The role of alginate lyases in the enzymatic saccharification of brown macroalgae, *Macrocystis pyrifera* and *Saccharina latissima*

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ABSTRACT

In this work, we have compared the carbohydrate content and the enzymatic saccharification of the brown algae *Macrocystis pyrifera* from Chile and *Saccharina latissima* from Norway. *M. pyrifera* contained 40% mannitol, 31% uronic acids and 15% glucose, while *S. latissima* contained 37% glucose, 30% uronic acids and 25% mannitol. Thus, the ratio between mannitol and glucose was much higher for *M. pyrifera*. Acid pre-treated and untreated algae were enzymatically saccharified in two steps; first at pH 7.5, 25 °C for 12 h with a blend of recombinant alginate and oligoalginate lyases, then the pH was changed to 5.2, a commercial cellulase cocktail was added and saccharification continued at 50 °C for 4 h. These experiments showed that the use of recombinant alginate lyases and oligoalginate lyases in combination with cellulases increased the release of glucose from untreated seaweed. However, for saccharification of pretreated algae, only cellulases were needed to achieve high glucose yields. Finally, it was shown that brown algae hydrolysates could be used as a growth medium to produce microbial ingredients, such as *Candida utilis* yeast.

1. Introduction

Marine macroalgae such as brown algae are currently receiving a lot of attention as a source of renewable biomass for the production of a wide range of products, including food, nutraceutical, pharmaceutical, fine chemical, biofuel, fertilizer, as well as feed [1]. Macroalgae are among the fastest growing organisms in the world, and produce large amounts of biomass without the use of fresh water, agricultural land, fertilizer, or pesticides [2]. The cold-temperate growth conditions in Norway and Chile offer large potential for cultivation of macroalgae for the production of sustainable biomass. The brown algae *Macrocystis pyrifera* and *Saccharina latissima* are phylogenetically closely related and belong to the family Laminariaceae [3]. *M. pyrifera* is the dominant brown macroalgae found at the coast of south of Chile, and the largest and fastest-growing seaweed species on earth [4]. *S. latissima* is one of the main seaweeds found along the Norwegian coast and preparations for large scale cultivation are currently underway [5,6]. The main carbohydrates found in brown algae are alginate, cellulose, laminarin, fucoidan and mannitol. Alginate is a *co*-polymer of the uronic acids α-L-

guluronate (G) and β-D-mannuronate (M), being arranged as homo-polymeric G blocks, homopolymeric M blocks, alternating GM blocks or random heteropolymeric G/M stretches [7]. Both laminarin and cellulose are polysaccharides composed of glucose; laminarin consists of a β(1 → 3)-glucan backbone with β(1 → 6)-branches, while cellulose is a linear chain of β-1,4-linked D-glucose units that shows an x-ray diffraction pattern similar to that of native cellulose from land plants [8]. These polysaccharides from algae can be converted to fermentable sugars by enzymatic saccharification [9,10] that can be further used as a carbon and energy source in the fermentation of yeast. The main enzymes used for saccharification of cellulose are endoglucanases (EC 3.2.1.4), which randomly cleave internal glucosidic linkages of this polysaccharide, cellobiohydrolases (EC 3.2.1.91); β-glucosidase, which hydrolyze cellobiose to glucose (EC 3.2.1.21); and lytic polysaccharide monooxygenases (LPMOs), which cleave cellulose by an oxidative mechanism [11]. Mannuronate lyases (EC 4.2.2.3) and guluronate lyases (EC 4.2.2.11) cleave alginate within the chain producing unsaturated uronic acid oligomers with a double bond between C4 and C5 at the non-reducing end. Oligoalginate lyases (EC 4.2.2.-) cleave these

oligomers to produce monosaccharides (unsaturated uronate) [12]. The enzymatic saccharification of the macroalgae was carried out using a combination of a commercial cellulase cocktail and recombinant alginate lyases and oligoalginate lyases [13].

The aims of this study were: (a) comparison of the chemical composition of the brown algae *M. pyrifera* from Chile and *S. latissima* from Norway, (b) evaluation of five different recombinant alginate lyases for their effect on enzymatic liberation of glucose from the macroalgae, and (c) evaluation of the growth of the yeast *Candida utilis* in algae hydrolysate for the production of microbial ingredients.

