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Biorefinery of red seaweed *Palmaria palmata* for production of bio-based chemicals and biofuels

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ABSTRACT

The seaweed *Palmaria palmata* was used as feedstock for production of chemical/fuel precursors (acetone, butanol, ethanol) and biogas. *Palmaria palmata* consists of xylan as major cell wall polysaccharide, as well as galactan and glucan. First, the biomass was subjected to acid hydrolysis at laboratory scale to solubilise fermentable sugars using acetic or hydrochloric acid as catalysts. Differences were observed in composition and amenability to hydrolysis of seaweeds from different harvests. Highest saccharification yields were obtained using HCl (pH 1.7, 120 °C, up to 80 % of xylose). Hydrolysates were fermented to acetone-butanol-ethanol by *Clostridium beijerinckii* reaching yields of 0.28 g products/g consumed sugars. The process by-products (solids after acetic acid hydrolysis and spent fermentation broth) were used as feedstocks for anaerobic digestion showing biogas yields between 310 and 650 L/kg dry organic material when mixed in different ratios with sugar beet pulp. Subsequently, the hydrolysis and fermentation processes were upscaled up to a 100-L pilot volume where nanofiltration was implemented to increase sugar concentration and remove salts. While high monomeric sugar yields were replicated during upscaling, fermentation inhibition was observed in the upscaled process. This paper shows *Palmaria palmata* to be a suitable feedstock for the co-production of bio-butanol and biogas and highlights process development needs to desalt and detoxify seaweed hydrolysates prior to fermentation.

1. Introduction

As one of the fastest growing biomass at European latitudes, seaweeds or macroalgae have a great potential as feedstocks for the production of food/feed ingredients, bio-based chemicals, biofuels and bioenergy [1–3]. Cascading biorefinery concepts, in which multiple products are obtained, hold the best prospect for economically and environmentally feasible utilization of marine biomass [4]. Near- or offshore cultivation of seaweeds does not compete for arable land or fresh water use. Seaweeds have a great potential for large-scale cultivation due to their high productivities, reported to be 30–110 dry t/ha/y compared to 10–30 dry t/ha/y for common terrestrial crops [5,6]. Finally, seaweeds are a potential source for (specialty) chemicals including functional polymers (e.g., alginate, fucoidan), uronic acids (e.g., mannuronic acid, guluronic acid), sugar alcohols (e.g., mannitol), aldoses (e.g., glucose, xylose, galactose), deoxy sugars (e.g., rhamnose, fucose), proteins (e.g., Rubisco), amino acids/peptides (e.g., glutamic acid, aspartic acid), pigments/colorants (e.g., fucoxanthin), nutrients (e.g., K, Ca) and other minerals [4,7,8].

Palmaria palmata, also known as (red) dulse and dillisk, belongs to the *Rhodophyta* group and displays a reddish-brownish colour due to natural pigments. It grows on northern coasts of the Atlantic and Pacific

Abbreviations: ABE, Acetone-Butanol-Ethanol; AD, Anaerobic digestion; dw, dry weight; HPLC, High performance liquid chromatography; ICP-AES, Inductively Coupled Plasma Atomic Emission Spectroscopy; LS, Liquid-to-solid ratio; PAD, Pulsed Amperometric Detection; RI, Refractive Index; VFA, Volatile Fatty Acids.

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oceans, and has been consumed as a food for centuries [9,10]. *Palmaria palmata* consists primarily of xylan, representing up to 35 % of the dry weight (dw) of the biomass [1]. Other main constituents of *Palmaria palmata* are floridoside (2-O-D-glycerol-α-D-galactopyranoside with trace amounts of L- and D-isofloridoside), polymeric glucose, lipids, proteins, and ash [11,12]. As for all seaweeds, the content of carbohydrates and proteins present in *Palmaria palmata* depends on cultivar, season and growth location [12]. *Palmaria palmata* harvested from wild stocks has been mostly known for its value as food supplement in particular in Western countries. However, open-sea and closed-systems cultivation of *Palmaria palmata* has been studied in order to increase the supply in addition to wild harvest [13–15]. Scalable and sustainable *Palmaria palmata* biomass production via cultivation provides also the opportunity to expand on applications other than food, including the recovery of high value chemicals (e.g. phycoerythrin, mycosporine-like amino acids) and biorefining to biofuels and chemicals [15].

Production of biofuels and chemicals from seaweeds has been widely studied and various reviews have been published recently [3,16,17]. An overview with studies that use seaweeds as feedstock for fermentation and/or anaerobic digestion is included in the Supplementary materials. Most studies on liquid fuel production from seaweed focus on fermentation of the carbohydrates to ethanol or butanol. In case of ethanol production from seaweeds, the state-of-the-art reported ethanol yields for red and brown seaweeds seem to be of the same order of magnitude, ~105–114 L/ton dry seaweed [18–21]. Methane productivity through anaerobic digestion of seaweed seems to vary between 130 and 300 L/kg of volatile solids, while being promoted by implementing pre-treatment technologies such as wet milling [8,22]. Production of acetone, butanol and ethanol (ABE) by anaerobic fermentation of seaweed has been reported for brown and green seaweeds with yields between 0.23 and 0.48 g ABE per g consumed sugars, depending strongly on the degree of saccharification achieved through acid and/or enzymatic hydrolysis as well as the degree of optimisation of the fermentation conditions and used strains [23–27].

In this manuscript, we describe the use of *Palmaria palmata* biomass as feedstock for ABE and biogas production (Fig. 1). In the presented biorefinery approach, seaweed biomass is first hydrolysed to release monomeric sugars that were subsequently fermented to ABE. Anaerobic digestion of resulting residues, i.e. solid remaining after acid hydrolysis and the remaining aqueous stream from fermentation, was explored as a means to produce biogas for bioenergy purposes. The acid hydrolysis and fermentation processes were further demonstrated at larger scale (100*L*), in order to validate the feasibility of the biorefinery process.

2. Materials and methods

2.1. Materials

All chemicals were used as obtained from the supplier: H_2SO_4 (72 %, p.a.) from Boom B.V.; HCl (32 % analytical grade), acetic acid (100 %, analytical grade), and $BaCO_3$ (analytical grade) from Merck; HCl (25 % technical grade) and NaOH (30 % technical grade) from VWR. Mannitol (Sigma Aldrich, ACS reagent), glucose (Sigma Aldrich, >99.5 %), xylose (Fluka >98 %), floridoside (CarboSynth), fucose, arabinose, galactose, and rhamnose (L-rhamnose monohydrate) (Fluka, HPLC grade, >99 %) were used as sugar standards for analysis.

For screening experiments of the acid hydrolysis, four samples of fresh *Palmaria palmata* (samples I, II, III and IV) were used. These samples were kindly provided by Ocean Harvest Technology (Galway, Ireland) and harvested from natural stocks in Galway, Ireland, between late spring and summer, stored in polystyrene boxes with ice packs for transportation. After transit (2–3 days), the biomass was stored at 4 °C and used within a week of collection. For larger scale experiments, both fresh and dry flakes *Palmaria palmata* (samples V and VI) were used, since there was insufficient fresh biomass available to reach the targeted amount of sugars to be produced. Dry seaweed flakes were provided by Ocean Harvest Technology (Galway, Ireland) and used without further treatment. Fresh *Palmaria palmata* was kindly provided by The Scottish Association for Marine Science (summer, North Luing, Scotland), frozen prior to transportation and further stored frozen at −20 °C upon arrival until use. Free water from all fresh seaweeds was drained off manually before use (see Table 1 for dry matter content).

