A comparative investigation of non-catalysed versus catalysed microwave-assisted hydrolysis of common North and South European seaweeds to produce biochemicals.

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Abstract

Using a circular economy approach, this study investigated microwave assisted non-catalysed (water only), acid catalysed (H_2SO_4), and metal cation catalysed (Pb^{II} and Al^{III}) hydrolysis of brown and red seaweed biomass prior to and following extraction of high value products. Results show a wide variety of organic acid products is attained following each hydrolytic method, which are heavily depending on initial quantity and type of carbohydrates in seaweed as well as process conditions, such microwave reactor temperature and catalyst properties. The best results were achieved with extracted *Gracilaria gracilis* residues, which resulted in 20.1% w.t. levulinic acid, produced at 180 ˚C after 10 minutes with an overall carbohydrates conversion rate over 95%. Metal ion catalysed hydrolysis of the same extracted seaweed also yielded interesting quantities of lactic acid (5% w.t. at 240 ˚C), 5-HMF (6% w.t. at 220 ˚C) with Pb(II) and propionic acid (5% w.t. at 220-240 ˚C) with Al(III), bringing seaweed residues closer to full bioresource valorisation.

Keywords: Seaweed; Platform Chemicals; Chemo-Catalysis; Microwave; Hydrolysis.

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1 Introduction:

The world needs low emission, renewable, sustainable and economical fuel sources to replace fossil fuels. Aquatic plants (seaweed or macroalgae) could play a very important role in the international renewable energy scenario in the coming years as a carbon-neutral feedstock for biofuels and bioenergy [1, 2]. Macroalgae have several advantages when compared with terrestrial plants, with the most important being their cultivation does not occupy arable land. Furthermore, their extractable chemicals and bioactives are currently used as food supplements and additives, cosmetics, nutraceuticals, and drugs [3]. According to FAO Stats [4], the global seaweed industry is worth more than USD 6 billion per annum (approximately 12 million tonnes per annum in volume), of which 85% is used for human consumption with about 40% making the world's hydrocolloid market (seaweed extracts). Recently, the role of seaweed biorefineries for co-production of high value chemicals such as fatty acids, oils, natural pigments, antioxidants, biological components and others [5] has attracted a lot of attention as pre-step to biofuels production. In addition, when processed for commercial bioproducts extraction, a significant amount of residues is generated (20-25% w./w.) [6], which is still carbohydrate-rich [7] and could be used for high-value commodity chemicals or biofuels production, where custom pretreatments can play a crucial role at maximising the extraction value chain [8, 9].

Hydrothermal processing is a common method [10] for extracting complex carbohydrates from wet marine biomass and for breaking these down to sugar monomers [11] that can be subsequently used for biofuels. Acid, alkaline, autoclaving, microwave and enzymatic hydrolysis have been investigated by Hernández et al. [12] for saccharification of microalgal biomass as pretreatment prior to bioethanol production, where the acid, microwave and autoclave conversion route were found to enable a poor sugar release. Poor sugar release was species dependant and attributed mainly to the need for disruption of microalgal lipidic cell wall, which is not found in macroalgae (seaweed). Some studies [13, 14] on seaweed thermal hydrolysis reported the method would induce the formation of inhibitor-compounds, which are toxic for biofuels production via fermentation. Park et al. [14] identify particularly 5-HMF, levulinic acid and formic acid formation from hydro-processing of seaweed *Gelidium amansii*, with inhibiting concentrations rising with treatment time. Nonetheless, some of these compounds classed as 'inhibitors', when followed by biological technologies for biofuels, have a rising market value as commodity chemicals for a wide range of applications. Levulinic acid, for instance, is a building block for making fuel additives, solvents and polymers [15]. Lactic

acid can be produced from seaweed hydrolysates [16] and has very recently been commercialized into seaweed-based biopolymers opening an emerging field of investigation for the generation of more sustainable bioplastics. In this respect, recent advances in the conversion of cellulose into lactic acid found that the latter can be chemically synthesised in hot water by simply adding a metal cation [17]. An outstanding yield of lactic acid (68%) was attained in the presence of diluted Pb(II) in a concentration of 7 mM for the conversion of ball-milled cellulose at 190 °C after 4 h. The Lewis acidity of Pb(II) plays a key role in the formation of alkyl lactates. This may be due to their catalytic functions in the isomerization and retro-aldol reactions, which are key steps in the conversion of glucose to alkyl lactates. If found technically and commercially viable, this and similar biorefining concepts can be easily retrofitted into existing hydrolysing systems with minimal upgrade cost to suite macroalgal substrates.

The literature lacks studies where different systems for seaweed hydrolysis have been investigated and compared, therefore the scope of this study was to evaluate the nature of the biochemical products attainable from seaweed in relation to the hydrolysis conditions (non-catalysed, acid catalysed, and metal cation catalysed). Two of the most prevalent Northern European brown seaweeds, i.e. *Aschophyllum nodosum* (commonly used in biotechnology for extraction of phycocolloids) and *Laminaria digitata* (currently used mainly as ingredient in kelp supplements or cosmetics), and a Mediterranean red seaweed *Gracilaria gracilis* were selected for this study, which aimed to determine whether the type of hydrolysis process affected the production of biochemicals from seaweed. *Gracilaria* has been chosen as representative of the Mediterranean region, as it is one of the dominant indigenous species with a recently recognised potential for phycobiliproteins production [18]. These species also hold a great economic potential in medical diagnostic and natural colorants markets for food and cosmetics, proteins for food and feed [19] and agar polymer as mesoporous material [20].

2 Methods

2.1. Source and storage of seaweed samples

Samples harvesting (fresh) occurred in 2015. Brown seaweeds *Laminaria digitata* (LD) and *Aschophyllum nodosum* (AN) were collected on-shore in June in Tracth beach (Co. Galway, Ireland) during low tide. The red seaweed *Gracilaria gracilis* (GG) was collected in the Lesina Lagoon (Southern Adriatic Sea, Italy) at the beginning of August of the same year. The seaweed biomass was immediately dried after collection at 105 °C, until constant weight was achieved, then stored in a desiccator until use. The samples were coded based on species and labelled 'Raw' and 'Res', respectively referring to use 'as is' or 'extracted' for bioproducts, prior to hydrolysis and chemo-catalysis trials. More details are provided in section 2.3. Due to small quantity available at the collection site, limited trials could be performed on AN-Raw seaweed, which was solely used in metal cation-catalysed hydrolysis.

2.2. Analytical methodologies

2.2.1. Proximate and ultimate analysis of seaweed biomass

Dried samples were taken from the desiccator, manually ground (pestle and mortar) to suitable particle size then sieved to fine powder (≤1 mm).