2. Materials and methods

2.1. Feedstock and compositional analysis

Two brown algae species from the family Laminariaceae were evaluated in this study performed at the Norwegian University of Life Sciences: *M. pyrifera* from Chile and *S. latissima* from Norway. *M. pyrifera* samples were identified based on blade and holdfast morphology (Buschmann A., personal communication). *S. latissima* was identified based on overall morphology, and verified by genetic analysis (Seaweed Energy Innovations, personal communication). Both algae are closely related phylogenetically [1] and they were collected in the summer months of each country. *M. pyrifera* were collected by scuba diving 30 km southwest of Puerto Montt in Chile. The seaweed was harvested in January 2015 (average temperature: 16.5 °C), by Professor Buschmann, University of Los Lagos, Chile. *S. latissima* was harvested in the Trondheims fjord in Norway In July 2014 (average temperature 15.0 °C), by Seaweed Energy Solutions. The fresh and unwashed seaweed was shipped, frozen and stored at −20 °C until use. Both seaweed were dried at room temperature, grounded by an electric grinder and passed through a 1-mm sieve. Elemental analysis of the untreated, sulfuric acid pre-treated, and enzymatic residue of *S. latissima* and *M. pyrifera* samples were performed by a Vario EL cube elemental analyzer (Hanau, Germany). All samples were dried at 105 °C and homogenized before analysis.

The carbohydrate composition of *M. pyrifera* and *S. latissima* were analyzed using a two-step sulphuric acid hydrolysis method [14] modified from the one developed by NREL [15]. Dried grounded samples were exposed to 72% (w/w) H_2SO_4 at 30 °C for exactly 1 h and then further hydrolyzed in 4% (w/w) sulphuric acid at 121 °C in an autoclave for 40 min. After complete hydrolysis, the hydrolysates were filtered with syringe filter (0.22 μm). The supernatant was analyzed for sugar content by High Performance Liquid Chromatography (HPLC) and High Performance Anion Exchange Chromatography (HPAEC).

2.2. Pre-treatments and enzymatic saccharification

For the pretreatment, 30 g of dry *M. pyrifera* and *S. latissima* were pretreated in a 1:3 ratio (wt) with 2% (v/v) sulfuric acid diluted in milli-Q water. Algae and solvent were placed into tubes followed by incubation in a thermostated oil bath at 120 °C for 1 h. After incubation, the tubes were removed from the oil bath and the algae were first washed six times using milli-Q water, then two times with McIlvaine Buffers [16] at pH 7.5 (for neutralization), later centrifuged and finally dried at 37 °C for 3 days.

For enzymatic saccharification, 80 mg dry algae (both native and pretreated with diluted sulphuric acid) were incubated at 25 °C for 12 h in a 0.45 M McIlvaine buffer pH 7.5 with 0.2 mg protein of recombinant alginate lyases (alginate lyase H from *Pseudoalteromonas elyakovii* [17], alginate lyase G from *Pseudoalteromonas sp.* [18], alginate lyase I from *Microbulbifer sp.* 6532A [19], alginate lyase K from *Sphingomonas sp.* [20] or alginate lyase L from *Sphingomonas sp.* [21]), and 0.2 mg protein of a blend of recombinant oligoalginatelyases; oligoalginatolyase Atu from *Agrobacterium tumefaciens* strain C58 [12], oligoalginatelyase Q from *eubacterium* SCB49 and oligoalginatelyase O from *Lewinellapersica*.

Then, the pH was adjusted to 5.2 using 6 M of HCl and 0.4 mg protein from cellulases (CellicCTec2 from Novozymes) was added to the samples which were incubated at 50 °C for 4 h. Thus, a 50:50% ratio of alginases (25% alginate lyase + 25% oligoalginate lyase) and cellulases on a protein-basis was applied. The biomass loading in the reaction was 5% (w/v). Four control incubations were performed; a blank without enzymes, a control with a commercial alginate lyase (from Sigma) and two controls with only cellulases (Celluclast (from Novozymes) + β-glucosidase (from Megazyme) or CellicCTec2 (from Novozymes). In each control, 0.4 mg cellulases were used. After enzymatic saccharification, the samples were incubated at 100 °C for 10 min, centrifuged and filtered through a 0.2-μm centrifuge filter. All experiments were performed in duplicate.