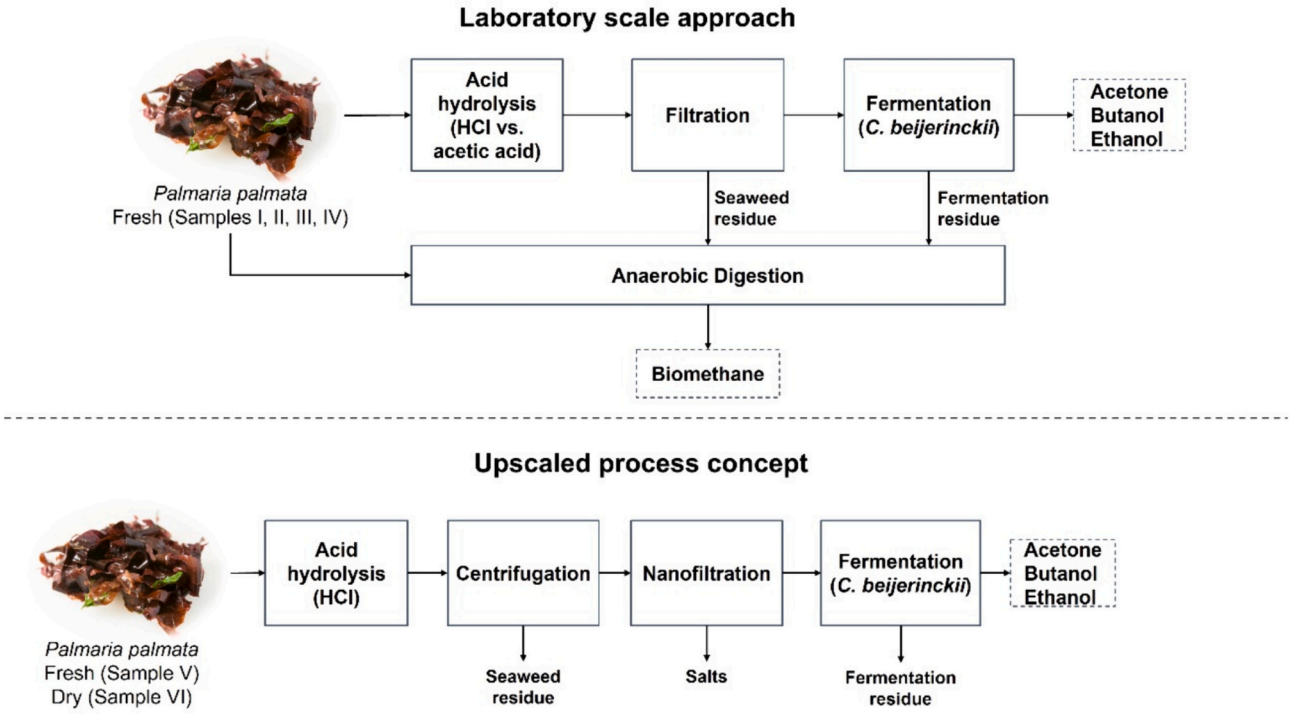

Fig. 1. Laboratory scale approach and upscaled process concept followed for the biorefinery of *Palmaria palmata* biomass.

Table 1
Composition of *Palmaria palmata* biomass harvests used in this study and the solid residues after acid hydrolysis.[c]

Sample	Harvest month and location	Dry weight (%)	Biochemical composition (% dw)[a]				
			Galactan	Glucan	Xylan	Protein[b]	Ash
P. palmata I (fresh)	July - Ireland	17.7	12.8 ± 0.0	6.2 ± 0.0	26.7 ± 0.0	10.8	12.8 ± 0.5
P. palmata II (fresh)	May - Ireland	15.7	6.4 ± 0.0	2.6 ± 0.0	24.7 ± 0.1	15.7	27.3
P. palmata III (fresh)	September - Ireland	22.0	10.4 ± 0.1	6.5 ± 0.0	26.8 ± 0.6	9.9	20.3 ± 0.3
P. palmata IV (fresh)	Augustus - Ireland	15.6	9.9 ± 0.1d	4.7 ± 0.1	27.3 ± 0.2	12.6	22.6 ± 0.4
P. palmata V (fresh)	September - UK	16.8	11.8 ± 0.1	4.5 ± 0.1	26.3 ± 0.0	12.3	21.1 ± 0.1
P. palmata VI (dry flakes)	Commercial - Ireland	93.8	13.3 ± 0.0	4.8 ± 0.1	24.8 ± 0.1	10.2	21.7 ± 1.4
Solid residue - Experiment 1	–	14.3	8.2 ± 0.1	7.5 ± 0.1	24.0 ± 0.3	19.2[b]	10.3 ± 0.8
Solid residue - Experiment 2	–	16.6	7.5 ± 0.0	7.0 ± 0.1	22.7 ± 0.1	n.d.[c]	9.6
Solid residue - Experiment 3	–	15.1	3.7 ± 0.1	4.2 ± 0.1	17.4 ± 0.3	n.d.	16.2
Solid residue - Experiment 4	–	15.3	3.2 ± 0.0	5.0 ± 0.0	16.8 ± 0.1	n.d.	n.d.
Solid residue - Experiment 5	–	24.3	2.1 ± 0.1	7.6 ± 0.2	11.1 ± 0.1	n.d.	8.0
Solid residue - Experiment 6	–	15.4	9.4 ± 0.0	7.4 ± 0.1	24.4 ± 0.3	n.d.	18.8 ± 0.1
Solid residue - Experiment 7	–	16.1	5.9 ± 0.0	8.7 ± 0.1	27.4 ± 0.2	22.3[b]	12.0
Solid residue - Experiment 8	–	16.6	5.7 ± 0.0	8.5 ± 0.0	20.1 ± 0.0	21.8[b]	11.7

[a] Carbohydrates expressed as monomeric anhydrous equivalents. Arabinose was not detected. Fucose, rhamnose and mannitol were < 1 % wt.
[b] Based on nitrogen-to-protein factor of 4.92 [34].
[c] Not determined.

2.2. Acid hydrolysis of Palmaria palmata biomass at laboratory scale

Hydrolysis experiments were performed in a 20 L autoclave reactor (Kiloclave, Büchi Glas Uster AG, Switzerland). In this process, 2.5–5 kg fresh seaweed (ca. 0.5–1 kg dry matter) was mixed 1:1 *w/w* with an acid solution resulting in a liquid-to-solid (LS) ratio of 9 kg/kg dry seaweed at a specific acid concentration. The reactor was heated to the treatment temperature while being mixed, kept isothermal for 2 h and, finally, cooled down to below 40 °C. The temperatures tested were 100 and 120 °C, and acetic acid (0.1 and 0.2 M) or HCl (0.1 M) were used as acid catalyst. The slurry after reaction was filtered quantitatively over a Whatman glass microfiber filter (grade GF/D). Optionally, a screw press was applied to increase the liquor recovery. The filtered process liquor, called hereafter as hydrolysate, was stored frozen before analysis and further processing. Solid residue and sugar (monomeric or oligomeric) yields were calculated as follows:

Solid residue (%) = Dry weight solid residue/Initial dry weight biomass × 100.