Proximate analysis (moisture, ash, volatile solids and fixed carbon) of biomass and extraction residue was performed using a thermogravimetric analyser TGA 701 (LECO), following ASTM D7582 method [21]. The dry matter fraction in total solids is reported as TS (%), while the organic volatile solids is reported as VS (% d.w.).

The elemental analysis of the samples (C, H, N, S and O) was achieved with the method LECO-ASTM-D 5291 [22], which was performed with an elemental analyser CHNS-LECO 680. The % of O was indirectly calculated as complementary percentage to the sum of each element's percentage and the ash content. These tests were conducted in triple repetition.

2.2.2. Carbohydrates and organic chemicals analysis

Carbohydrates and organic platform chemicals content of the dried-ground seaweed biomass was determined prior to and following all hydrolysis trials, using a HPLC Agilent 1200 (Agilent, USA) equipped with a Refractive Index Detector (Agilent, USA) and a Aminex HPX-87H organic acids column (Bio-Rad, USA), which was thermostated at 60 °C. The injection volume was set at 20 µL and H_2SO_4 0.005 M fluxed isocratically at 0.7 mL min^{-1} was used as the mobile phase. The amount of each product was determined using calibration curves derived from standard solutions. Analytical-grade standard monosaccharides cellobiose, d-glucuronic

acid, d-galacturonic acid, d-glucose, d-galactose, d-xylose, d-mannose, d-rhamnose, d-mannitol and d-arabinose were purchased from Sigma-Aldrich Co. Short chain fatty acids, lactic (LA), formic (FA), acetic (AA), levulinic (LVA), propionic (PA), isobutyric and butyric (BA), isovaleric and valeric, 5-HMF, furfural and caproic, were purchased from Sigma Co., Ltd. Sugars and product yields were determined via standard procedure (NREL/TP-510-42618, NREL/TP-510-42623) [23, 24]. The carbohydrate conversion X (wt.%) and organic acid yield Y_i (wt%) were defined according to eq.1 and eq. 2 respectively:

$$X = \left(1 - \frac{Mass\ of\ unconverted\ carbohydrates}{Structural\ structural\ carbohydrate\ contenet}\right) \text{x100}\ (eq.1)$$

$$Y_i = \frac{mass\ of\ organic\ acid\ i}{mass\ of\ seaweed\ reacted} \text{x100}\ (eq.2)$$

2.2.3. Extraction of high-value products from *Ascophyllum nodosum*

High value bioproducts (laminarin, mannitol and alginate) were extracted and retained as "AN-Res". The extractions were performed in series by soaking 200 g of algae pieces (2 cm) in three separate buckets, respectively, containing 3L of ethanol 99.9% pure for pigments, then a mild acid solution (acetic acid pH 5.5) for laminarin/mannitol and finally a 5L solution of 10% w/w Na_2CO_3 (pH 9.5) for alginate at room temperature for 3 hours per step, according to [25]. Samples were squeezed and dried at 105ºC until constant mass weight was achieved.

2.2.4. Extraction of phycobiliproteins from *Gracilaria gracilis*

GG-Raw underwent extraction of phycobiliproteins as performed by [18], which is thereafter referred as GG-Res. For the extraction of phycobiliproteins, the GG-Raw dried-sample (0.5 g d.w.) was ground manually with pestle and mortar to <1mm, then the mixture was suspended in 10 mL of 1 M acetic acid–sodium acetate buffer (pH 5.5) with 0.01% of sodium azide for 30 min in the dark. After the incubation with buffer, samples were ground for 5 minutes, using a Potter homogeniser (Marconi, model MA099). The mixture was transferred in a centrifuge glass tube and centrifuged at 5 °C at 15,000 rpm for 20 min. Supernatant was separated from the solid fraction, which was then dried and stored as described in section 2.2.1.

2.3. Hydrolysis and chemo-catalysis of seaweed samples

As illustrated in Figure 1, the dried raw macroalgae biomass (AN-Raw, LD-Raw and GG-Raw) and the residue obtained from two different extraction processes (AN-Res and GG-Res) were

characterized (as described in 2.2.1 and 2.2.2.), then hydrolysed using the approaches described in Table 1. The experimental variables tested were catalyst (water only, H_2SO_4, $Pb(NO_3)_2$ and $AlCl_3$) and reaction temperature, whilst treatment time was kept constant at 10 minutes. LD was the only species that was studied as raw substrate and, therefore, not subjected to any bioproduct extraction prior to the hydrolysis trials.

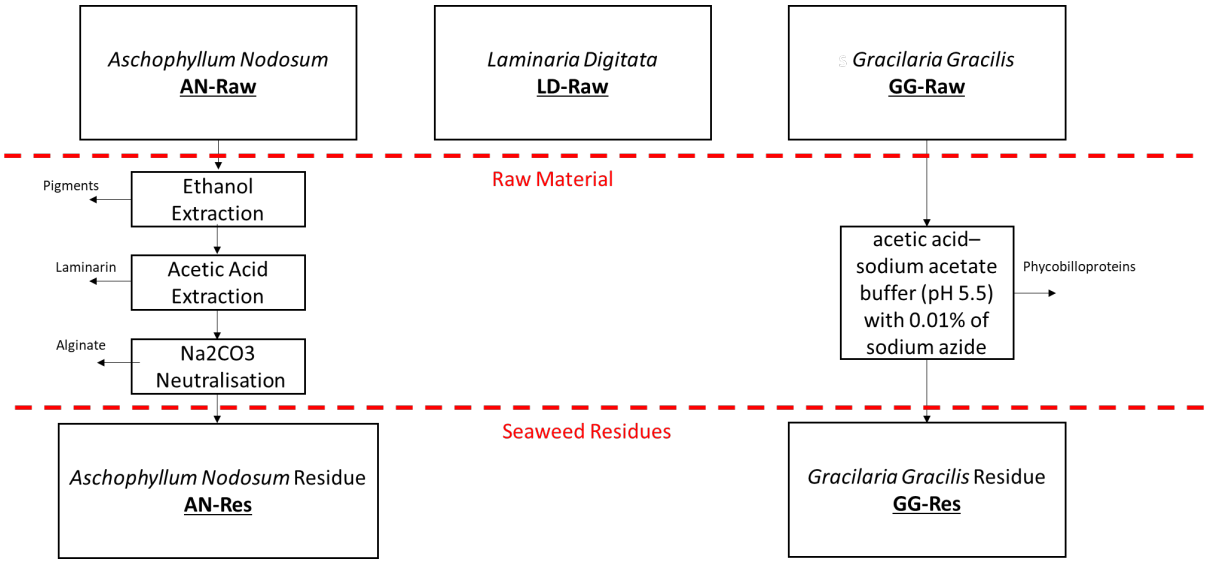

Figure 1 – Flow diagram of the pre-treatment steps of the macroalgae samples

Table 1 Experimental conditions of non-catalysed and catalysed hydrolysis

Process		Coded sample	Catalyst concentration	Microwave reactor Temperatures
Non-catalysed (water only)		AN-Res LD-Raw GG-Raw/Res	-	160 - 180 ˚C
Acid-catalysed (H_2SO_4)		AN-Res LD-Raw GG-Raw/Res	3 %wt.	
Metal cation-catalysed	Pb^{II}	AN-Raw/Res LD-Raw GG-Raw/Res	7 mM	160 - 240 ˚C
	Al^{III}			

