the beads to hop and traverse around the bowl, exposing all of their surfaces to the flux of coating material, such that they were approximately uniformly coated during the course of a 1-hr deposition run. Coatings were subjected to post-deposition annealing in air at 673 K for 30 min and then allowed to cool gradually (5–6 hr.) to avoid the formation of thermal stresses in the coatings. Following deposition and annealing of the coatings, their crystallographic properties were studied with X-ray diffraction (XRD) (Panalytical Xpert powder diffractometer with CuKa1 radiation at 0.154 nm in grazing incidence mode at 3° over a scan range from 20° to 70° 20; the accelerating voltage and applied current were 40 kV and 30 mA, respectively). The coating thickness and elemental composition were studied with SEM / EDX (EDAX Trident, Edax Co. installed on a Zeiss Supra 40 VP-FEG-SEM, Edax. Co., USA). The surface area and surface roughness of the coating were analyzed with atomic force microscopy (AFM) (Horiba XPlora Plus, Horiba UK Ltd, UK, scan area of 30 µm ×30 µm, the surface parameters were determined as mean values of 5 scans). The optical properties of the coatings used for band gap value calculation were obtained with UV-visible spectrometry (Ocean Optics USB4000 spectrometer, Ocean Optics, USA). The band gap value of bismuth oxide was calculated by the Tauc plot method (Tauc et al., 1966), by plotting  $(\alpha hv)^{1/2}$  as a function of hv and extrapolating the linear region to the abscissa (where  $\alpha$  is the absorbance coefficient, h is Plank's constant, v is the frequency of vibration).

## 2.2 Antimicrobial effects of bismuth oxide photocatalytic beads on Cyanobacteria

Cultures of Microcystis aeruginosa (CCAP 1450/16) and Anabaena sp. (CCAP 1450/13A), both capable of causing environmentally and economically negative algal blooms, were obtained from the Culture Collection of Algae and Protozoa. Batch cultures (40 mL) were grown using BG11 growth media (Stanier, 1971), in a 45 cm<sup>2</sup> Nunc EasYFlask with filter cap (ThermoFisher Scientific, Loughborough, UK) inside a growth cabinet (Fitotron, Weiss Gallenkamp, UK) set to 23°C, with a 16 hr./8 hr. light/dark cycle using a 60 W daylight bulb (integrated irradiance value in the wavelength range 400-800 nm was 190 W/m<sup>2</sup>). A 24-hr control was studied due to the microorganisms being photosynthetic, meaning they require light in order to survive and grow; however, a previous study has demonstrated that without irradiation, this bismuth oxide coating does not demonstrate antimicrobial activity (Ratova et al., 2018). In order to quantify cell numbers, a standard curve relating optical density (measured at  $680 \text{ nm} - \text{OD}_{680}$  - using a spectrophotometer plate reader) to cell numbers (counted using a hemocytometer - Hawksley, Sussex) was created (data not shown). Prior to starting any experiments, preliminary data demonstrated that following a 250 µL inoculation from a dense culture into 40 mL of fresh BG11 media, a ten-day incubation period was required to ensure cells were actively growing (i.e., optical density measures of the culture were increasing).

Following the preparatory 10-day incubation period described above, 6 mL aliquots were transferred to sterile 45 cm<sup>2</sup> Nunc EasYFlasks. Three different test conditions were created by either adding 1 g of bismuth oxide-coated glass beads (BOGB), 1 g of uncoated glass beads (GB) or no beads. All conditions were tested in triplicate. To assess differences in cell numbers, the OD<sub>680</sub> of 200  $\mu$ L from each test condition was measured daily over 16 days. The experiment was repeated once. Additionally, to assess the potential of the bismuth oxide coating to prevent growth of cyanobacteria in a low-density culture, beads were added at day 0 (i.e., without the 10-day period to establish the growth phase).

#### 2.3 Effect of bismuth oxide thin films on the degradation of free-floating genomic DNA

Lambda DNA (ThermoFisher Scientific, UK) was diluted to a concentration of 5 ng/ $\mu$ L (measured using Qubit 3.0 High Sensitivity dsDNA assay kit) in DNAase-free water, to a volume of 6 mL, in a 45 cm<sup>2</sup> Nunc EasYFlask. One gram of beads (either BOGB or GB) was added, and the mixture was illuminated under the same light source described above. Five  $\mu$ L was aseptically removed (using DNase-free filter tips) and measured for DNA quantity using the same Qubit assay kit every 20 min in triplicate. The experiment was repeated once.