Sugar yield (%) = (Hydrolysate weight × Sugar concentration)/(Initial dry weight biomass × Sugar content) × 100.

2.3. Fermentation of Palmaria palmata hydrolysates at laboratory scale

Clostridium beijerinckii NCIMB 8052 (a laboratory strain) was stored as spore suspensions and cultivated as described elsewhere [28]. For pre-culture preparation, spores were heat-shocked and placed into CM2 medium, which is composed of 2.5 g/L yeast extract, 1 g/L KH_2PO_4, 0.8 g/L $K_2HPO_4{\cdot}3H_2O$, 2.9 g/L ammonium acetate, 0.1 g/L *p*-aminobenzoic acid, 1 g/L $MgSO_4{\cdot}7H_2O$, and 6.6 mg/L $FeSO_4{\cdot}7H_2O$. Sugar stock solutions were sterilised separately before inoculation in control media. Prior to fermentation, the pH of the solutions was adjusted to 6.5 with 4 M NaOH. Fermentation tests were run over the span of 6 days. After fermentation, the fermentation broth from each culture was pooled and kept at 4 °C. The broth was placed in a rotavapor (R-200, Büchi, The Netherlands), and the ABE product mixture removed at 85 °C under vacuum for 2 h. The broths depleted of ABE were combined and used in anaerobic digestion tests. Optionally, enzymatic hydrolysis was performed prior to fermentation as an additional treatment of the acetic acid hydrolysate. For this purpose, a commercial cellulase cocktail (GC220, Genencor) was used at a dose of 34 FPU/g of dry weight biomass, as it has xylanase side-activity [29]. The pH of the hydrolysate was adjusted to 4.8 (with NaOH) prior to enzyme addition. Enzymatic hydrolysis was performed in a shaking water bath at 50 °C for 24 h, and the hydrolysate was stored at −20 °C until further use.

2.4. Anaerobic digestion of residues resulting from laboratory scale trials

Anaerobic digestion (AD) of the process residues were carried out in a 5 L reactor at 37 °C (mesophilic conditions) by means of a thermostated water bath. Operation of the reactor was initiated with sugar beet pulp as sole feed, and once stable operation was achieved, co-feeding of the process residues was initiated. The digesters were fed once per day and stirred manually after feedings. The volume in the digester was kept constant by draining the same volume as the volume of the feed. Total solids in the reactor were kept between 2 and 2.5 % wt. The volume of produced gas was measured through collection in bags twice a day. AD tests were run for 5 to 32 days as indicated in Table 4, with different mix ratios of process residues and sugar beet pulp depending on the amount of sample available. Residual solids, ashes, pH, conductivity, volatile fatty acids (VFA) and ammonium were measured according to standard analysis protocols [30]. Biogas yields are reported as litre per kg volatile solids in the feed.

2.5. Upscaling of hydrolysis and ABE fermentation

For scaled-up hydrolysis, a 100 L autoclave (Techema BV) was used to process dry and fresh *Palmaria palmata* in deionised water at 120 °C with constant stirring. pH was adjusted before the hydrolysis with HCl in concentrations that were between 0.17 and 0.40 M (see Supplementary materials). After the reaction was finished, the system was cooled down to <40 °C and the pH adjusted to 3.5–4.0 before handling. The seaweed slurry was centrifuged (Evodos 10, Evodos), filtered over glass fibre filter (GF/D, Whatman, 2.7 μm) and stored at a low temperature (4 °C) before conditioning. The collected hydrolysates were conditioned through nanofiltration to simultaneously increase the sugar concentration and remove minerals. The filtration system consisted of a feed vessel, a circulation pump and a nanofiltration membrane module. The latter contained a spiral element with a cut-off diameter of 10 Å to retain molecules >150 Da and permeate minerals. The system was operated continuously at 30–40 °C until achieving permeation of 70–75 % *w*/w of the starting hydrolysate.

The fermentation was carried out in a stainless steel bioreactor with a working volume of 150 L (Applikon Biotechnology, The Netherlands and Biostream International, The Netherlands). The conditioned hydrolysate (50 kg) was diluted in demineralised water (39 L) with glucose (3 kg) and anti-foam (Sigma 204, 10 mL). The pH was adjusted to 6.4 (32 % wt NaOH) prior to sterilisation (121 °C, 20 min). Next, the broth was made anaerobic by flushing with N_2. Concentrated medium nutrients, from anaerobic stock solutions, were added to reach their concentration in CM2 medium (2.5 g/L yeast extract, 1 g/L KH_2PO_4, 0.8 g/L

$K_2HPO_4{\cdot}3H_2O$, 2.9 g/L ammonium acetate, 0.1 g/L *p*-aminobenzoic acid, 1 g/L $MgSO_4{\cdot}7H_2O$, and 6.6 mg/L $FeSO_4{\cdot}7H_2O$). The fermentation was started by adding approx. 10 L of an overnight preculture of *C. beijerinckii* grown in CM2 medium with glucose (50 g/L). The start pH of the fermentation was 5.8. Cultivation was carried out at 37 °C for 5.75 days, with stirring at 100 rpm, an overpressure of 0.2 bar, and a N_2 flow of 2 L/min over the headspace.

2.6. Analytical methods

Solid samples were freeze-dried, homogenized and milled to <250 μm before analyses. For monosaccharide analysis in solids, a sample of 0.3 g was hydrolysed with sulphuric acid in two steps as described elsewhere [31]. After hydrolysis, the samples were neutralized with $BaCO_3$ and analysed using a High-Performance Anion Exchange Chromatography system with Pulsed Amperometric Detection HPAEC-PAD (ICS3000, Dionex, Sunnyvale, CA). This system was equipped with a CarboPac PA1 column, a guard column and post-column addition of 0.1 mL/min 0.25 M NaOH. Gradients of NaOH and sodium acetate were used as eluent (0.25 mL/min). Lactose was used as internal standard. Hydrolysates were analysed via HPAEC-PAD both directly and after hydrolysis (100 °C, 1 M H_2SO_4, 3 h). Ash content of solid samples was determined by combustion at 550 °C for 5 h. Nitrogen content was measured with an elemental analyser (Carlo Erba Instruments FLASH EA 1112, Wigan, UK). The metal contents of calcium, potassium, sodium and sulphur were measured using inductively coupled plasma atomic emission spectroscopy ICP-AES (Thermo ICAP 6000) after digestion with $HNO_3/HClO_4/HF$.

Organic acids, solvents and sugars in the fermentation broth were analysed by High Performance Liquid Chromatography (HPLC). An aliquot was diluted 1:1 *v*/v with internal standard solution (250 mM propionic acid in 1 M H_2SO_4), centrifuged for 5 min at 14,000 rpm (Centrifuge 5417C Eppendorf desktop) and filtered (0.2 μm, Spartan 13/0.2 RC). Separation was carried out in a Waters HPLC system equipped with an autosampler (Waters 717) and a Shodex KC-311 column (Shodex, Japan). The column was kept at 80 °C, with 3 mM H_2SO_4 as eluent (1 mL/min). A refractive index (RI) detector (Waters model 2414) and a UV absorbance detector (Waters model 2487) were used in series. The concentrations of most metabolites were determined from the RI chromatograms; with the exception of butyric acid, which was determined from the UV absorbance (210 nm). This method cannot separate xylose and galactose, so when no additional measurements were performed, the two sugars are reported combined.