Three aqueous diluted catalyst solutions were prepared respectively with H_2SO_4 at 3 %wt. for the acid catalysed hydrolysis and Pb(II)/Al(III) at a concentration of 7 mM for the metal cation catalysed hydrolysis. Such metal catalyst concentration and range of reactor temperature was selected as a recent study [17] reported high concentration of lactic acid formation (~70% wt.) from cellulose-derived glucose, which could be effective for the conversion to lactic acid of the real biomass matrices investigated here. Non-catalysed and acid-catalysed reactor

temperatures were instead investigated within 160 - 180 ˚C, as this thermal interval is used by most studies and would therefore provide results for comparison with existing literature.

For each type of microwave hydrolysis, 0.05g sample was added to 2 mL of catalyst in a closed vessel microwave equipment (CEM-Discover). The vessel was sealed with a septum, placed in the microwave apparatus, and heated to the desired temperature under magnetic stirring (300 rpm) for 10 minutes. A ramp temperature of 5 min was set for each experiment. Temperature in the vessel was measured by means of an IR and fiber optic sensor. The microwave frequency used for the reaction was 2450 MHz. The graphs presented in the results section (3.2), exclusively report yields (%wt.) for seaweed species and type (coded -Raw or -Res) of sample that exhibited appreciable differences when varying thermal conditions.

2.4. Statistical Analysis

All the experiments were repeated three times (n=3). Unless otherwise stated, all data were expressed as mean ± standard deviation (SD). The means of all the parameters were examined for significance by analysis of variance (ANOVA) using the software JMP version 9 (SAS Institute Inc., Cary, North Carolina, USA). When F values showed significance, individual means were compared using Tukey's honest significant difference (HSD). Significant differences were considered when $p < 0.05$.

3 Results and discussion

3.1 Seaweed biomass composition and Summary of findings

The compositional characterisation summary is provided in Table 2. The structural carbohydrates content varies significantly between seaweed feedstocks, with GG being the richest in cumulative carbohydrates content among raw seaweed species. Following phycobiliprotein extraction, GG-Res showed the highest sugar content at nearly 57% wt. compared to the raw form at 38.41% wt. This indicates that about a third of the present sugars are insoluble and were not degraded by the mild extraction process. Therefore, sodium azide extraction may be a useful pre-treatment for red seaweeds to concentrate the sugars.

AN-Raw presents the lowest sugar content of all the raw seaweeds with the dominant fraction being rhamnose/mannitol, which is believed to be primarily mannitol [26]. During the multistage extraction process most of the rhamnose/mannitol fraction was removed (which dropped from 7.1% to 1.8%) indicating that the substrate is not tightly bound to the polysaccharide matrix and can be easily dissolved. Both AN-Raw and AN-Res exhibited <15% carbohydrates content despite the elemental carbon content is comparable to unextracted GG and LD. This indicates that there is an unidentified carbon source that is not fully hydrolysed, most likely alginate of fucoidan, which normally requires enzymes addition [27].

LD (raw only) also has significant amount of rhamnose/mannitol at 19.3%, with mannitol believed to be the primary fraction. It is interesting to note that mannitol is a sugar alcohol that may react significantly differently to normal sugars. LD-Raw also included a glucose fraction of 10.4%, which is low compared to that reported by other literature sources [28].

The sulphur content is high across species and sample type with all of them in excess of 1% with the exception for LD-Raw. Sulphur can be part of the polysaccharide matrix as either fucoidan and carrageenan forms in brown and red seaweeds respectively. It has been proposed [29] that the sulphur is bound to uronic acid polymers and sulfonates, and it is difficult to dissociate. The oxidation of the sulphur was not investigated further here, but as it is a known catalysts poison and it is important to point out that it may interact with homogenous catalysts.

Significant amounts of FA, AA, LVA and 5-HMF (Table 2) were detected in AN-Res and GG-Res, attributed to prior extractive processing. However, GG-Raw exhibits important amounts of organic acids, which are believed to result from the water-extractives procedure, indicating this species is very susceptible to degrade even at extremely mild conditions.