2.3. Analysis of soluble sugars

The release of monomeric sugars was quantified by a HPLC. The separation was made using a 300 × 7.8-mm Rezex ROA-Organic Acid H^+ analytical column fitted with a cation-H cartridge guard column at 65 °C with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 ml/min. Calibration standards were made for glucose, xylose, mannitol and fucose in the concentration range 0.1 to 2.5 g/l. Dionex software Chromeleon 7.2 had been used for identification and quantification of the sugars.

Quantification of uronic acid (mannuronic acid and guluronic acid) were performed by HPAEC. The mobile phase was 0.1 M NaOH and 1 M NaAc at a flow rate of 0.25 ml/min. The standards of guluronic acid and mannuronic acid (Carbosynth, Berkshire, UK) were made in the concentration range 0.1 to 2.5 g/l.

2.4. Ash content

Ash content of *M. pyrifera* and *S. latissima*, both native and pretreated, were determined by a NREL method [22], weighing samples before and after heating in a furnace at temperature of 600 °C for 4 h. This analysis was performed in duplicates.

2.5. Recombinant alginate lyases

Previously, an *E. coli* expression protocol has been developed for the expression of alginate lyases from polysaccharide lyases families; PL7 and PL18 according to Carbohydrate-Active enzymes database [23]. These alginate lyases are highly active and soluble enzyme preparations [13]. These same parameters were utilized for expressing the enzymes in this work. Endo-alginate lyases were expressed using vector pETG41 containing an N-terminal His-tag/Maltose-binding protein (MBP) partner [24]. Exo-alginate lyases were cloned into vector pNative, which has no fusion protein partner (modified pet22b+ with a stop codon before the His-tag, Merck, MA, USA). After chemical transformation of BL21 (DE3) Rosetta2, the complete mix was transferred to grow overnight in 7 ml Luria-Bertani (LB) liquid medium plus 100 μg/ml ampicillin and 34 μg/ml chloramphenicol. The OD600 of the cultures were then recorded, and the expression cultures were inoculated to a 0.05 final OD600. Expression cultures consisted of 20 ml of ZYM-5052 autoinduction medium [25] in 60 ml Erlenmeyer flasks, grown at 25 °C, and 200 RPM orbital shaking for 14 h. 20 ml of the autoinduction cultures were centrifuge and resuspended in 6 ml Tris-buffered saline (TBS) pH 7.5. Samples were sonicated three times for 45 s alternating with incubations on ice, with a 1/8″ tip at 20 W power using a XL-2000 sonicator (Misonix, Farmingdale, NY, USA). Samples were then centrifuged at 9289 g for 10 min at 4 °C. The clear supernatant was then recovered and used for the enzymatic assays. A description of each recombinant alginate lyase is shown in Supplementary Table S2.

2.6. Fermentation for microbial ingredient production

To obtain hydrolysates as suitable media for fermentation experiments *M. pyrifera* (pretreated) and *S. latissima* (native) were enzymatically saccharified, using the best conditions determing in 2.2. The saccharification was carried out at 15% Dry Mass (DM) loading in a 0.45 M McIlvaine buffer pH 7.5 with alginate lyase G for pretreated *M. pyrifera* and with alginate lyase H for native *S. latissima* at 25 °C for 12 h. Then the pH was changed to 5.2 using 6 M of HCl and the samples were incubated with 0.4 mg protein of CellicCTec2 at 50 °C for 4 h.

The obtained hydrolysates were centrifuged and the resulting liquid fractions were used for the yeast fermentation experiments. To increase the nitrogen content of the hydrolysates, ammonium sulphate was added as per yeast elemental composition ($C_1H_{1.64}N_{0.16}O_{0.52}P_{0.01}S_{0.005}$). A pre-culture of *Candida utilis* was grown in yeast extract-peptone-dextrose (YPD) broth at 30 °C for 24 h. Fermented hydrolysates were produced by combining 2 ml of the pre-culture with 20 ml of hydrolysate media followed by incubation at 30 °C for 42 h. After that, the fermented hydrolysates were centrifuged and the resulting pellets were washed once using milli-Q water. The pellets were then dried in a freeze drier (0,042 mbar to − 80 °C) for two days prior to nitrogen quantification by Kjeldahl nitrogen analyzer (Kjeltec™ 8400, Sweden).

2.7. Scanning electron microscopy

Scanning electron microscopy (SEM) of native, pretreated and saccharified *S. latissima* and *M. pyrifera* were analyzed by a Zeiss Evo (50EP, UK) instrument at excitation voltage of 10 kV. The dried samples were prepared by sputter coating with a thin layer of gold-palladium. The micrographs were taken at 5000 × magnification.