3. Results and discussion

3.1. Composition of Palmaria palmata biomass

Six different samples of *Palmaria palmata* biomass were used in this study in either fresh or dried form. These were collected in different times of the year in Ireland and the United Kingdom. Table 1 shows the biochemical composition of the *Palmaria palmata* samples and the solid residues after acid hydrolysis tests. The seaweed predominantly consisted of carbohydrates (xylose, galactose and glucose), proteins and ash. The total sugars, proteins and ash content of the *Palmaria palmata* samples accounted to approx. 69–77 % of their dry weight (dw). Other components that were not measured herein may include glycerol, fatty acids and pigments [11,12]. The main carbohydrate moiety present was xylose, corresponding to 25–27 % dw as xylan. While the xylan content did not differ substantially among the various *Palmaria palmata* samples, large differences were observed for galactan (6–13 % dw), glucan (3–6 % dw) and ash (13–27 % dw). In general, these variations are due to seasonal changes in the seaweed composition. Seasonal variations in the sugar content have been observed for other seaweeds in prior studies [32,33]. Galactose in *Palmaria palmata* seaweed is commonly found in the form of floridoside (2-O-D-glycerol-α-D-galactopyranoside) [32]. Hydrolysis of floridoside would therefore yield glycerol in addition to monomeric galactose. The presence of glycerol in the hydrolysates from *Palmaria palmata* was confirmed by liquid chromatography but not quantified (data not shown).

The protein content in the *Palmaria palmata* samples varied between 10 and 16 % dw, as estimated by the nitrogen content. This fraction is of interest as a food and/or feed ingredient due to its high content in essential amino acids [35]. Co-extraction of protein could be attained via enzymatic hydrolysis or alkaline treatment; however, this was out of the scope of the present study. *Palmaria palmata* samples also contained high amounts of ash (21–23 % dw), except for the sample collected in July which contained a much lower ash content. Salts that are part of the ash fraction are readily extracted during hydrolysis and can interfere with downstream process steps such as fermentation, as later discussed in Section 3.3. While recovery of salts was not part of the present work, hydrolysate conditioning could be implemented in such a way that salts can be recovered for application [26].

3.2. Acid hydrolysis of Palmaria palmata biomass

Table 2 presents the conditions applied and solid/liquid recoveries obtained in laboratory-scale acid hydrolysis experiments of fresh *Palmaria palmata*. Initially, acetic acid was used as a reagent in the acid hydrolysis of *Palmaria palmata* biomass, as it can be utilized as substrate in the downstream ABE fermentation (experiments 1–4 and 6–7) [23]. Pre-hydrolysis of seaweeds using acetic acid has been reported before for other *Rhodophyta* seaweed (agarose-rich) to produce galactose [36]. Next, HCl was used for additional acid hydrolysis tests (experiments 5 and 8).

The seaweed biomass partially lost its physical structure during the hydrolysis process. When using acetic acid as catalyst, 35–55 % dw of the starting material was obtained as solid residue. The hydrolysate showed a high viscosity (not measured) and a final pH of 4.3–4.6. As the pH of 0.1 M acetic acid is 2.9, it was evident that neutralization took place once the seaweed was mixed in, presumably by the action of minerals from the seaweed. In tests performed at 120 °C (experiments 4, 5 and 8), the structure of the seaweed was completely broken resulting in a fine slurry and lower solid residue yields. A direct correlation between the solid residue yield and the liquid recovery after filtration was observed, which suggested that conditions leading to lower solid residue yields (high temperature, low pH), also lead to lower liquid retention in the residual biomass.

Fig. 2 shows the effects of acid concentration and temperature on the yields of solid residue and of the three main carbohydrates from *Palmaria palmata* in the liquid hydrolysate. When the hydrolysis was performed at 100 °C with 0.1 M acetic acid, a larger fraction of sugars was released from the *Palmaria palmata* samples II and III than from samples I and IV. Furthermore, the solid residue yield was higher for *Palmaria palmata* samples I and IV, which was consistent with their lower sugar yields. The amount of monomeric xylose released from *Palmaria* using acetic acid was negligible at the conditions tested. In this case, the hydrolysis of xylan resulted almost completely in xylo-oligosaccharides. When using 0.1 M acetic acid with *Palmaria palmata* sample II, the increase of temperature from 100 to 120 °C decreased the solid recovery from 45.5 to 34.9 % dw and the seaweed lost entirely its structure, indicating increased solubilization of the seaweed. However, lower total sugar yields were observed in the hydrolysate at this higher temperature. A small increase in monomeric galactose was observed; however, monomeric xylose and glucose remained negligible at 120 °C.

The biomass behaved differently when performing the acid hydrolysis with 0.1 M HCl. Given the strength of the mineral acid, the initial and final pH of the hydrolysis tests were lower (pH <3) using HCl than using acetic acid, despite the partial neutralization also observed with HCl. Furthermore, the solid recovery yields decreased from 34.9 % dw using acetic acid to 21.8 % dw using HCl for *Palmaria palmata* sample II. The total xylose yield as monomeric and oligomeric sugars was

Table 2
Laboratory-scale acid hydrolysis experiments: Conditions and mass yields.

Exp	*Palmaria palmata* biomass sample	T (°C)	Time (h)	Acid	Initial pH (−)	Final pH (−)	Solid residue yield (% dw)[a]	Hydrolysate recovery (%)[b]
1	I	100	2	0.1 M acetic acid	ND	ND	51.6	66.8
2	I	100	2	0.2 M acetic acid	ND	4.1	54.9	70.3
3	II	100	2	0.1 M acetic acid	4.0	4.6	45.5	79.1
4	II	120	2	0.1 M acetic acid	4.0	4.6	34.9	85.7
5	II	120	2	0.1 M HCl	1.5	2.8	21.8	97.4
6	III	100	2	0.1 M acetic acid	4.4	4.7	53.9	58.9
7	IV	100	2	0.1 M acetic acid	4.0	4.3	55.6	73.4
8	IV	120	2	0.1 M HCl	1.5	2.2	35.9	86.6

[a] Dry weight of solid residue after acid hydrolysis compared to dry weight of *Palmaria palmata* feedstock.
[b] Amount of product hydrolysate recovered compared to total amount of initial available water in test (i.e., moisture in feedstock plus added water).

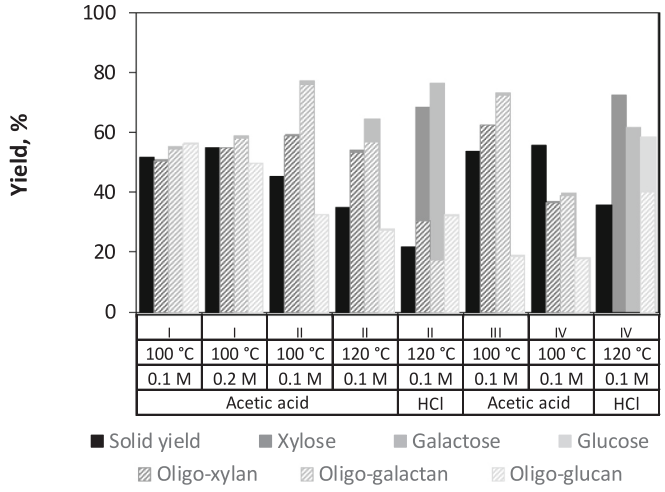

Fig. 2. Yields of solid residue, xylose, galactose and glucose after the hydrolysis of *Palmaria palmata* I-IV with 0.1 M and 0.2 M acetic acid at 100 and 120 °C, and 0.1 M HCl at 120 °C for 2 h.

improved from 54 % to 68 %, respectively for acetic acid and HCl applied to *Palmaria palmata* sample II. Similarly, galactose and glucose yields increased from 65 to 77 % and from 28 to 36 %, respectively.