*Table 2 Compositional properties of seaweed samples**

Composition [% w/w]	AN-Raw	AN-Res	GG-Raw	GG-Res	LD-Raw
Cellobiose	-	-	-	-	-
Glucuronic acid	1.57±0.11	0.13±0.02	0.42±0.08	0.53±0.11	4.16±0.27
Galacturonic acid	0.82±0.04	0.54±0.04	0.69±0.09	2.55±0.17	2.95±0.19
Glucose	2.03±0.13	1.66±0.10	12.14±0.41	18.89±1.02	10.42±0.52
Galactose-Xylose- Mannose	1.79±0.08	2.82±0.19	20.47±0.57	29.25±1.14	0.84±0.04
Rhamnose-Mannitol	7.13±0.48	1.77±0.14	3.25±0.12	5.38±0.31	19.29±0.81
Arabinose	0.00±0.00	0.00±0.00	1.44±0.09	0.00±0.00	0.00±0.00
Total carbohydrates	**13.34±0.84**	**6.91±0.49**	**38.41±1.36**	**56.59±2.75**	**37.67±1.83**
Lactic acid	-	-	-	-	0.02±0.01
Formic acid	-	-	3.82±0.26	1.81±0.16	-
Acetic acid	-	2.66±0.22	-	4.27±0.41	-
Levulinic acid	-	-	2.20±0.19	1.16±0.13	-
Propionic acid	-	-	-	-	-
Isobutyric acid	-	-	-	-	-
Butyric acid	-	-	-	-	-
Isovaleric acid	-	-	-	-	-
5-HMF	-	-	2.96±0.26	4.03±0.31	-
Valeric acid	-	-	-	-	-
Furfural acid	-	-	-	-	-
Caproic acid	-	-	-	-	-
Total of carbohydrates and organic acids	**13.34±0.84**	**9.58±0.71**	**47.38±2.07**	**67.85±3.76**	**37.69±1.84**
Moisture	6.85±0.15	4.88±0.07	6.58±0.11	1.33±0.01	2.35±0.03
Ash	16.75±0.01	19.57±0.16	19.85±0.47	8.99±0.17	17.3±0.13
Fixed Carbon	7.95±0.43	3.13±0.09	11.43±0.05	13.38±0.56	7.12±0.47
Volatile	68.44±0.27	72.42±0.01	62.15±0.31	76.31±0.39	73.31±0.30
Volatile Dry	73.48±0.41	76.13±0.06	66.52±0.41	77.34±0.40	75.05±0.33
Ash Dry	17.99±0.03	20.58±0.16	21.25±0.48	9.11±0.17	17.66±0.15
Fixed Carbon Dry	8.53±0.44	3.29±0.1	12.24±0.08	13.56±0.56	7.29±0.47
C	37.79±0.01	37.43±0.05	33.16±0.06	42.20±0.40	34.29±0.05
H	5.24±0.02	5.37±0.13	5.72±0.01	6.23±0.03	5.63±0.07
N	1.46±0.11	1.26±0.00	4.50±0.13	3.03±0.05	1.58±0.01
S	1.54±0.05	2.47±0.11	1.42±0.08	1.14±0.05	0.36±0.08
O	20.2±0.02	17.1±0.01	55.2±0.03	38.4±0.02	40.89±0.02

**Data reported as mean ± SD, n=3 repetitions.*

Table 3 summaries the overall results of catalysis shown in Figures 2-8. A detailed justification with comparison with literature results is provided in the following sections, dedicated to uncatalyzed hydrolysis (3.2), acid hydrolysis (3.3) and metal ion catalysed (3.4) reaction trials. Moreover, section 3.4 is further divided into sub-sections, which discuss the results in relation to the seaweed species investigated: *Ascophyllum nodosum* (3.4.1), *Laminaria digitata* (3.4.2) and *Gracilaria gracilis* (3.4.3).

Table 3 Major products achieved by microwave assisted hydrolysis reaction*

	Non-catalysed	Acid	Lead	Aluminium
AN-Raw	Not conducted	Not conducted	FA	AA
AN-Res	FA, AA	FA, AA, LVA	FA	AA, FA
LD-Raw	FA	LVA, FA	FA	AA
GG-Raw	FA, PA	LVA, FA	FA, 5-HMF/PA	FA, AA
GG- Res	AA, FA, 5-HMF	LVA	5-HMF, LA, FA	LVA, PA, FA

*** Acid products acronyms:** Acetic (AA), Butyric (BA), Formic (FA), Lactic (LA), Levulinic (LVA), Propionic (PA), 5-Hydroxymethylfurfural (5-HMF).

3.2 Non-Catalysed Microwave Assisted Hydrolysis

The main organic acid yields from the non-catalysed aqueous (microwave assisted) hydrolysis of all seaweed samples are reported in Figure 2 in relation to testing conditions. It is clearly observed that the process conditions are sufficient to initiate the degradation of seaweed, with significant yields of FA formed for all seaweed species at 180ºC. For AN-Res, the temperature data indicate a slightly higher FA formation (by 6%) at 180ºC compared to 160ºC, and an increased level of AA is also observed, with 180ºC yielding around 1.5% more AA. The apparent temperature dependence of all samples is an indication of organic acids being the products of the hydrolysis reaction and not previous processing. No other organic acids were observed in significant concentrations, but small quantities of LA, LVA and PA were detected, while 5-HMF was only recorded for GG-Res at 180ºC. These minor components may be indicative of either sugar, polysaccharide or protein hydrolysis, though cannot be made definitive without further work.

The LD-Raw and GG-Res samples showed the highest conversion to a variety of commodity chemicals, with AA and FA being the primary hydrolysis products, followed by lactic acid and 5-HMF, respectively. For both types of GG samples there were noticeable temperature dependent increases in product yields, with GG-Res showing a very different set of organic acids (from 160 to 180 ºC), possibly caused by the activation of different reaction pathways, as there is minimal difference in carbohydrate conversion. The latter was noticeably higher for the red seaweeds (GG-Raw and GG-Res in excess of 90% conversion); however, product yield was not significantly higher than the other seaweed varieties. This high carbohydrates conversion with minimal yields may be caused by the process conditions not being harsh enough to hydrolyse the sugars into their free forms, as also reported by [12].

One of key polysaccharides of brown seaweed is alginate, whose decomposition under hydrothermal [30] and acidic [31] conditions was investigated in detail by Jeon et al. The authors reported a large concentration of dicarboxylic acids from alginate decomposition, including succinic and glycolic acids, which were not detected in this study. The lack of these dicarboxylic compounds could indicate that natural alginate in seaweed is far more resistant to breakdown. Alginate was extracted in AN-Res, which explains the higher carbohydrates conversion compared to AN-Raw (from 27% to 45% approximately). Alginate and carrageenan are both anionic polysaccharides [32] and a similar trend in carbohydrates conversion can be observed for GG-Raw and GG-Res at 180 ºC.