3. Results and discussion

3.1. Chemical characterization of macroalgal biomass

Carbohydrates, protein and ash were quantified for the algal biomasses (Fig. 1). The values are given as dehydrated monomers (by applying a conversion factor for dehydration on polymerization:

glucose, fucose, guluronic, mannuronic = 0.9; xylose = 0.88; and mannitol = 1). Supplementary Table S1 shows the concentration of carbohydrates (g/kg of DM) and percentage of carbohydrates from *S. latissima* and *M. pyrifera* biomass without pretreatment and pretreatment with 2% sulphuric acid. The composition of *S. latissima* biomass used in this study consisted of 37% glucose and 25% mannitol. For *M. pyrifera* biomass consisted of 15% glucose and 40% mannitol. Fig. 1 shows that the pretreatment changed the composition of both algae biomasses. The pretreated *S. latissima* contained 247 g glucose/kg of DM and 94.3 g alginate/kg of DM. Pretreated *M. pyrifera* contained 217.8 g glucose/kg of DM, 167.9 g alginate/kg of DM. The main carbohydrate lost during pretreatment was mannitol. This sugar alcohol is free in algae or it is part of laminarin [18]. The pre-treatment reduced the ash content in both algae biomass, and in particular for *M. pyrifera*, where the ash content became 23 times lower. Interestingly, the pretreatment led to a more than doubling of the crude protein content in *S. latissima*.

Pretreatment changed the α-L-guluronic acid (G) and β-D-mannuronic acid (M) ratio. The alginates are made up of different blocks of guluronic and mannuronic acids, the blocks are referred to as MM blocks, GG blocks and MG blocks [7]. The change of G:M ratio could be because the MM blocks are more exposed to pretreatment and, therefore, the amount of M decreases after the pretreatment

M. pyrifera and *S. latissima* are closely related phylogenetically, belonging to the family Laminariaceae [1]; however, their chemical composition differs and both respond differently to the applied pretreatment.

3.2. Enzymatic saccharification and released of glucose

Native and pretreated *S. latissima* and *M. pyrifera* were saccharified with recombinant alginate lyases plus oligoalginate lyases at 25 °C for 12 h; then a cellulase cocktail was added to the reactions and further incubated for 4 h at 50 °C. Five different recombinant alginate lyases were used to compare their effect in the release of glucose. Figs. 2 and 3 show the enzymatically released glucose from native algae and pretreatment algae, respectively. The highest glucose release (209 g/kg DM; 94.3% of theoretical yield) for *S. latissima* (native) was obtained
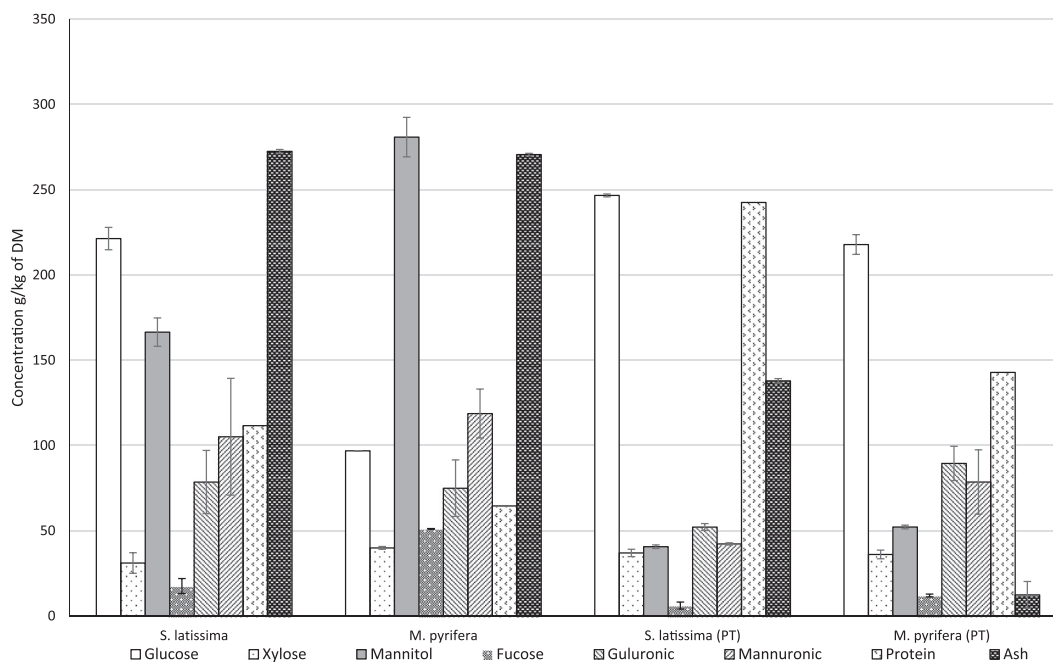

