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Characterization of *Nizimuddinia zanardini* macroalgae biomass composition and its potential for biofuel production

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Abstract

Nizimuddinia zanardini macroalgae, harvested from Persian Gulf, was chemically characterized and employed for the production of ethanol, seaweed extract, alginic acid, and biogas. In order to improve the products yields, the biomass was pretreated with dilute sulfuric acid and hot water. The pretreated and untreated biomasses were subjected to enzymatic hydrolysis by cellulase (15 FPU/g) and β-glucosidase (30 IU/g). Hydrolysis yield of glucan was 29.8, 82.5, and 72.7 g/kg for the untreated, hot-water pretreated, and acid pretreated biomass, respectively. Anaerobic fermentation of hydrolysates by *S. cerevisiae* resulted in the maximum ethanol yield of 34.6 g per kg of the dried biomass. A seaweed extract containing mannitol and a solid residue containing alginic acid were recovered as the main byproducts of the ethanol production. On the other hand, the biogas yield from the biomass was increased from 170 to 200 m^3 per ton of dried algae biomass by hot water pretreatment.

Keywords: Alginic acid; Biogas; Ethanol; Macroalgae; Mannitol; Pretreatment

1. Introduction

Production of biofuels is undoubtedly one of the best solutions for declining the crude oil reserves and global warming due to excessive greenhouse gasses emissions (Harun & Danquah, 2011). Bioethanol, biogas, and biodiesel are the most important biofuels in terms of market share. Production of ethanol from sugars and starch-containing materials, referred to the first generation of ethanol, suffers from the debates in competing with human food. In contrast, the second generation of ethanol, produced from lignocellulosic materials, does not have a direct negative impact on food resources, although it may indirectly affect it by using agricultural lands for preparation of the lignocellulosic materials (Harun & Danquah, 2011). In addition, due to the recalcitrant structure of lignocellulosic materials, different costly pretreatment techniques are required to make the materials susceptible to biological conversions (Kumar et al., 2013; Shafiei et al., 2013; Taherzadeh & Karimi, 2008).

Macroalgae have recently received considerable attentions as a substrate for biofuels production, since they have higher growth rates compared to the plants. Furthermore, they do not need fresh water and land for growth and do not compete with food resources (Borines et al., 2011; Kumar et al., 2013; Mussgnug et al., 2010; Singh et al., 2011; Taherzadeh & Karimi, 2008). Generally, macroalgae (red, brown, and green) are obtained from natural and cultivated resources. The harvested macroalgae is mainly used for production of different hydrocolloids, e.g., agar, alginate, and carrageenan. Limited portions of these materials are also used for production of food, namely in Asian countries (Jung et al., 2013).

In addition to hydrocolloids (10-40%), macroalgae typically contain high concentration of carbohydrates (35-74%) and proteins (5-35%) together with low concentration of lipids (0.2-3.8%) (Ito & Hori, 1989). Algal carbohydrates, obtained from non-arable lands, are promising alternatives substrates for production of bioethanol. Nearly all macroalgae do not contain lignin since unlike plants, they do not need rigidity. Therefore, process of bioethanol production from algal carbohydrates does not need a complex lignin removal step that is typically necessary to increase digestibility of lignocellulosic materials (Jung et al., 2013). It should be mentioned that brown algae contain different concentrations of polyphenols (Horn, 2000), which are usually referred to as lignin-like materials. The lignin-like materials, which are detected in different macroalgae by the standard methods of biomass characterizations, do not exhibit plant lignin properties (Lewis & Yamamoto, 1990; Sluiter et al., 2008b).

Biorefineries integrate different biomass conversion processes for simultaneous production of several value added products including biofuels, chemicals, power, and heat. Macroalgal biorefineries have recently received growing attentions (Jung et al., 2013; Kumar et al., 2013; Mussgnug et al., 2010). Kummar et al. (2013) introduced a biorefinery for conversion of red algae to agar and ethanol. Khambhaty et al. (2012) used red algae for production of ethanol and a biofertilizer. Nkemka and Murto (2012) demonstrated an efficient method for pretreatment of a mixture of a brown and a red algae for biogas production. Golberg et al. (2014) proposed a design for macroalgal biorefineries. The design was examined on a biorefinery for conversion of *Ulva* biomass, a green macroalgae, into ethanol.

This work is a preliminary step for development of a potential biorefinery based on the biomass of *N. zanardini,* a brown macroalgae harvested from Persian Gulf. The biomass was characterized and used for production of bioethanol, biomethane, alginate, and mannitol. Furthermore, the impact of hot water and dilute acid pretreatments on improvement of the products yields were examined.