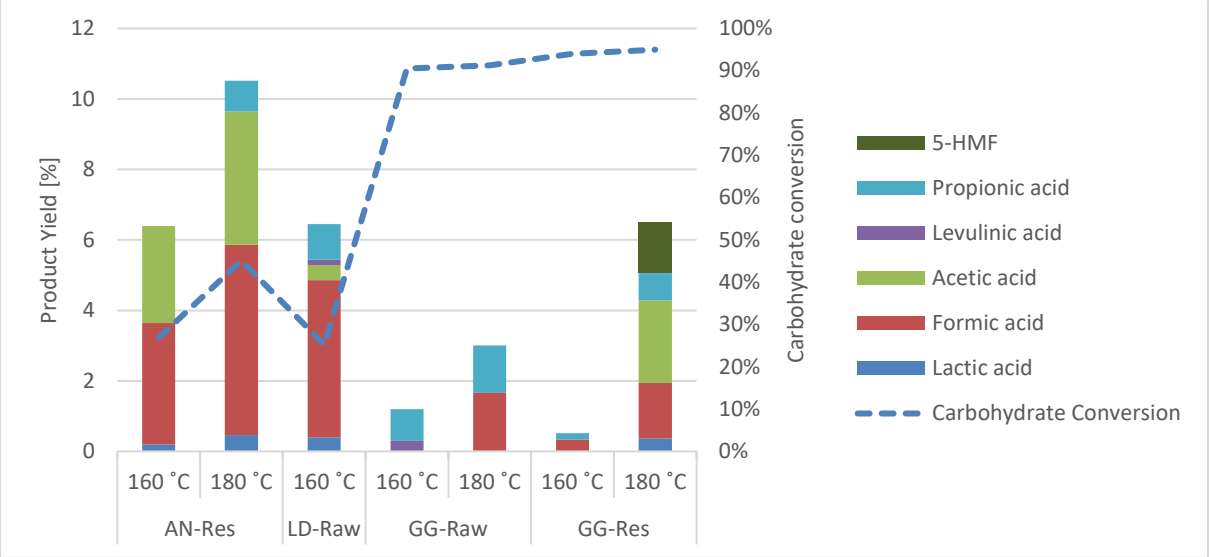

Figure 2- Non-catalysed treatment (microwave assisted) of seaweeds. Numeric data (mean±SD, n=3) are reported in Supplementary Material (Table S1)

3.3 Acid Microwave Assisted Hydrolysis

Saccharification of macroalgae using sulfuric acid hydrolysis was successfully reported by Jang et al. [33]. The sulphuric acid-catalysed hydrolysis of seaweed yielded significant quantities of levulinic acid (LVA), which confirms what was suggested in another, very similar study conducted by Yuan and Macquarrie [34]. When reporting on the effect of temperature in a hydrolytic microwave system fed with AN-raw, the authors noted a monosaccharide yield decrease at 180 ˚C, which was allegedly attributed to formation of LVA, though this was not measured. From Figure 3, it can be clearly seen that acidic conditions are sufficient to promote a significant production of LVA, formic acid (FA) and acetic acid (AA) in descending order. The red seaweeds (GG) resulted in the highest organic acid yields and GG-Res at 180˚C had a LVA yield of 21.1%, value in line with a similar study [35] which found a yield of 16.3% (normalised product-to-carbohydrate and organic acid ratio of 0.31 compared to 0.23) from *Gracilaria lemaneiformis.* Such yield is very high compared with that obtained here from AN-Res at the same temperature. This difference can be primarily attributed to the initial carbohydrate and organic acids content (in fact the normalised conversion ratio for AN-Res is ~0.3). These preliminary results indicate that GG is the most promising of the three species for LVA production, supporting the findings reported by Francavilla et al. [36] in microwave assisted conditions (10 min, 180 °C) with 1% v/v sulfuric acid.

The LD-Raw sample presented particularly high conversion of sugars, yet had the lowest organic acid yield during acid hydrolysis, with the largest being LVA. It may be possible the mannitol sugar alcohol could be alternatively dehydrated into isomannide under the acidic conditions, masking the overall yields [37]. Though this was investigated further in another study [38], which found that isomannide and its stereoisomer isosorbide are promising polymer precursors with the potential to replace bisphenol A.

It was observed that for AN-Res the organic acid yields exceeded that of the initial sugars content. This suggests that the sugars are not the only reactant in the seaweed substrate and that the uncharacterised alginate or other polysaccharide is also reacting. However, there was no dicarboxylic acid detected, which is normally associated with full alginate hydrolysis [39]. This suggests alginate degradation is inhibited, by either cross-linking or ionic interference. In the case of AN-Res, the primary products were FA and LVA with molar ratio exceeding 3.8, which is higher than that stoichiometrically predicted for the formation of LVA. While the formation of acetic and propionic acid is uncommon during acid hydrolysis of cellulosic biomass [40], it consistently occurred under all non-catalysed and catalytic conditions as shown by Figures 2-

8. Therefore, the inhibited degradation of the alginate may primarily lead to formic, acetic and propionic acid.

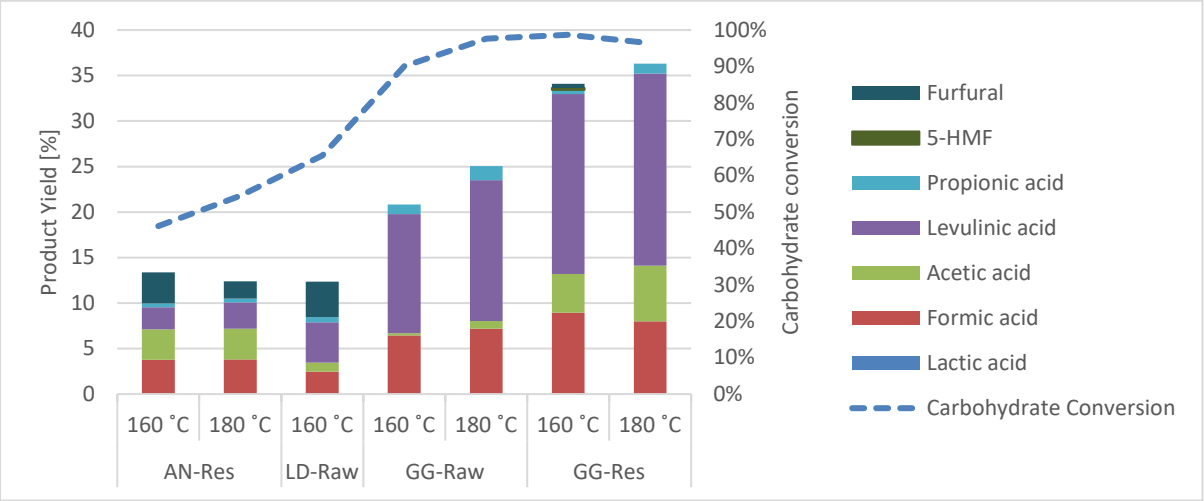

Figure 3 – Acid catalysed (microwave assisted) treatment of seaweeds. Numeric data (mean±SD, n=3) are reported in Supplementary Material (Table S2)

3.4 Lead- and aluminium-catalysed microwave-assisted hydrolysis

3.4.1 *Ascophyllum nodosum*

Figures 4 and 5 show the conversion data for the AN-Raw/Res samples, hydrolysed at different temperatures in the presence of the two metal cation catalysts under investigation. With both catalysts there appears to be synergy between temperature and product yields, with the dominant product being AA at all conditions except for Pb(II) at 240˚C using either AN samples. In the temperature region between 220-240˚C with Pb(II) as well as Al(III), lactic acid (LA) becomes a constant by-product of the reaction clearly at increased proportions than those detectable at lower temperatures. This is consistent with the findings of Deng et al. [41], which studied the transformation of cellulose and its derived carbohydrates into LA via bifunctional Al(III)–Sn(II) hydrothermal catalysis at ~190 ˚C. These suggest that Al(III) mainly catalyses the isomerisation of glucose or the C_3 intermediates, which is the first in a tandem of steps for the synthesis of LA. Similarly, the formation of butyric acid (BA) can be noticed in Figures 4 and 5 to appear exclusively in this temperature interval with both metal catalysts, though in low concentrations (<1%), which has not been previously reported in the literature. The formation of BA at these conditions is recurrent for all species, particularly with Al(III)-catalysed reactions. BA is a carboxylic acid mostly used as industrial solvent, but it is also ranked amongst the most promising biofuels for replacing gasoline in the future, particularly in its role to forming biobutanol [42].