Fig. 1. Carbohydrate, protein and ash contents (g/Kg of dry mass) in *S. latissima* and *M. pyrifera* native and pretreated form. Monosaccharide content was analyzed after of acid hydrolysis by high performance High-performance Liquid Chromatography (HPLC) and High-performance Anion Exchange Chromatography (HPAEC). Each data point represents the mean value of independent duplicates ± SD.
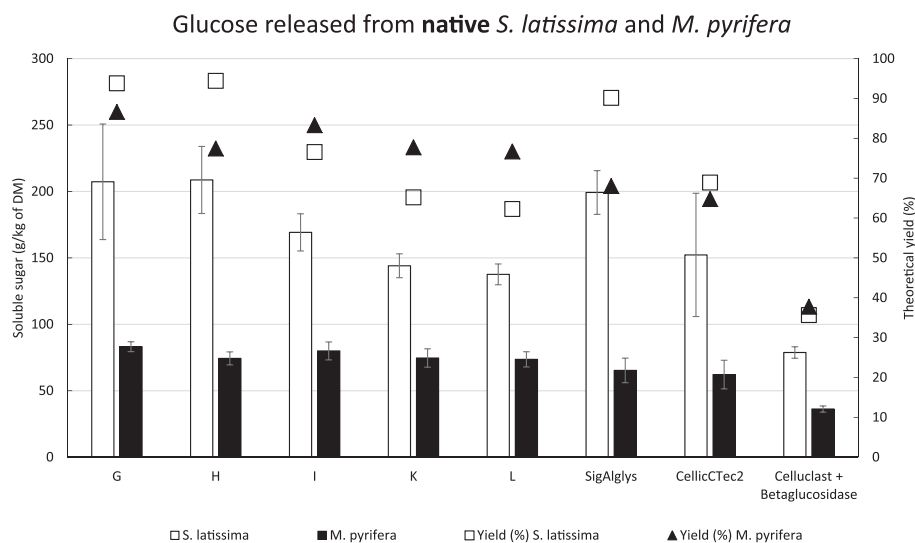
using the recombinant alginate lyase H (from *Pseudoalteromonas elyakovii*) plus oligoalginate lyases and CellicCTec2. However, the reactions with alginate lyase G and the Sigma alginate lyase showed glucose release at similar high levels. When the *S. latissima* was saccharified with only CellicCTec2, it released 68.8% of the glucose. The reactions with the alginate lyases I, K and L showed similar levels of glucose release as CellicCTec2 alone. Thus, only two of the recombinant lyases increased the glucose release. For native *M. pyrifera*, the results were very different. As expected, the glucose concentrations obtained after enzymatic processing of *M. pyrifera* were much lower than for *S. latissima* (Fig. 2) as *M. pyrifera* has lower initial content of glucose. It has also been shown that all alginate lyases had a positive effect on glucose release, where the highest yield was achieved with alginate lyase G.

Fig. 3 shows that the saccharification results were very different for pretreated algae. In this case, *M. pyrifera* also showed high yields of glucose release. This reflects the high glucose content in the pretreated *M. pyrifera* (more than twice the concentration found in the untreated *M. pyrifera*; Fig. 1). The highest glucose yield (207.2 g/kg of algae, 95.1%) was obtained with the recombinant alginate lyase G (from *Pseudoalteromonas sp.*) plus oligoalginate lyases and CellicCTec2. However, all incubations showed high yields, even the reaction with CellicCtec2 alone. This was also the case when *S. latissima* was degraded, showing that the cellulase cocktail alone can efficiently

saccharify pretreated algae. Figs. 2 and 3 show that alginate lyases are only helpful for saccharification of native macroalgae, while the effect of this enzyme is limited in pretreated algae. Interestingly, enzymes G and H seemed to be better suited for saccharification of non-pretreated *S. latissima*. This could be due to their particular preference for the different glycosidic bonds within alginate, although all *endo*-lyases in this work where selected for their flexibility to cleave GG, MM, and GM glycosidic bonds (Table S2). On the other hand, these two endo-lyases are the only ones from the Polysaccharide Lyase family 18 (Table S2), which could be related to a structural or catalytic feature that confers them an advantage for degrading alginate molecules that are not readily exposed (non-pretreated biomass), although this would have to be tested through structure/function studies.