2. Materials and methods

2.1 Algal biomass, materials, and fermenting microorganism

The biomass of *N. zanardini,* was harvested from the southern coasts of Qeshm Island (Persian Gulf, Iran) in July. It was identified by the Off-Shore Fisheries Research Center (Chabahar, Iran). After washing with distilled water, it was dried in an oven at 40°C for 24 h. Moisture content of the dried biomass was measured at 105°C until a constant weight. The dried biomass was milled using a hammer mill, screened to achieve a size below 1 mm, and stored at -4°C until use. Two commercial enzymes, cellulase (Celluclast 1.5 L, Novozyme, Denmark) and β-glucosidase (Novozym 188, Novozyme, Denmark) were used for enzymatic hydrolysis. The cellulase and β-glucosidase activities were respectively measured according to methods presented by Adney and Baker (2008) and Eduardo et al. (1996), and their corresponding values were 60 FPU/ml and 190 IU/ml. A flocculated strain of *Saccharomyces cerevisiae* CCUG 53310 obtained from Culture Collection of University of Gothenburg (Sweden) was used for ethanolic fermentation. It was grown on agar slants containing (g/l): yeast extract, 10; peptone, 10; glucose, 20; and agar, 20, at 30°C for 48 h and then stored at 4°C. A solution containing 50 g/l glucose, 7.5 g/l $(NH_4)_2SO_4$, 3.5 g/l

K_2HPO_4, 1 g/l $Cacl_2.2H_2o$, 0.75 g/l $MgSO_4.7H_2O$, and 5 g/l yeast extract was prepared and autoclaved for 20 min at 121°C. After cooling to room temperature, the solution (200 ml) was inoculated with the yeast cells in 500 ml Erlenmeyer flasks. The cells were grown at 30°C and 110 rpm for 24 h in cotton plugged flasks (Karimi et al., 2006). The biomass was separated by centrifugation for 5 min at 7000 rpm under aseptic conditions and used for ethanol production under anaerobic conditions.

2.2 Algae biomass characterization

Biomass of *N. zanardini* was analyzed for extractives (Sluiter et al., 2008c), carbohydrates, lignin-like materials, acetate (Sluiter et al., 2008b), ash (Sluiter et al., 2008a), and protein (Hames et al., 2008) contents based on the procedures described in the standard biomass analytical procedures. Ash was also analyzed for its macro-mineral contents (sodium, potassium, and calcium). These elements were extracted from the ash with 70 and 60% w/v solutions of HNO_3 and $HClO_4$, respectively, and analyzed by an atomic absorption spectrophotometer (Perkin-Elmer, UK) (Fleurence & Coeur, 1993). The sulfur content was analyzed by a CHNSO thermo analyzer (Perkin-Elmer, UK). Alginic acid content was determined through an alkaline extraction process at 80°C according to the method presented by Basha et al. (2011).

2.3 Dilute acid and hot water pretreatments of *N.zanardini* dried biomass

Hot water and dilute sulfuric acid pretreatments were used to improve ethanol production from the algal biomass. Macroalgal biomass was mixed with dilute sulfuric acid solution or

distilled water in 118 ml glass bottles. Pretreatments were performed at 121°C and different solid loadings (5 and 10 %w/v), retention times (30, 45, and 60 min), and sulfuric acid concentration (7.0 %w/w). After the pretreatments, solids were separated from the solutions by vacuum filtration and washed several times with distilled water to reach neutral pH.

2.4 Separate saccharification and fermentation for ethanol production

Pretreated and untreated macroalgal biomasses were added to a citrate buffer solution (0.05 M), and the enzymatic hydrolysis was initiated by adding cellulase (15 FPU/g of dried macroalgae biomass) and β-glucosidase (30 IU/g of dried macroalgae biomass). The hydrolysis was performed at 45 °C and 100 rpm for 48 h in 118 ml reactors with 50 mL working volume. The liquid samples were periodically taken and analyzed for sugar contents.

After 24 h enzymatic hydrolysis, the hydrolysates were separated from the insoluble residues by centrifugation at 5000 rpm for 10 min. Then, 40 ml of the hydrolyzates were supplemented with 7.5 g/l $(NH_4)_2SO_4$, 3.5 g/l K_2HPO_4, 1 g/l $CaCl_2.2H_2O$, 0.75 g/l $MgSO_4.7H_2O$, and 5 g/l yeast extract (Karimi et al., 2006). The pH of the solution was adjusted to 4.8 using sulfuric acid (1M) and sodium hydroxide (1M) solutions. Afterward, the solution was autoclaved at 121°C for 20 min and after cooling to room temperature, inoculated with 1 g/l (dry weight) of *S. cerevisiae*. The bottles were sealed with butyl rubbers and aluminum caps. Anaerobic condition was attained by purging the fermentation flasks with pure nitrogen. The fermentations were performed at 32 °C and 100 rpm for 48 h.