## 2.4 Determination of photogenerated species via trapping reactions

Investigation on reactive species generated on the surface of photocatalytic bismuth oxide under visible light irradiation was carried out using scavenger experiments in a manner similar to earlier published work (Grao et al., 2020). The following scavengers were used: isopropanol (IPA) for •OH, 4-hydroxy-TEMPO (TMP) for  $O_2^{-}$ , sodium oxalate (SO) for  $h^+$  and sodium nitrate (SN) for e<sup>-</sup> (all chemicals used were of analytical purity and purchased from Sigma Aldrich, UK, unless stated otherwise). Since some of the scavengers themselves (e.g. IPA) may have detrimental effects on living media growth, the scavenging experiments were carried out using the model pollutant dye Rhodamine B (RhB), which is frequently used for evaluation of the photocatalytic activity of materials (Ratova et al., 2018, Grao et al., 2020). The dye degradation experiment has been described in detail in earlier work (Ratova et al., 2018); in brief, the RhB absorbance peak height at 554 nm was monitored in real time using an Ocean Optics USB4000 spectrometer. Prior to the experiments, adsorption-desorption equilibrium was achieved by placing the 4 g load of beads into 50 mL of a conditioning solution of RhB dye (the same concentration as the testing solution) and keeping it in the dark for a total time of 1 hr. Then the bismuth oxide-coated beads were placed in a quartz cuvette containing 50 mL of RhB aqueous solution (concentration 2 µmol/L) with continuous magnetic stirring, while being irradiated with the visible light source described in Section 2.2. A series of reference tests was performed, including the uncoated beads in dark and light conditions (no meaningful effect on RhB concentration); tests of the coated beads in the dark; and dye solution photolysis rate tests under the irradiation source with no photocatalyst present. For scavenger tests, 1 mmol of each scavenger at a time was added to the RhB solution.

## 2.5 Cytotoxicity of bismuth oxide thin film leachate on human hepatic stellate cells

One gram of BOGB was incubated in a 45 cm<sup>2</sup> Nunc EasYFlask containing 6 mL of sterile phosphate buffered saline (PBS – ThermoFisher Scientific), under constant illumination for seven days using the aforementioned light source. To assess whether the presence of the BOGB transferred any cytotoxic potential into the surrounding liquid, the PBS was assayed for its ability to damage human hepatic (liver) cells. To achieve this, immortalized human hepatic stellate cells (Lx-2 cells developed by Scott Friedman, Mount Sinai, NYC (Xu et al., 2005)) were cultured in a monolayer at 5% CO<sub>2</sub> and 37°C in Dulbecco's modified Eagle's medium plus L-glutamine, Na pyruvate, and antibiotics supplemented with 10% fetal bovine serum (Sigma, UK). For experiments, Lx-2 cells were plated in 24-well plates in serum-free conditions for 24 hr. PBS that had been incubated with BOGB was added to the cells at a concentrations of 1:10,

1:100 or 1:1000 (with each dilution being replicated in triplicate) and incubated for a further 24 hr. The medium was then replaced with a tetrazolium dye known as MTT (EMD Millipore, Billerica, MA), a colorimetric assay used to assess cytotoxicity of potentially toxic materials (Mosmann, 1983), and incubated for a further 4 hr. Purple formazan crystals, formed by active mitochondrial enzymes (indicative of viable cells), were dissolved in 0.04 mol/L HCl in isopropanol (Fisher, UK) and the intensity was measured at 560 nm (n = 3 experiments), with sterile growth medium used as a blank control.

## 2.6 Statistical analysis

Comparisons between treatments (bismuth oxide glass coated beads) and the control were determined using ANOVA and Tukey's post hoc test. All tests were carried out using Prism Graphpad 7.