Additionally, mannitol released from native as well as pretreated *S. latissima* and *M. pyrifera* with the theoretical yields are shown in Figs. S2 and S3.

Usually, saccharification of brown macroalgae is carried out by a two-step process of biomass treatment. In a first step, biomass is processed with acid and subsequently treated with enzymes. Acid for the first step is generally sulfuric acid [26–31], but cases of pretreatment with hydrochloric acid have also been reported [32]. In general, conditions for acid hydrolysis with sulfuric acid vary between 0.1 and 2 wt % at 120–121 °C for 30 to 60 min. Considering that the acid is not
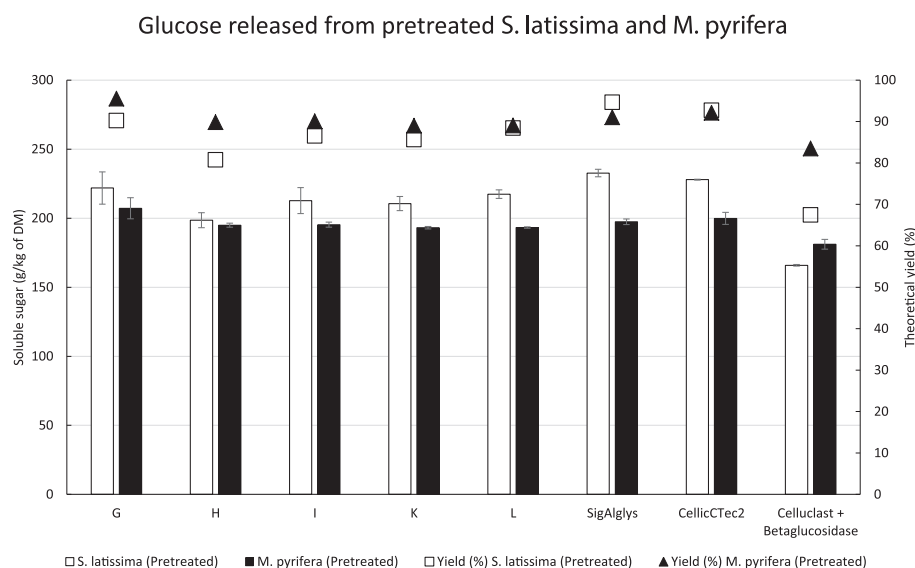
Table 1

Elemental and ash (in % weight dry mass basis) analysis of brown macroalgae in native form, sulfuric pretreated, residue and supernatant after enzymatic saccharification.

Brown algae	Carbon	Hydrogen	Nitrogen	Sulfur	Oxygen[a]	Ash
S. latissima	29.8	4.9	1.6	1.1	35.3	27.2
M. pyrifera pretreated	38.8	5.4	2.3	0.7	51.6	1.3
S. latissima (*Residue after saccharification*)	18.3	2.8	1.7	0.6	33.8	42.8
M. pyrifera pretreated (*Residue after saccharification*)	35.8	4.5	4.0	0.5	37.8	17.3
S. latissima (*Supernatant after saccharification*)	48.5	NA	1.0	0.1	NA	4.8
M. pyrifera pretreated (*Supernatant after saccharification*)	45.1	NA	6.4	0.06	NA	5.6

NA is not applicable.

[a] Calculated oxygen value by subtracting all other elements by 100%.

selective, it is difficult to identify the optimal parameters for acidic saccharification and to control the experimental conditions. As a result, there is a non-negligible risk of either partial release of some monosaccharides, or production of toxins derived from the toughness of the treatment. Even so, acid hydrolysis is still chosen as a good option to be used in combination with enzymes and to reduce their use.