2.5 Anaerobic digestion

Anaerobic digestion of the untreated and hot water pretreated macroalgae was performed according to the method presented by Hansen et al. (2004). For the hot water pretreatment, a solution of 5% macroalgae biomass in water was heated at 121°C for 30 min in an autoclave. For the untreated sample a 5 % solution of the original biomass was used. The obtained mixtures were then subjected to the anaerobic digestion after cooling to room temperature.

Mesophilic inoculum was obtained from a 7000 m^3 biogas plant (Isfahan south wastewater plant, Isfahan, Iran) and used in all experiments. An amount of 20 ml microbial inoculum was added to each bottle, and the bottles were closed using rubber seals and aluminum caps. At the beginning of the digestions, anaerobic condition was obtained by purging the system with nitrogen as an inert gas. The digestion was performed at 37 ºC for 40 days. The biogas production from inoculum without substrate was also monitored as a reference over the 40 days of digestion.

2.6 Analytical methods

A high-performance liquid chromatograph (HPLC) equipped with UV/VIS and RI detectors (Jasco International Co., Japan) was used to analyze all the liquid samples. Concentrations of sugars were determined by an Aminex HPX-87P column (Bio-Rad, USA) at 80 °C using deionized water as eluent (at a flow rate of 0.6 ml/min). Acetic acid, furfural, HMF, and ethanol were analyzed by an Aminex HPX-87H column (Bio-Rad, USA) at 60 °C with 0.6 ml/min eluent of 5 mM sulfuric acid. Concentrations of glucose, mannitol, arabinose,

mannose, ethanol, and acetic acid were determined by RI detector, while furfural and HMF were quantified using UV chromatograms at 210 nm.

A gas chromatograph (GC) (Perkin-Elmer, UK) equipped with a packed column (3 m length and 3 mm internal diameter, stainless steel, Propak Q column, Chrompack, Germany) and a thermal conductor was used to analyze composition of the biogas during the anaerobic digestion. The carrier gas was helium (20 ml/min), and the temperature of the column, injector, and detector was 50, 90, and 140 °C, respectively. The gas samples were taken using a 250 µl pressure-tight syringe (VICI, Precision Sampling Inc., USA) and injected directly to the GC. The excess gas was released after each sampling from the bioreactors using a needle. Pure methane and carbon dioxide were used as standards. All biogas production results are presented at standard conditions.

All experiments were performed in duplicated except the biogas production experiments which were triplicated.

3. Results and Discussion

Production of ethanol and biogas from macroalgae is suggested to be one of the best solutions for management of fuel versus food conflict problems (Jung et al., 2013). Furthermore, simultaneous production of other value-added materials such as mannitol and alginate, via a biorefinery, can improve the economy of the biofuel production processes.

So far, more than 119 macroalgal species have been collected and identified from Persian Gulf (Sohrabipour et al., 2004), which have a high potential for production of biofuels. In this work, glucan fraction of the macroalgal biomass was converted to ethanol, while

mannitol and alginic acid remained intact. The latter two materials can be used to produce ethanol (Enquist-Newman et al., 2014), in addition, they have several applications in food and medical industries. The alternative route was subjection of the whole ingredients of the algal biomass to anaerobic digestion for biogas production.

3.1 Characterization of *N. zanardini* biomass

The chemical composition of *N. zanardini* was characterized, based on the standard methods, and the results are presented in Fig. 1. Mannitol, alginic acid, potassium, proteins, and glucan were the major ingredients of the algal biomass with contribution of 171.2±11.5, 150.0±10.0, 115.2±8.0, 100.0±7.1, and 90.0±5.0 g per kg of dried biomass, respectively. Furthermore, water and ethanol extractives represented 495.3±21.0 and 35.6±2.1 g/kg of the biomass, respectively. Moreover, total amount of mannitol together with other polysaccharides including arabinan, glucan, xylan, mannan, and galactan was 320.6±20.2 g per kg of the biomass. Free mannitol, which was water extractable, comprised 15.5±1% of the total mannitol in biomass. Polyphenols, i.e., lignin-like materials, were the other important biomass ingredient available at high concentrations (123.2±8.5 g/kg). Furthermore, the biomass contained 73±3.0% volatile solids. The chemical composition of the *N. zanardini* biomass was comparable with that of the *Saccharina latissima,* a brown macroalgae according to Scullin et al. (2014). The glucan, mannitol, and alginate content of the *S. latissima* biomass harvested in July was more than biomass harvested in December and August, and they were 17.9%, 26%, and 25%, respectively. Shyamali et al. (1988)

reported that ethanol extractives, polysaccharides, lignin like materials, and ash content of the *Turbinaria conoides*, a brown macroalgae, were 38.0, 31.8, 19.2, and 34.3 %, respectively. The results obtained in this study are in the line of the previous reports.