When using HCl, hydrolysis of the *Palmaria palmata* sample II resulted in lower yields of sugars compared to that of *Palmaria palmata* sample IV. Also, the hydrolysate from *Palmaria* sample II contained a higher fraction of xylo- and galacto-oligosaccharides. *Palmaria palmata* samples II and IV showed inconsistent behaviour when comparing acetic acid (100 °C, 0.1 M) and hydrochloric acid (120 °C, 0.1 M) as catalysts. While sample II showed lower sugar yields than sample IV with acetic acid, the opposite was observed for the HCl-catalysed tests. While the reason for this is not entirely evident, structural changes during the biomass growth cycle may play a role. For instance, the water-soluble fractions of xylose and galactose in *Palmaria palmata* have been shown to decrease from spring to autumn, in spite of the overall increase of carbohydrate content of the biomass [32,37]. Further detailed investigation of the composition and hydrolysis kinetics in this type of biomass can support the understanding of the variability of seaweeds from same species harvested at different locations and times. While this study displays trends and responses in terms of sugar yields with HCl and acetic acid, comprehensive process optimisation should rely on a representative single feedstock batch to avoid any influence from inherent seasonal variations in the biomass.

3.3. Fermentation of Palmaria palmata hydrolysates

The hydrolysates resulting from experiments 1, 7 and 8, using acetic acid and HCl, respectively, were used as substrate for ABE fermentation by *C. beijerinckii*. These hydrolysates were selected as representative of the hydrolysis with acetic and sulphuric acid to compare the performance of the fermentation for the same and different starting biomass substrate via two different hydrolysis routes. *C. beijerinckii* is a well-known ABE-producing strain that uses both hexoses and pentoses as carbon source [38]. Table 3 presents a summary of the sugar composition of the hydrolysates used for fermentation and the product concentrations obtained after their fermentation. Fig. 3 presents the sugar and product concentration profiles during fermentation using hydrolysate from experiment 8 (120 °C, 0.1 M HCl). Concentrations of total monosaccharides in the hydrolysate from experiments 1 and 7 were lower than 3 g/L, and it was expected that this was not sufficient to support fermentation [24]. Therefore, these hydrolysates were further treated with a cellulase cocktail (GC220) that is known to show high xylanase activity, even in hydrolysing isolated xylan fractions from grass [29]. Incomplete hydrolysis of the oligomeric sugars to monomers was observed under the enzymatic hydrolysis conditions applied herein for the *Palmaria palmata* acetic acid hydrolysates (Table 3). This may be related to various reasons. For one, the presence of enzyme inhibitors in the extracts can lead to lower activity, due to binding of other substrates like galactan or soluble proteins to the enzyme. Another possible reason may be related to enzyme activity that is non-specific for the various types of xylan found in *Palmaria* (β-(1 → 3)/β-(1 → 4)-D-xylans) compared to the glucuronoarabinoxylans typically seen in terrestrial biomass [39]. Kabel et al. [29] have shown the significant differences between standard activity for commercial enzymes and the activity towards enriched carbohydrate fractions from lignocellulose biomass. Furthermore, prior to fermentation the cultures of *Palmaria* hydrolysates were supplemented with nutrients as in the CM2 medium to reach the same levels as in the control culture (also in CM2 medium). However, the fermentation profiles in hydrolysates with and without supplementation were similar (data not shown), suggesting that *Palmaria palmata* hydrolysates were rich in nutrients to support fermentation, and that no significant inhibitors were present in these cultures (inhibitors were not measured).

Almost no formation of ABE is observed after more than six days of fermentation of raw hydrolysate obtained with acetic acid. The concentrations of acetic acid (initial = 6.1 g/L) and of galactose (initial = 0.2 g/L) decreased after this time. Furthermore, the xylose concentration increased to 7.8 g/L after 6 days (Table 3), corresponding to approx. 48 % of the total xylose content in the hydrolysate. The xylose monomers are most probably resulting from the hydrolysis of xylan oligomers due to the action of *Clostridial* enzymes and/or as a result of the acidification of the medium during the fermentation. As a control, samples of hydrolysate without inoculum were incubated at the same conditions of the cultures. In these samples, the pH was adjusted to the end pH of the cultures with or without addition of butyric acid. Monomeric xylose was not detected in these control tests, supporting the possibility of being formed by action of clostridial xylanases. However, the release of monomeric xylose was most probably not rapid enough to support the metabolic switch from acid to ABE production in the hydrolysate cultures. On the other hand, the enzymatically-treated hydrolysate from experiment 7 was more active towards fermentation. ABE formation

Table 3
Sugar and product concentrations in cultures of *C. beijerinckii* on *Palmaria palmata* hydrolysates, enzymatically post-hydrolysed hydrolysate and control medium (CM2 medium with sugars). Hydrolysates were supplemented with nutrients as in CM2 (Experiment 7 and 8) or used as such (Experiment 1). The total saccharide content (monomeric and oligomeric) is presented in brackets for the raw hydrolysates from experiments 1 (prior to inoculation) and 7 (at $t = 0$). Average data of duplicate cultivations.

	Control medium	Experiment 1 *Palmaria palmata* I 100 °C, 0.1 M acetic acid		Experiment 7 *Palmaria palmata* IV 100 °C, 0.1 M acetic acid		Experiment 8 *Palmaria palmata* IV 120 °C, 0.1 M HCl
		Raw hydrolysate	After enzymatic hydrolysis	Raw hydrolysate	After enzymatic hydrolysis	Raw hydrolysate
Composition t = 0 days (g/L)						
Glucose	3.3 ± 0.4	0.0 ± 0.0 (1.3)	2.7 ± 0.4	0.0 ± 0.0 (0.6 ± 0.0)	1.2 ± 0.0	0.9 ± 0.0
Xylose	22.9 ± 2.4	0.4 ± 0.5 (20.5)	8.3 ± 1.0	0.4 ± 0.0 (16.1 ± 1.1)	11.7 ± 0.5	23.2 ± 0.7
Galactose	7.7 ± 0.9	0.6 ± 0.2 (10.9)	0.4 ± 0.0	0.2 ± 0.0 (5.9 ± 0.4)	0.3 ± 0.0	7.4 ± 0.5
Total sugars	33.9 ± 3.8	1.0 ± 0.8 (32.7)	11.4 ± 1.5	0.6 ± 0.0 (22.2 ± 1.6)	13.2 ± 0.6	31.5 ± 1.3
Acetic acid	2.1 ± 0.0	4.0 ± 0.0	3.6 ± 0.2	6.1 ± 0.0	6.1 ± 0.0	2.6 ± 0.0
Composition $t = 6$ days (g/L)						
Glucose	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Xylose	7.2 ± 0.0	12.2 ± 0.0	2.6 ± 0.4	7.8 ± 0.2	4.7 ± 0.7	9.9 ± 0.9
Galactose	4.3 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	2.3 ± 2.9
Total sugars	11.5 ± 0.5	12.2 ± 0.0	2.7 ± 0.4	7.8 ± 0.2	4.9 ± 0.5	12.2 ± 1.0
Acetic acid	0.6 ± 0.0	0.7 ± 0.0	1.7 ± 0.0	1.6 ± 0.0	4.7 ± 0.1	1.1 ± 1.0
Acetone	1.4 ± 0.1	<0.1	1.2 ± 0.1	0.1 ± 0.0	0.4 ± 0.0	1.2 ± 0.0
Butanol	5.7 ± 0.1	<0.1	2.6 ± 0.3	0.9 ± 0.0	0.9 ± 0.1	4.2 ± 0.1
Ethanol	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Butyric acid	1.8 ± 0.1	6.3 ± 0.6	3.0 ± 0.2	8.8 ± 0.1	5.0 ± 0.3	4.9 ± 2.7
Product yields t = 6 days						
ABE yield, g/g sugar consumed	0.32 ± 0.04	n.d.	n.d.	n.d.	0.17 ± 0.02	0.28 ± 0.05
Butanol yield, g/g sugar consumed	0.25 ± 0.03	n.d.	n.d.	n.d.	0.11 ± 0.01	0.22 ± 0.04