For AN samples, the overall organic acids were low with yields not exceeding 6% for AN-Raw using Al(III) and Pb(II), with yields lower than acid-catalysed and non-catalysed hydrolysis. The primary observed organic acids were FA and AA with high carbohydrate conversions exceeding 80% and 60% for AN-Raw and AN-Res respectively. The catalysts are both metal ions that have been found to be absorbed by living seaweed in the alginate matrix. The acidic alginate has been found [43] to absorb and bind to cations reducing the number of active sites free for hydrolysis, which may be responsible for the lower organic acids yield. Though unproven this may indicate that metal contamination of seaweeds could inhibit catalysis and future catalyst selection criteria should identify possible heavy metal contamination.

It should also be noted that at lower temperatures (<180˚C) the Al(III) catalyst had higher organic acid yields than Pb(II) in stark contrast to the LD and GG samples, indicating Pb(II) effectiveness is kick-started by higher temperatures for this substrate.

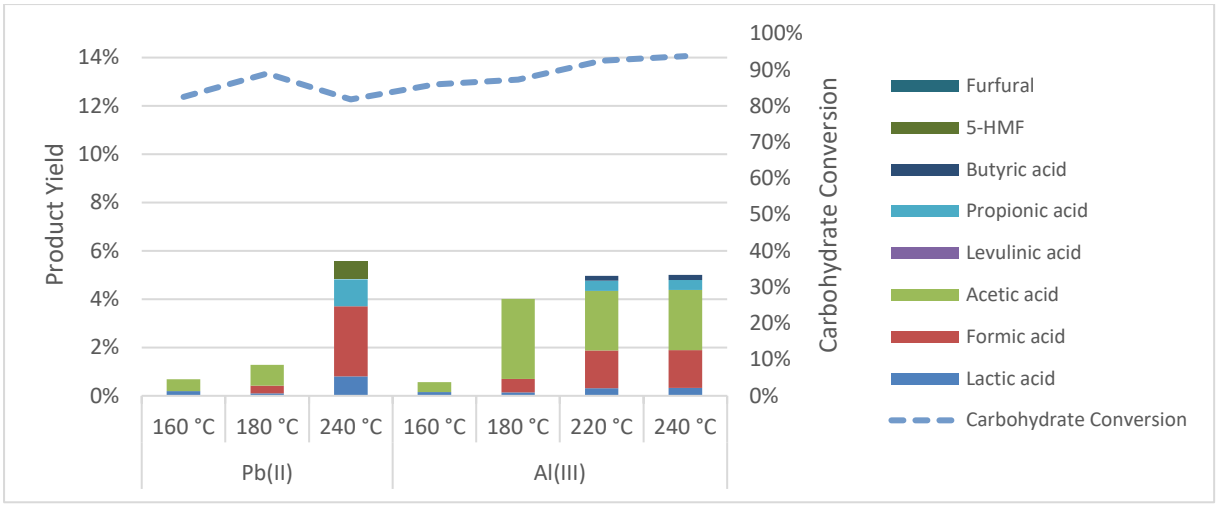

Figure 4 – AN-Raw lead- and aluminium-catalysed (microwave assisted) hydrolysis. Numeric data (mean±SD, n=3) are reported in Supplementary Material (Table S3)

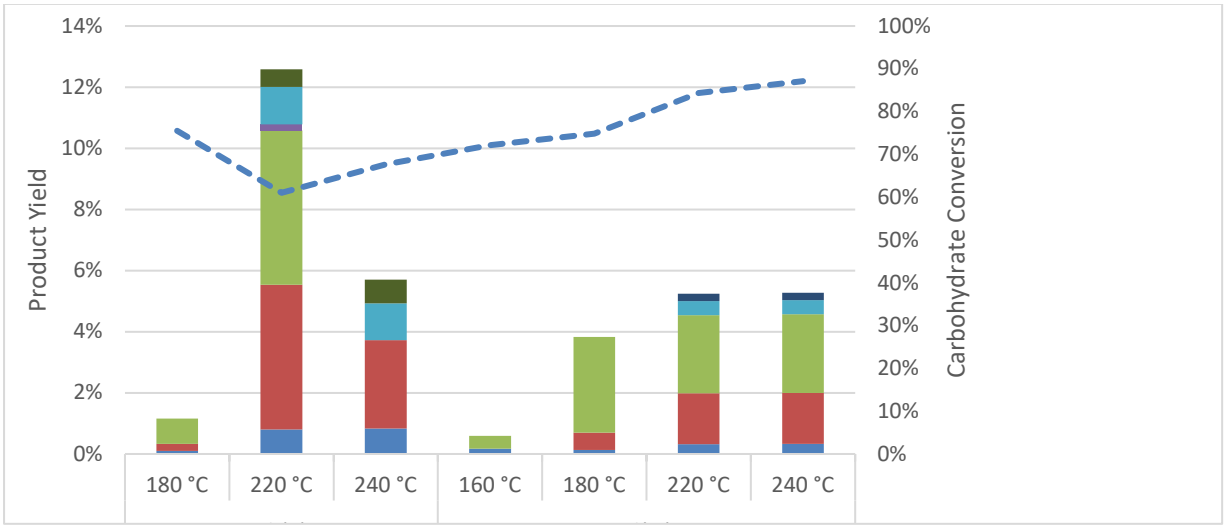

Figure 5 – AN-Res lead- and aluminium-catalysed (microwave assisted) hydrolysis. Numeric data (mean±SD, n=3) are reported in Supplementary Material (Table S4)