After pretreatment, hydrolysis of the seaweed biomass is necessary to release the sugars locked up in the structural polysaccharides. Enzymatic treatment of brown algae is best reported for Laminaria sp. For instance, cell walls of *Laminaria hyperborea* and *Laminaria digitata* were successfully saccharified using laminarinase after acid pre-treatment [33], releasing 92.5% glucose/g algae. On the other hand, to address heterogeneity of algal carbohydrates, multienzymatic preparations containing predominantly cellulase and cellobiase were successfully applied. That is the case for *Laminaria japonica*, which was saccharified with a mixture of fungal cellulase and cellobiase [30]. In the case of *Saccharina japonica*, acid pretreatment and Thermamyl 120 l, a commercial cocktail containing a thermostable amylase, released 70% of total carbohydrates in the biomass [27], but when pretreated with acid and then with cellulase and glucosidase, 84% of carbohydrates were extracted [31].

With the appearance of the biorefinery concept, in the last few years, much interest has been placed on recovery of non glucose-

producing carbohydrates. That is how the use of alginate lyases combined with oligoalginate lyases became popular in order to take advantage of monomers of alginate. The alginate lyases have been applied either individually [26,34] or combined with cellulolytic enzymes [14,35–36]. The new generation of cellulolytic enzymes represented by Cellic CTec2 and Cellic HTec2 from Novozymes, have also contributed to increase the recovery of carbohydrate components present in seaweed algae polysaccharides, such as, laminarin and cellulose [14,37]). The use of alginate lyase improves the cellulase catalyzed degradation of laminarin and cellulose in the material by selective removal of alginate, as was suggested by results showing that cellulase alone released only half of the available glucose [14].

Table 1 shows elemental and ash (in % weight dry basis) analysis of brown macroalgae in native form, sulfuric pretreated, residue and supernatant after enzymatic saccharification. The increase in carbon, hydrogen, nitrogen and partial sulfur content have been observed in the pretreated macroalgal compared with the native one, resulting from the release of the ash in the hydrolysate. It must be noted that the carbon and hydrogen content of *S. latissima*, in the residue after enzymatic saccharification was less than the native *S. latissima* due to the release of sugars during saccharification. However, the trend of elements present in *M. pyrifera* in the residue after saccharification was similar to the *S. latissima*. Additionally, Supplementary Fig. S1 shows the mass balance and process scheme for *S. latissima* and *M. pyrifera* (pretreated by sulfuric acid) during enzymatic saccharification. This Fig. S1 shows the amount of dry matter and carbon in the substrates (*S. latissima* and *M. pyrifera* pretreated), the enzyme hydrolysates (solubilized) and the solid residues.

3.3. Scanning electron microscopy analysis

Scanning electron microscopy is an analytical technique that has great versatility and importance when studying biomass structure. The results of this analysis are shown in Fig. 4. The images of particles of *S. latissima* native (SL), *S. latissima* pretreated (SLP), enzymatic residue of *S. latissima* (SLS), *M. pyrifera* native (MP), *M. pyrifera* pretreated (MPP), and enzymatic residue of *M. pyrifera* pretreated (MPPS) were obtained by Scanning electron microscopy (SEM) at 5000 ×. The analysis of the images shows a contrast between before and after enzymatic saccharification, also a contrast between untreated and pretreated and between algae species, where the saccharified biomass has a rough surface with a lot of holes demonstrating enzymatic removal of parts of the biomass, possibly glucose was removed. As expected, the results show greater
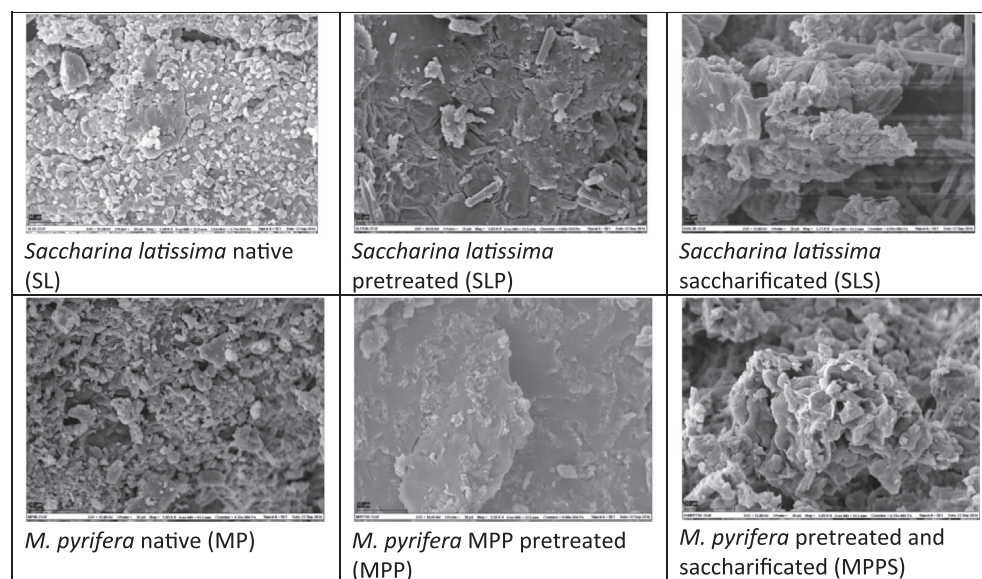