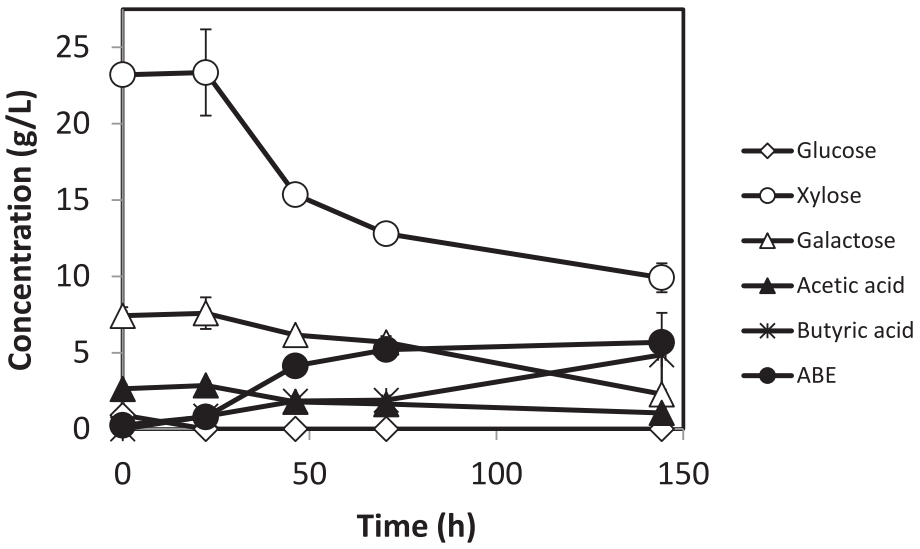

Fig. 3. Sugar and product concentration profiles during fermentation by *C. beijerinckii* on *Palmaria palmata* hydrolysate from experiment 5 (0.1 M hydrochloric acid as catalyst at 120 °C). Average data of duplicate cultivations.

started after 18 h, reaching a titre concentration of 1.3 g/L after six days. While galactose was completely converted, xylose and acetic acid concentrations decreased to 4.7 and 4.7 g/L, respectively, after six days. ABE formation was more rapid and evident using the hydrolysate from experiment 8 as substrate. This occurred due to the immediate availability of higher amounts of monomeric sugars (xylose and galactose) than in the hydrolysates from experiments 1 and 7 (with or without enzymatic treatment).

When grown on *Palmaria palmata* hydrolysates, *C. beijerinckii* produced mainly butyric acid as end product of the fermentation (Table 3). This is most probably due to the low concentration of monosaccharides in the medium, and to the relatively high concentrations of salts in the hydrolysates. An excess of monosaccharides in the medium is normally required for the production of solvents by solventogenic Clostridia. ABE

production was also observed from the enzymatically-treated hydrolysate (Experiment 7), although the levels of butyric acid in the medium were higher (5.0 g/L) than in the control medium (1.8 g/L, Table 3). A similar butyric acid concentration was obtained in hydrolysate from experiment 8, obtained from the acid hydrolysis with HCl.

It has been reported that accumulation of salts in the medium can switch the bioconversion pattern from solventogenic to acetogenic fermentation in solventogenic Clostridia due to stress response mechanisms [40]. When the level of salts is high, it can completely inhibit growth. It has been reported a reduction of growth of 50 % for *C. carboxydivorans* when using medium containing approx. 11 g/L NaCl and electrical conductivity of approx. 29.9 mS/cm [41]. In a previous study on hydrolysates from *Saccharina latissima*, concentrations of 14.5 g/L K^+, 7.3 g/L Na^+ and 3.3 g/L SO_4^{2-} have been determined in a non-fermentable hydrolysate [42]. In the cited study, the hydrolysate became fermentable when it was diluted two times; however, the major product of the fermentation was butyric acid and no ABE was produced. The content of inorganic elements was measured in solid samples from *Palmaria* sample II and the residues from experiments 2 and 5. Large amounts of K^+ and Na^+ were found in the feedstock (49 and 33 g/kg dw, respectively). Acid hydrolysis using HCl at 120 °C released ca. 80 % of these alkali metals which were thus available in the hydrolysate. Taking into account the quantification of these inorganics in the feedstock and residue, it is estimated that approx. 7 g/kg of K^+ and Na^+, or 16 g/kg of equivalent KCl and NaCl could be found in the hydrolysate from experiment 8. These concentrations are of the same order as the amounts of NaCl reported to be inhibitory in previous studies [41,42] and therefore could explain the relatively slow growth of *C. beijerinckii* and low product formation. These elements are found abundantly in seaweed as a result of the sea salinity and the preferential transport of K and Na through seaweed cell walls. A recent study on fermentability of *Saccharina latissima* hydrolysates shows that not only salts, but also phenolic compounds affect fermentation with *C. acetobutylicum* [27]. In the *Palmaria palmata* hydrolysates, phenolic compounds are also expected [10,43]. However these have not been characterized, and their effect on the fermentation by *C. beijerinckii* needs yet to be determined.

In all cases, the acetic acid in the media was consumed by the bacteria. The highest ABE concentration was achieved with the hydrolysate from experiment 8, in which 5.5 g/L ABE was observed after 140 h. This corresponded to a butanol yield of 0.22 g/g of sugars consumed, 12 % lower than that of the control medium. *C. beijerinckii* utilized all three sugars present in the control medium (Table 3), although xylose and galactose were not fully consumed.