3.2 Formation of inhibitors and release of sugars during the pretreatments

The objectives of the dilute-acid and hot water pretreatments were enhancement of ethanol and biogas productions from the macroalgal biomass. Generally, pretreatments resulted in release of different sugars together with formation of some inhibitors. Products of sugar degradation such as HMF and furfural, which can be formed during the pretreatments at high temperatures, may inhibit ethanol production by yeast and bacteria (Klinke et al., 2004). Accordingly, in this work, the goal was choosing appropriate pretreatment conditions resulting in minimal inhibitory compound formation. Table 1 shows furfural and HMF yields during hot water and acid pretreatments at two different solid loadings of 5 and 10%. HMF was not significantly formed at 5% solid loading. In contrast, at 10% solid loading, 0.1-0.4 g HMF per kg of biomass was formed during the acid pretreatment, but not during the hot water pretreatment. On the other hand, no detectable furfural was formed during hot water and 0.5% acid pretreatments, while pretreatment with higher acid concentrations (i.e. 3.5 and 7%) resulted in the formation of 0.1-0.6 g furfural per kg of biomass regardless of the solid loading. Since HMF was not produced at considerable amounts at 5%, this level of solid loading was chosen for the rest of experiments.

The pretreatments resulted in releasing different monosaccharides and acetic acid from the macroalgae (Table 2). The sugar alcohol mannitol was the most important liberated

ingredient in terms of concentration. Hot water pretreatment for 30 min released 92.5 g mannitol from each kg of the macroalgae, corresponded to 54% of the total mannitol available in the biomass. Mannitol yield decreased to 75 g/kg after 45 min pretreatment, while increased to 100 g/kg after 60 min pretreatment. The same trend was observed during all acid pretreatments in which a subsequent decrease and increase was observed in the yield of liberated materials by prolonging the pretreatment from 30 to 45 and 60 min.

For all pretreatment times, increasing the acid concentration from 0 to 3.5% accompanied with enhancement of mannitol yield. However, further increase in the acid concentration to 7% resulted in either no change (for 30 and 45 min treatments) or slight reduction (for 60 min treatment) in the mannitol release (Table 2). The level of polysaccharides branching with mannitol in the macroalgal biomass may considerably affect the mannitol yield (Kim, 2011). Highly branched mannitol was released to the pretreatment solution after 60 min, while release of free mannitol and less branched mannitol occurred earlier. On the other hand, higher acid concentrations may lead to mannitol degradation.

Release of acetic acid during the course of pretreatment is an indication of progress of the hydrolysis of hemicellulosic-like carbohydrates. As shown in Table 2, regardless of the pretreatment time, increasing the sulfuric acid concentration from 0 to 0.5% ended with a sharp increase in acetic acid liberation (more than 4.5 folds enhancement). Acetic acid release was not significantly increased at higher concentrations of sulfuric acid (Table 2).

Generally pretreatment time and acid concentration did not show significant impact on galactose liberation and on average 12.4g galactose per kg of biomass was released (Table 2)

Pretreatment with hot water and 0.5% acid did not hydrolyze more than 4.6 g arabinose per kg of biomass (Table 2), which was less than 22% of initial arabinan available in the biomass, whereas treatment with 3.5% sulfuric acid for 30 min resulted in 8.3g/kg arabinose. The yield increased to 15.2 g/kg after 45 min, while it declined to 11.2 g/kg after 60 min treatment. Glucan and mannan hydrolyses did not considerably take place at pretreatment with acid concentrations of less than 7%. By using 7% acid, however, 2.9-5.0 g/kg mannose was released and the yield was reduced by increasing the pretreatment duration (Table 2). Among different polysaccharides, glucan showed the lowest degree of hydrolysis at the applied conditions. This indicated the low level of glucan in hemicellulosic like materials in the biomass. Furthermore, the pretreatments did not lead to significant hydrolysis of the cellulosic-like materials content of the biomass. At 7% acid concentration, by prolonging the pretreatment from 45 to 60 min, glucose yield was enhanced from 6.3 to 10.5 g/kg (lower than 11% hydrolysis of the total glucan content). This enhancement suggested that hydrolysis of the cellulosic-like carbohydrates was started by 60 min pretreatment with 7% acid solution.