3.4.2 *Laminaria digitata*

Figure 6 shows the results for the lead- and aluminium-catalysed hydrolysis of LD samples. The lead catalyst clearly favours FA, which also appears to be the highest yield product from alginate degradation in the temperature range of 160-220˚C identified by another study [31] when using metal ion catalysts. The higher temperatures also coincided with formation and/or an increase in LVA and 5-HMF yield, which is indicative of a higher acidity of the solution, which however did not result in higher sugar conversion, despite Pb(II) yielded the most detectable organic acids, with a total cumulative organic acids yield of 7-9%. The carbohydrate conversion in fact remained below 40% with Pb(II) with the majority of the unreacted carbohydrates being composed of rhamnose-mannitol. This clearly shows that Pb(II) cannot catalyse reactions of sugar alcohol mannitol unlike Al(III), which exhibited carbohydrate conversions in excess of 90% (Figure 6). As a strong Lewis acid Al(III) appears sufficient to catalyse the conversion of mannitol but its concentration (7mM) is evidently insufficient to further catalyse other sugars into levulinic acid, somewhat favouring AA formation compared with both the water-only as well as sulphuric acid conditions. In addition, it can be seen that Al(III) is shifting the degradation of uncharacterised polysaccharides towards other products (up to 50% wt. of the converted carbohydrates could not be identified), which need to be appraised by follow-on research using methods other than ion-exclusion column separation. As discussed previously, this catalyst was found to yield LA, also this case in negligible quantities (<1%).

Finally, propionic acid (PA) levels almost doubled when using Pb(II), though it is produced from all seaweed species (Figure 4-8). A very recent work by Liu et al. [44] reported high conversion yields from bio-based LA to PA using alkali metal catalyst NaI. However, there is significant lack of literature on catalytic production of PA from seaweed. Therefore, more research to determine the kinetic mechanisms leading to its formation/increased concentration are needed, due to its relevance in the food and feed industry.

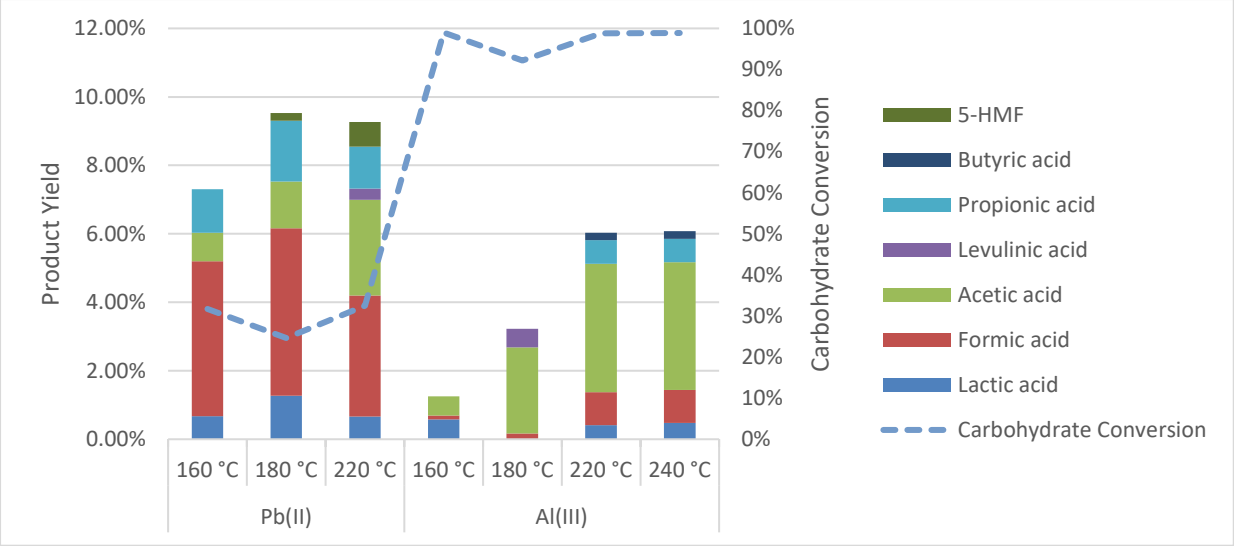

Figure 6: Lead- and aluminium catalysed (microwave assisted) hydrolysis of LD seaweed. Numeric data (mean±SD, n=3) are reported in Supplementary Material (Table S5)

3.4.3 *Gracilaria gracilis*

Figures 7 and 8 show the lead and aluminium catalysed conversion of GG-Raw and GG-Res samples. The processed GG-Res exhibited significantly higher carbohydrate conversion yields than the raw sample, indicating at the explored conditions the removal of high value compounds such as phycobiliproteins has a positive impact on the overall platform chemical yields. The extraction process using a sodium acetate-sodium azide buffer is not particularly harsh, however the centrifugal separation method used could have affected macro-structures indicating a possible benefit of mechanical pre-treatment of red seaweeds for producing organic acids.

GG-Raw hydrolysis with Pb(II) catalyst produced noticeable amounts of FA, PA and 5-HMF at temperatures 180-220˚C. However, at the highest temperature setting (240˚C) was accompanied by a significant increase in LA, LVA and PA yield at the expense of the complete disappearance of FA. It appears this high temperature may significantly alter the reaction kinetic as there was no noticeable change in carbohydrate conversion. The decreased carbohydrate conversions of GG-Raw with Al(III) could instead be potentially resolved with longer reaction times, although this would carry little benefits as the primary products were FA and AA, which are of relative economic value. A few investigations [14, 35, 45, 46] performed hydrolysis of GG species within a temperature range comparable with this study and used homogeneous and heterogeneous catalysts, reporting high yields of LVA and FA (in decreasing order) with small quantities of 5-HMF as the main reaction products, mostly in range with these results at unoptimized conditions. However, the major reaction product amongst those mentioned above for all of these investigations appears to be catalyst dependent.

As previously mentioned the GG-Res resulted in significantly higher yields of organic acids than the unextracted sample, with nearly a 5% yield of LA at 240˚C with Pb(II), alongside high yields of LVA and FA. Similarly to the GRA-Raw conversion, there was a sharp reduction in total organic acid yields in conjunction with an increased selectivity between 220-240˚C with Pb(II), which further reaffirms the possibility of highly changed reaction kinetics. Al(III) catalysed reactions of GG-Res at 220-240˚C present high yields (~5% w.t.) of PA (Figure 8) and, as previously mentioned, more research is needed to clarify this aspect of the conversion pathway in its direction as well as that leading to the formation of vary small yield of BA at the highest temperatures as observed with both metal catalysts.

At 180˚C, a 3% LVA was found in correspondence of the lowest carbohydrates conversion of 77%, which suggest that Al(III) was sufficient to hydrolyse the formation of free sugars and produce LVA. While, at the higher temperatures, the minimal concentration of unconverted sugars points to a series of reactions with chemical compounds released during the extraction process. This highlights the lack of knowledge on the uncharacterised portion of seaweed that may directly affect catalysis. In all instances, Pb(III) showed superior conversion efficiency for platform chemicals than Al(III).