3.4. Anaerobic digestibility of residual streams

Anaerobic digestion tests were performed on residues resulting from acid hydrolysis using acetic acid and residues from ABE fermentation to evaluate their suitability for energy recovery. Fresh *Palmaria palmata* feedstock was also digested as a reference (Table 4). Co-feeding with sugar beet pulp was implemented for the acid hydrolysis residues due to the limited amounts of the residues produced at laboratory scale. Sugar beet pulp is a standard neutral feed used in the standard operating procedure of the AD reactors used in this study and relevant as bioenergy feedstocks for biogas production in the Netherlands. For one of the residues, a change in the mixing ratio with sugar beet pulp was performed to assess the impact of the residue concentration in the medium. The tested feedstocks or their mixtures with sugar beet pulp were all digestible and were not inhibited due to e.g. their high salt content. The conductivity of the digestion mixtures increased slowly during operation (5–7 mS) due to the inherent increase of salt concentration by feed addition to the digester. All digestion experiments operated at a pH between 7 and 7.5 and with high HCO_3-to-VFA ratios (>7 g VFA per g HCO_3). The latter is a positive property of the *Palmaria palmata* residues and it shows that seaweed-derived substrates increased the chemical buffering system inside the digester.

Table 4
Conditions and biogas production by anaerobic digestion of process residues.

Reactor feed			Test duration (days)	Biogas production (L/kg dry volatile solids in feed)
Substrate type	Dry weight input (%)			
	Substrate	Sugar beet pulp		
Palmaria palmata fresh biomass	100	0	5	440
Solid hydrolysis residue – Experiment 1 (Sample I, 100 °C, 0.1 M acetic acid)	30	70	32	650
Solid hydrolysis residue – Experiment 2 (Sample I, 100 °C, 0.2 M acetic acid)	40	60	21	380
Solid hydrolysis residue – Experiment 2 (Sample I, 100 °C, 0.2 M acetic acid)	66	34	26	310
Residue of fermentation broth after ABE evaporation[a]	100	0	5	650

[a] This was collected from the various fermentation tests reported in Table 3 (including microbial biomass) after ABE removal.

The biogas yield of process residues were between 310 and 650 L/kg dry volatile solids (VS), when mixed in different ratios with sugar beet pulp, compared to 440 L/kg dry VS from the fresh *Palmaria palmata* biomass (Table 4). For reference, feedstocks such as beet pulp and corn silage tested in the same AD reactor used in this study yielded 570 and 550 L/kg dry VS, respectively (data not shown). The biogas yields obtained were mostly similar to those obtained with such conventional feedstocks. However, the digestion of the solid hydrolysis residue was aided by the addition of sugar beet pulp. Addition of lower amounts of sugar beet pulp to process residues from acetic acid hydrolysis seems to lead to lower overall biogas yields. Furthermore, the methane content of the biogas formed from the *Palmaria palmata* residues was approx. 50–60 % *v*/v, corresponding with a lower heating value of 16–19 MJ/m^3. The methane production obtained from the *Palmaria palmata* feedstock (i.e., ~220–260 L/kg dry VS) was somewhat lower than reported the methane potential of *Palmaria palmata* (308 L/kg VS) reported by Jard et al. [8], but within the range reported for various seaweed species, 191 to 335 L/kg VS [22,44].

This data suggests that process residues from *Palmaria palmata* biorefinery can be used as co-feedstocks for AD. However, the reported biomethane yields might be an overestimation since residues from experiments 1 and 2 used for AD tests resulted from suboptimal hydrolysis treatments and thus contained quite significant amounts of remaining carbohydrates (Table 1). In addition, prolonged digestion tests as well as performance of process residues as sole feedstocks are needed to ensure their suitability in commercial digesters. The production of biogas from process residues would increase the energy efficiency of the whole biorefinery process chain and further exploration of its integration to a seaweed biorefinery is recommended.

3.5. Upscaling of acid hydrolysis and fermentation

Various hydrolysis experiments were carried out at 100 L scale to produce a sufficient amount of sugars for a larger scale fermentation test. Hydrolysis at this scale was performed using HCl at 120 °C to replicate the highest monomeric sugar yields observed at lab scale. Dry flakes and fresh *Palmaria palmata* with the composition presented in Table 1 (samples V and VI) were used in six batches at various LS ratios (18, 15 and 11 kg/kg dry seaweed). A description of the conditions and results of these tests is presented in the Supplementary materials. In this

configuration, it was observed that higher amounts of HCl (0.17–0.40 M) were required to maintain a pH between 1.6 and 2, compared to screening tests. Fig. 4 shows the sugar and solid residue yields of these tests.

Solid residue yields at larger scale (13–19 % dw) were lower than those obtained at laboratory scale (22–36 % dw). In agreement with this, the xylose and galactose monomeric yields were higher (88–94 % and 75–96 %, respectively) than at laboratory scale (73 and 62 %, respectively). Hydrolysis towards monomeric glucose was also improved by over 30 %. These differences can be related to differences in the heating rate of the reactors. Longer time (~108 min) was needed to reach the process temperature at 100 L scale compared to the time needed at 2 L scale (~70 min). Cooling was also quicker at the smaller scale. The extended heating/cooling time at active temperatures (100–120 °C) could have contributed to the higher monomeric yields. No evident effect on the sugar yields was observed as a function of the LS ratios at 100 L scale. However, the solid yield was higher when decreasing the LS ratio from 18 to 11.

To increase the sugar concentration prior to fermentation and remove minerals, hydrolysates from upscaling batches 1, 2, 3 and 4 were filtered through a nanofiltration element to remove approximately 67–75 % *w*/w water (permeate). In this setup, the retentate was recirculated to the feed of the filtration system and contained the bulk of sugars with remaining minerals, while the permeate stream contained removed water and minerals as well as some sugars. This treatment doubled the total monomeric sugar concentration in the collected retentates and removed 64–78 % K^+ and Na^+ and 44–59 % Ca^{2+} from the hydrolysates (variations as a function of the permeation). The produced retentates still contained significant amounts of minerals, e.g. 2.5–3.2 g/kg Na^+, 3.0–4.8 g/kg K^+ and 0.5–0.9 g/kg Ca^{2+} (see Supplementary materials). Furthermore, sugar losses of the order of 21–34 % of total sugars to the permeates were observed, indicating the need of implementing e.g. multiple filtration stages to minimise sugar losses in the nanofiltration process.

The retentates resulting from the nanofiltration trials were combined, resulting in contents of 3.4 g/L glucose, 25 g/L xylose and 14.2 g/L galactose and were used for fermentation. Initially, laboratory scale cultures were grown on this hydrolysate as such, and on hydrolysate diluted 1:1 and supplemented with glucose (Table 5). No growth was observed in the cultures with hydrolysate as such; while on hydrolysate diluted 1:1, growth and sugar consumption was observed (Table 5). Because the diluted hydrolysate contained a low concentration of sugars (18.6 g/L), additional tests were performed on cultures where the diluted hydrolysate was supplemented with 20 g/L of glucose.