Saccharina latissima native (SL)

Saccharina latissima pretreated (SLP)

Saccharina latissima saccharificated (SLS)

M. pyrifera native (MP)

M. pyrifera MPP pretreated (MPP)

M. pyrifera pretreated and saccharificated (MPPS)

Fig. 4. Images by Scanning electron microscopy (SEM) at 5000 × of: (a) particles of *S. latissima* native (SL), (b) *S. latissima* pretreated (SLP), (c) enzymatic residue of *S. latissima* (saccharified, SLS), *M. pyrifera* native (MP), *M. pyrifera* pretreated (MPP), and enzymatic residue of *M. pyrifera* pretreated (saccharified, MPPS).

Table 2
Candida utilis production from *S. latissima* native and *M. pyrifera* pretreated with 2% sulphuric acid and both brown algae enzymatic hydrolysate.

Brown algae	Initial concentration of glucose (g/l) C_0	Concentration of yeast (g/l)	Yeast nitrogen (% of DM)	Nitrogen to protein factor	Yeast protein (% of DM[*])
S. latissima native	15.4 ± 3.1	6.3 ± 0.2	5.72	5.38	30.77
M. pyrifera pretreated with 2% sulphuric acid	16.3 ± 1.6	8.2 ± 0.1	3.34	5.38	17.96

[*] DM: Dry Mass.

degradation of fibers when the algae were saccharified with different enzymes.

3.4. Biomass loading and S. latissima and M. pyrifera fermentation

Enzymatic hydrolysates were prepared by taking high solid content (15%) of pretreated *M. pyrifera* and native *S. latissima* in order to investigate the potential use of these macroalgae as sources of sugars, nitrogen and other nutrients in the production of yeast. The yeast *Candida utilis*, which has been shown to be a suitable protein source for farmed fish [38], was successfully grown aerobically for 48 h in these hydrolysates. The initial concentration of glucose, final concentration of yeast biomass and its protein content are shown in the Table 2. Algal biomass composed of non protein nitrogen (NPN) such as pigments, nucleic acids, nucleotides, inorganic nitrates (NO_3), ammonium (NH_4) and free amino acids could be produce an overestimation of their protein content value. The nitrogen-to-protein conversion factor of 5.38 for brown algae has been taken for this study [39]. Table 2 shows that 6.3 and 8.2 g of dried yeast per liter were produced in the *S. latissima* (native) and *M. pyrifera* (pretreated) hydrolysates, respectively. The concentration of yeast and protein content in the yeast is highly dependent upon the available carbon and nitrogen in the algae hydrolysates. While the pretreated *M. pyrifera* had a higher protein content than the native *S. latissima*, the yeast produced in the *S. latissima* hydrolysates achieved a much higher nitrogen content. This could be due to the pre-treatment and washing of *M. pyrifera* that may have removed easily available nitrogen.

4. Conclusions

This work shows that the chemical composition of brown algae from Chile (*M. pyrifera*) and Norway (*S. latissima*) differs; in particular, in the ratio between mannitol and glucose, which is higher in *M. pyrifera* than in *S. latissima*. The two brown algae species responded differently to acid pretreatment and washing, in when, for instance, most of the ash was removed from *M. pyrifera* while the ash content *S. latissima* was only slightly reduced. The use of recombinant alginate lyases and oligoalginate lyases in combination with cellulases increased the glucose release from untreated macroalgae. However, for saccharification of pretreated algae only cellulases were needed to achieve high glucose release. Finally, it was shown that algae hydrolysates could be used as growth medium for *Candida utilis* culture for the production of microbial ingredients for use in the diets for farmed fish.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.algal.2017.08.012.

Acknowledgement

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