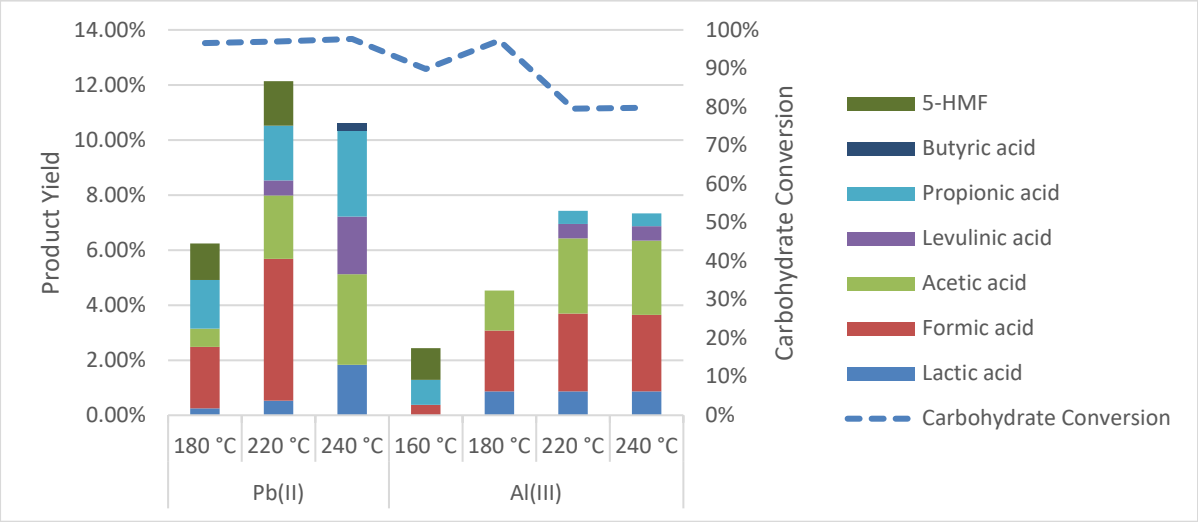

Figure 7: Lead- and aluminium-catalysed (microwave assisted) hydrolysis of GG-Raw seaweed. Numeric data (mean±SD, n=3) are reported in Supplementary Material (Table S6)

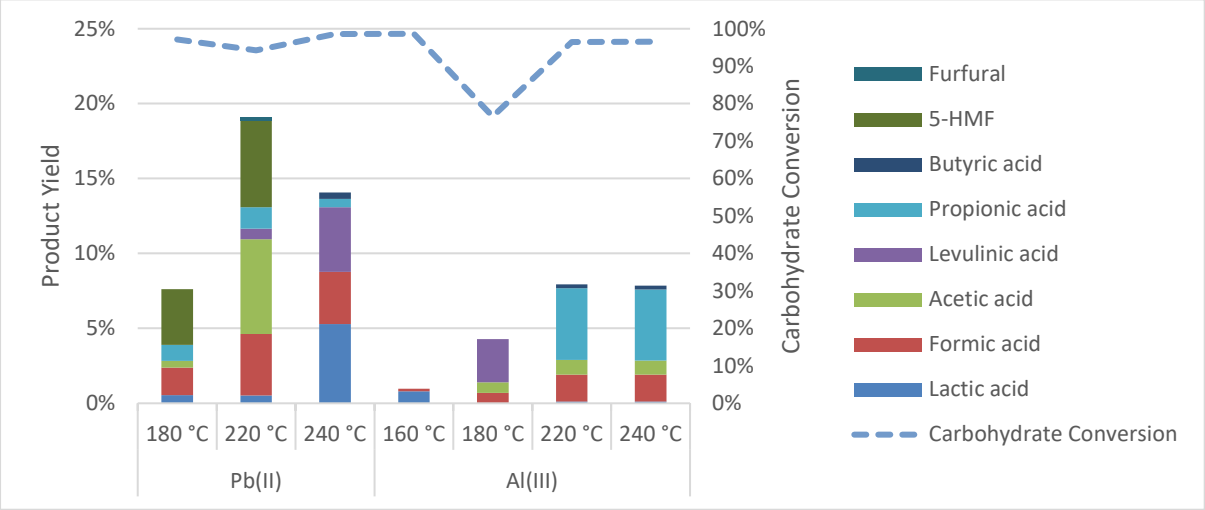

Figure 8 - Lead- and aluminium-catalysed (microwave assisted) hydrolysis of GG-Res seaweed. Numeric data (mean±SD, n=3) are reported in Supplementary Material (Table S7)

4 Conclusions

The microwave assisted production of platform chemicals from seaweed using non-catalysed and catalysed hydrolysis within a biorefinery concept remains a difficult proposition due to lack of understanding on how to comprehensively characterise seaweed biochemical composition. The use of homogenous catalysts (sulphuric acid) showed the production of levulinic would be possible in large quantities from seaweed and *Gracilaria gracilis*, with yields of nearly over 20% w.t. of biomass. Metal ion catalysts such as Pb(II) and Al(III) proved more challenging, indicating that specialised catalysts will be required to increase the target product selectivity as existing cellulosic catalysts are not universal to seaweeds. However, biorefinery integration by extraction of high value chemicals is promising as the mild extraction processes of high value products prior to microwave hydrolysis significantly improved yields compared with the raw (freshly harvested and unextracted) samples.

This work offers an interesting preliminary investigation of seaweed catalysis that will provide a good basis for future work while elaborating on potential issues. In details, as metal ion catalysed hydrolysis resulted in interesting quantities of lactic acid, 5-HMF, butyric and propionic, this work identified research gaps to be bridged to clarify aspects of the hydrolysis conversion pathway leading to these important by-products formation. Finally, acetic and formic acid were yielded in almost all microwave-assisted hydrolysis trials of both red and brown seaweed samples (extracted and not), with or in absence of a catalyst. These organic acids appear to be common by-products of seaweed microwave hydrolysis.

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'We wish to confirm the following CRediT roles for this work:

Dr Tedesco: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Writing - original draft; Writing - review & editing
Mr Hurst: Formal analysis; Writing - original draft; Writing - review & editing;
Dr Edward Randviir: Formal analysis; Investigation; Writing - review & editing;

Dr Matteo Francavilla: Conceptualization; Formal analysis; Investigation; Methodology; Supervision; Writing - review & editing'.

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