On these screening cultures, most sugars were consumed and 12.9 g/L total ABE were produced. This concentration of ABE is high enough to be recovered from a pilot bioreactor. Therefore, for the upscaling of the fermentation process, it was chosen to use *Palmaria* hydrolysate supplemented with glucose to assess fermentability of other *Palmaria* sugars (galactose and xylose). The upscaled fermentation showed a profile similar to the fermentations in flasks, achieving 14.6 g/L ABE, and a sugar consumption of 89 % including glucose and the *Palmaria* sugars xylose/galactose (Table 5). While the desalted hydrolysate used in upscaled fermentation contained significantly lower amounts of alkali and alkali-earth minerals (6–8 g/kg Na^+, K^+, Ca^{2+}) compared to laboratory scale hydrolysates, this concentration can still be inhibitory for the *Clostridium* strain. Amounts as low as 3.9 g/L of Na^+ have resulted in inhibition of *C. beijerinckii* P260 [45], which are similar to the concentrations observed in this study. Furthermore, nanofiltration of the hydrolysates may have resulted in the increase in concentration of other seaweed compounds such as polyphenolics [10]. Phenolic compounds typically found in hydrolysates from lignocellulosic biomass (vanillin, ferulic acid, *p*-coumeric acid) have been found to cause product and cell growth inhibition in *C. beijerinckii* NCIMB 8052 [46].

These results show that ABE can be produced from *Palmaria palmata* hydrolysates, although substantial improvements are required for an industrially feasible biorefinery. In particular, fermentable hydrolysates with higher sugar concentration (>60 g/L) need to be realised to minimise energy demands associated to concentrating the hydrolysate. The seaweed pre-treatment could focus on the use of lower liquid-to-seaweed ratios and downstream purification processes or combinations thereof. Since Clostridial strains can depolymerise xylose

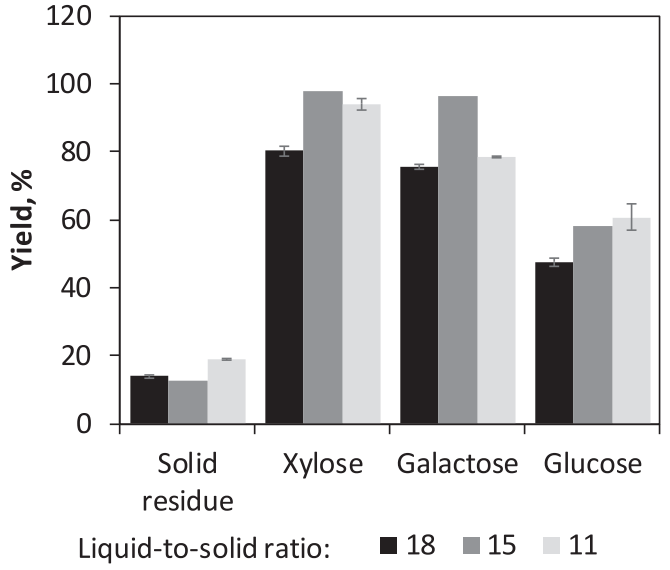

Fig. 4. Solid residue and monomeric sugar yields observed from acid hydrolysis of *Palmaria palmata* biomass in upscaled tests under various liquid-to-solid ratios (weight basis). Test conditions: 120 °C, initial pH 1.7 adjusted with addition of 0.25–0.30 M HCl, 2 h. Dry *Palmaria palmata* VI (93.7 % dw) was used in tests with 18 and 15 kg/kg dry seaweed, while fresh seaweed V (16.8 % dw) was used at liquid to solid ratio of 11 kg/kg dry seaweed.

Table 5
Fermentation of hydrolysates resulting from upscaled hydrolysis of *Palmaria palmata* and nanofiltration.

	Screening experiment (25 mL)		Upscaled experiment (100 L)
	Hydrolysate 50 % diluted	Hydrolysate 50 % diluted + glucose	Hydrolysate + glucose
Composition $t = 0$ days (g/L)			
Glucose	1.7	18.4	26.5
Xylose/galactose*	16.9	19.7	15.6
Total sugars	18.6	38.1	42.1
Acetic acid	2.4	2.5	1.5
Composition $t = 5.75$ days (g/L)			
Glucose	1	0.6	Nd
Xylose/galactose*	0.4	6.1	4.6
Total sugars	1.4	6.7	4.6
Acetic acid	0.7	0.5	0.3
Acetone	1.5	3.2	4.5
Butanol	4.6	8.6	9.3
Ethanol	1.1	1.2	1.1
Butyric acid	2.2	0.2	0.2
Product yields t = 5.75 days			
ABE yield, g/g sugar consumed	0.37	0.39	0.39
Butanol yield, g/g sugar consumed.	0.26	0.27	0.25

* HPLC method used for sugar determination did not separate xylose and galactose, therefore these sugars are measured combined for the data presented in this table. Nd, not detected.

oligosaccharides (Table 3, raw hydrolysate culture from Experiment 2) and utilise xylose as carbon source, the enzymatic hydrolysis of the seaweed biomass could be adapted to the use of lower enzyme loadings or hydrolysis time, for example. Other types of downstream treatments can be potentially explored to address inhibitory compounds in resulting hydrolysates, for instance, active carbon adsorption for removal of polyphenols and electrodialysis for removal of salts.

4. Conclusions

This work studied a biorefinery concept for the production of ABE and biogas from *Palmaria palmata*. Differences were observed in composition and amenability to hydrolysis of seaweed from different harvests. HCl treatment led to higher monomeric sugar yields than acetic acid treatment, resulting in higher amounts of ABE produced per kg of biomass. Acetic acid-hydrolysates required enzymatic post-hydrolysis prior to ABE fermentation. Hydrolysates with low sugar contents (~13 g/L) gave low ABE yields (0.16 g/g total sugars) and high levels of butyric acid. HCl-derived hydrolysates (~30 g/L sugars) produced higher amounts of ABE (0.28 g/g total sugars). Process residues were anaerobically digested mixed in different ratios with sugar beet pulp and gave biogas yields between 310 and 650 L/kg. The mineral acid hydrolysis and fermentation processes were scaled up to 100 L pilot scale. While high monomeric sugar yields were replicated during upscaling, higher mineral acid consumption was required indicating the large buffering capacity of the seaweed. Although nanofiltration was implemented to increase sugar concentration sufficiently for fermentation (42 g/kg) and to remove salts, fermentation inhibition was still observed. Thus, further process development is needed to optimise salt removal and detoxification of *Palmaria palmata* seaweed hydrolysates prior to fermentation.

Palmaria palmata biomass was found to be a suitable feedstock for the co-production of bio-butanol and biogas within an integrated biorefinery concept. As such, this work gives an example of the broad application potential and the challenges and process needs of the use of seaweeds as feedstocks for biorefinery.

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CRediT authorship contribution statement

Karla Dussan: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Data curation, Conceptualization. **Wouter J.J. Huijgen:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Data curation, Conceptualization. **Truus de Vrije:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Data curation, Conceptualization. **Hetty van der Wal:** Writing – review & editing, Writing – original draft, Validation, Methodology, Data curation. **Hector Capella:** Writing – review & editing, Writing – original draft, Validation, Methodology, Data curation. **Joep van Doorn:** Writing – review & editing, Writing – original draft, Validation, Methodology, Data curation. **Ana M. López-Contreras:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Funding acquisition, Data curation, Conceptualization. **Jaap W. van Hal:** Writing – review & editing, Visualization, Validation, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Karla Dussan, Jaap W. van Hal, Truus de Vrije, Ana M. Lopez Contreras reports financial support was provided by European Commission. Wouter J.J. Huijgen, Jaap W. van Hal, Truus de Vrije, Hetty van der Wal, Hector Capella, Joep van Doorn, Ana M. Lopez Contreras reports financial support was provided by Netherlands Enterprise Agency. Karla Dussan reports financial support was provided by Dutch Ministry of Economic Affairs. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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