## 2 Results and discussion

# 2.1 Characterization of bismuth oxide-coated beads

This work will only discuss those material properties that are of relevance to further presented results and essential for understanding and possible reproduction of the work discussed here. Overall, analysis of the coatings showed that they were identical to those described in earlier work prepared under the same deposition conditions (Ratova et al., 2018). A summary of the analysis is given Table 1. In brief, deposition of bismuth oxide resulted in uniform distribution of a pale-yellow coating on the beads' surface - the variation of coating composition analyzed with EDX at 5 points across the sample surface was no greater than 2%. The average thickness of bismuth oxide coating was 340 nm, obtained from a cross-sectional SEM micrograph (shown in Fig. 1). According to XRD results (XRD pattern is shown in Fig. 1), annealing at 673 K resulted in formation of tetragonal  $\beta$ -bismuth oxide, identified by comparison with crystallographic card JCPDS 96-901-2328. The mean values of the surface roughness and surface area of samples obtained via AFM are given in Table 1; it is evident that the bismuth oxide coatings were relatively smooth and dense. Optical band gap values of the deposited coatings were determined using UV-vis spectroscopy and the Tauc plot method (Tauc et al., 1966); the result is presented in Fig. 1. The band gap of the coatings was 2.40 eV, which corresponds to an absorbance limit of 515 nm, meaning that the material can be photoactivated with light of wavelength 515 nm or lower.

## 2.2 Antimicrobial potential of bismuth oxide photocatalytic beads

For both *M. aeruginosa* and *Anabaena* sp., the presence of bismuth oxide resulted in a significantly lower (p < 0.001) number of cyanobacterial cells 48 hr. after bead addition  $(3.4 \times 10^4)$ , when compared to the controls (addition of no beads or uncoated glass beads, with counts of  $4.32 \times 10^4$  and  $5.18 \times 10^4$  respectively) (Fig. 2). This demonstrates that the photocatalytic bismuth oxide coating can arrest an actively growing population of cyanobacteria and prevent further cell division. Cyanobacterial cells were also bleached, with a reduced level of chlorophyll-a (see Graphical Abstract). That this antimicrobial activity occurred with the 16 hr. light : 8 hr. dark culture conditions demonstrates that an overall antimicrobial effect on a living cyanobacterial culture is achievable even where the photocatalyst is not irradiated continuously (as would be found in the real world). The antimicrobial effect also occurs in the high nutrient conditions of the algal culture media. The literature is yet to come to a consensus on the impact of nutrients on photocatalysis, with some suggesting that nutrients having a role as photosensitizers (benefitting the photocatalytic process), and others suggesting that such nutrients act as a preferential hydroxyl radical scavenger and reduce the number of hydroxyl radicals available to damage microorganisms (Deng and Zhao, 2015).

If BOGB are present when cyanobacteria are inoculated (so cells are in the presence of the beads from day 0), no population develops. **Fig. 3** shows that the presence of BOGB results in a significantly (p<0.001) reduced population by day 16, with 1.606×10<sup>3</sup> cells/mL compared to both experiments with no beads and glass bead controls, with 9.9×10<sup>4</sup> and 9.31×10<sup>4</sup> cells/mL respectively. For *Anabaena* sp., the cell density was significantly (p<0.001) lower in the presence of BOGB at both day 10 (5.93×10<sup>4</sup>) and day 16 (6.2×10<sup>4</sup>) compared to either experiments with no beads (1.266×10<sup>6</sup> at day 10 and 3.29133×10<sup>6</sup> at day 16) or the glass bead control (1.424×10<sup>6</sup> at day 10 and 3.325×10<sup>6</sup> at day 16).

To assess the degradation of the glass beads in the presence of cyanobacteria, and the potential formation of cyanobacterial biofilms, bismuth oxide-coated glass beads were analyzed using SEM (**Fig. 4**). Beads that had been in the presence of cyanobacteria for 56 days revealed no degradation compared to unused BOGB, nor were they covered in a cyanobacterial biofilm.

A previous study has demonstrated the ability of BOGB to photocatalytically kill the Gram-negative bacterial species *E. coli* (Ratova et al., 2018), whilst this study demonstrates a bacteriostatic effect against cyanobacteria, which are similar to *E. coli* in that they are also classified as Gram-negative microorganisms. However, recent literature suggests that this typical classification is over-simplified, particularly where cyanobacteria are concerned, due to their more complex, thicker cell wall structure (Hoiczyk and Hansel, 2000). Similarly,

cyanobacteria have different mechanisms for responding to oxidative stress compared to E. *coli*, in part due to the wide range of environmental habitats (and therefore stressors and dangers) within which cyanobacteria exist (Latifi et al., 2009). This disparity in cell structure and stress response underlines the need to verify the antimicrobial efficiency of novel photocatalysts with a range of different microorganisms, and whilst testing against model microorganisms such as *E. coli* is important, considering the variety of microorganisms likely to be found in the environment is essential.

#### 2.3 Effect of bismuth oxide thin films on the degradation of free-floating genomic DNA

It is now widely recognized that DNA in the environment may prove to be a major reservoir of antibiotic resistance genes, which can persist longer than chromosomally encoded DNA, potentially allowing for transfer into microorganisms via horizontal gene transfer (HGT) (Mao et al., 2014; Nagler et al., 2018). Whilst there are enzymes found in the environment (DNases) that are capable of breaking down DNA, the recent focus on horizontal gene transfer and its role in the development of AMR (e.g., Watts et al., 2017) demonstrates the need for additional technologies to break down DNA in water. However, there is little available research describing the effect of photocatalysis on DNA, and that which does exist almost exclusively concentrates on UV-activated  $TiO_2$  (e.g., Kim et al., 2013).

The results from this study suggest that bismuth oxide-coated glass beads were able to degrade extracellular genomic DNA in solution at a steady and consistent rate, degrading 1 ng/ $\mu$ L in approximately 180 min (**Fig. 5**), and showing a significant decrease (p<0.05) compared to the control in 80 min. Degradation and damage of DNA by photocatalysis is caused by single-strand breaks and mutations in nucleic acid sequences (Chong et al., 2010), and when this activity occurs on DNA encoding for antimicrobial resistance, the gene may be damaged or destroyed. This study suggests that BOGB have the potential to efficiently break down DNA in a variety of forms such as genomic DNA, plasmids and mobile genetic elements.

## 2.4 Determination of photogenerated species via trapping reactions

To better understand the mechanism of photoexcitation of bismuth oxide, and determine the photogenerated species upon visible light excitation, a series of scavenger experiments was performed using a common model pollutant – Rhodamine B dye. The dye with chemical formula  $C_{28}H_{31}ClN_2O_3$  belongs to the thiazine dye family and is frequently used for assessment of bismuth compounds' photocatalytic properties and underlying mechanisms (Sood et al., 2015; Ratova et al., 2018; Wang et al., 2019). The plots of RhB degradation kinetics (where,

 $A_0$  and  $A_t$  are the absorbance values of RhB solution at 554 nm for time 0 and time of measurement, respectively) with no trapping agents, and four types of scavengers during each 1 hr. experiment are presented in **Fig. 6**, as well as photolysis rate (test under visible light with no photocatalyst). While the photolysis rate was relatively low (under 4% during a 1 hr. experiment), it is clear from the presented results that all types of photogenerated species tested participated in the photoreaction; hence, addition of scavengers resulted in much lower rates of RhB degradation, compared to experiments without addition of trapping agents. From the effect of scavengers on the reaction rate, it can be concluded that sodium nitrate and sodium oxalate had the highest quenching effect, meaning that electrons and holes play important roles in the photocatalytic process. However, the roles of •OH and O2 • were also significant, based on the quenching effects of isopropanol and 4-hydroxy-TEMPO, respectively. The obtained results are in good agreement with the work of Raza et al. (2018), who observed similar results for bismuth oxide-based photocatalysts with the use of different scavenging agents.

## 2.4 Cytotoxicity of bismuth oxide thin film leachate on human hepatic stellate cells

The above results suggest that a bismuth oxide coating has potential for water treatment; however, it is necessary to show that bismuth oxide does not have any adverse effects on human health. To date, there has been no literature assessing the toxicity of bismuth oxide coatings, although a study has suggested that bismuth oxide nanoparticles may be toxic to human cell lines (Abudayyak et al., 2017). This study assessed the liquid within which a bismuth oxide coating was incubated, mirroring the potential toxicity of water that may be consumed if BOGB had been used as an antimicrobial. MTT assays showed there was no change in cell viability (i.e., no significant increase in optical density) when cells were treated with PBS that had been incubated with BOGB (**Fig. 7**). **Fig. 7** shows the viability of cells at three different dilutions of the bismuth oxide-exposed PBS. There was no difference in viability across the three dilutions, indicating that the BOGB had no toxic effect. Additionally, cell viability in the presence of active beads (illuminated) was not significantly different from that with inactivated beads (not illuminated). These data suggest that bismuth oxide coatings do not pose the risk of cytotoxicity observed with bismuth oxide nanoparticles; however, further leachate studies are required to understand other impacts of the leachate.

# **3** Conclusions

Visible-light-activated photocatalytic bismuth oxide-coated glass beads provide a chemicalfree, non-toxic technology for photocatalytic water treatment that only requires sunlight for activation. Treatment of a water-body can arrest the growth of cyanobacterial populations, or even prevent cyanobacterial populations from being established, whilst potentially reducing the amount of free-floating genomic DNA (an essential issue in the face of AMR) – all of which should be essential considerations in emerging water treatment technologies. Combined with the ease of preparation and ease of removal of the beads when no longer required, bismuth oxide coatings demonstrate significant potential as a visible light photocatalyst for use in water treatment.

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**Table 1** Overview of compositional, surface, phase and optical properties of bismuth oxidecoatings deposited onto 2 mm glass beads and annealed at 673 K.

Analyzed parameter	Analytical technique	Result
Coating thickness	SEM (cross-sectional)	340 nm
Coating composition	EDX	44 at.% Bi / 56 at.% O
Crystal phase	XRD	Tetragonal $\beta$ -bismuth oxide
Surface roughness	AFM	8.3 nm
Surface area	AFM	$904 \ \mu m^2$
Band gap	UV-Vis spectroscopy / Tauc plot	2.40 eV
Photoactivation	UV-Vis spectroscopy / Tauc plot	$\leq$ 515 nm
wavelength		



**Fig. 1** Selected results of analysis of bismuth oxide-coated glass beads: XRD pattern (a), cross-sectional SEM (b), Tauc plot method band gap calculation (c). WD = Working distance.



**Fig. 2** Cell density of the cyanobacteria *Microcystis aeruginosa* (a) and *Anabaena* sp. (b) following addition at day 10 of either bismuth oxide-coated glass beads, glass beads or no beads. Cyanobacteria were actively growing at the time of treatment. Values represent means (n=3) and error bars represent ±1 standard deviation. Days at which the number of cells exposed to bismuth oxide are statistically significantly lower (p<0.001) than controls are indicated by \*.



**Fig. 3** Cell density of *Microcystis aeruginosa* (a) or *Anabaena* sp. (b) inoculated into fresh medium containing either bismuth oxide-coated glass beads, glass beads or no beads. Values represent means (n=3) and error bars represent ±1 standard deviation. Days at which the number of cells exposed to bismuth oxide are statistically significantly lower (p<0.001) than controls are indicated by \*.



**Fig. 4** Scanning Electron Microscopy image of Bismuth Oxide Glass Bead that had no exposure to *Microcystis aeruginosa* (a), compared to one that had been exposed to a culture of *Microcystis aeruginosa* for 56 days showing no degradation or biofilm formation (b),

photograph of bismuth oxide-coated glass beads showing visual appearance (c) WD: Working distance.



Fig. 5 Quantification of free-floating Lambda genomic DNA after treatment with either bismuth oxide-coated glass beads or glass beads. Values are mean (n=3) and error bars represent ±1 standard deviation. Time points where the amount of DNA is significantly lower (p<0.05) are represented by \*.



**Fig. 6** Reaction kinetics of Rhodamine B dye on bismuth oxide photocatalyst under visible light irradiation with addition of different trapping agents.



Fig. 7 Absorbance readings from MTT assay performed on LX-2 human hepatic cells in the presence of PBS that had previously been incubated with photocatalytically active bismuth oxide-coated glass beads. Inactivated beads (incubated in darkness) were used as a control. There were no statistically significant differences in absorbance at any dilution, demonstrating no additional cytotoxicity for the solution. Values represent means (n=3) and error bars represent ±1 standard deviation.