3.3 Recovery and characterization of pretreated biomass

In total, 26 and 40% of the algal biomass was recovered after 45 min pretreatment by 7% sulfuric acid solution and hot water, respectively. Glucan (80.0-89.1g/kg), alginic acid (109.9-150.0g/kg), and lignin-like materials (70.5-96.5g/kg) were the major ingredients of the recovered solids. The pretreatment with acid removed almost all hemicelluloses, mannitol, and potassium from the biomass, whereas these components made 16% (65.5 g/kg) of the biomass remained after hot-water pretreatment (Fig. 2a).

3.4 Characterization of seaweed extract after the pretreatments

According to the solid recoveries, 74 and 60% of the macroalgae biomass were expected to be released after the acid and hot water pretreatments, respectively. The analysis of the liquid phase after the pretreatment in both cases showed that mannitol, potassium, protein, and lignin-like materials were correspondingly the major liberated ingredients (Fig. 2b). Other monosaccharaides, alginic acid, and acetic acid were also released during the course of acid pretreatment (42.1, 40.0, and 31.8 g/kg, respectively), though they were released at lower portions during the hot water pretreatment (17.4, 0.0, and 5.8 g/kg, respectively).

As a whole, the pretreatments resulted in alginic acid enrichment in the remaining solids and mannitol extraction from the biomass. During pretreatment, hemicellulosic-like carbohydrates were hydrolyzed and monosaccharaides were released to the solution while they were degraded by increasing the acid concentration from 0 to 7% acid.

3.5 Enzymatic hydrolysis of the macroalgal biomass

Glucan of microalgae biomass is believed to be surrounded by other carbohydrates as well as lignin-like materials which limit the action of hydrolytic enzymes, i.e., cellulases

(Borines et al., 2011; Okuda et al., 2008). Therefore, a pretreatment step, which increases the accessibility of glucan, was suggested to improve the performance of the enzymes. Okuda et al. (2008) showed that hydrothermal pretreatment can improve the rate of enzymatic hydrolysis of glucan in red and green macroalgae. Ge et al. (2011) showed that dilute sulfuric acid pretreatment can improve the yield of enzymatic hydrolysis of an algal biomass residue, after extraction of alginate. In addition, glucan was not significantly released from biomass of *N. zanardidni* during the hot water and acid pretreatments in this study. Therefore, the untreated biomass and solid residues of the pretreatments were subjected to hydrolysis by cellulase and β-glucosidase enzymes, and the results are presented in Fig. 3. Glucose yield from the untreated biomass did not exceed 30 g/kg after 24 h enzymatic hydrolysis, which represents 29.8% of the theoretical yield. Pretreatment with hot water for 30 min enhanced the yield of glucose production to 41 g/kg, whereas increasing the pretreatment time to 60 min increased the yield to 82.5 g/kg (over 80% of the theoretical yield). Using 7% acid pretreatment, 47.9 g/kg glucose was released from the 30 min pretreated biomass. This was improved to 72.7 g/kg after 45 min pretreatment, while reduced to 30 g/kg by prolonging the pretreatment time to 60 min. The highest yields were 82.5 and 72.7 % of theoretical yields, which were obtained after pretreatment with hot water for 60 min and 7% acid for 45 min, respectively. Therefore, these two conditions were selected for pretreatment of biomass prior to ethanol production.

The yield of enzymatic hydrolysis was not considerably increased by prolonging the hydrolysis time over 48 h (data not shown).

3.6 Production of ethanol from pretreated biomass

Hydrolysates of pretreated macroalgae were fermented to ethanol by *S. cerevisiae*, and ethanol yield of 0.42 g/g consumed glucose was obtained for both of the pretreatments. Considering the recovery of the biomass after pretreatments and the yield of enzymatic hydrolysis, ethanol yields after the hot water and acid pretreatments were 34.6 and 30.5 g/kg initial macroalgae biomass, respectively. Based on the initial glucan available in the biomass, these values correspond to 68 and 60% of the theoretical yields. The capability of *S. cerevisiae* strain for fermentation of mannitol to ethanol was also investigated, but no ethanol was produced (Table 3).

Kumar et al. (2013) also achieved a comparable ethanol yield of 0.43 g/g sugar obtained from the residue of a red macroalgae after agar extraction. Horn (2000) employed *Pichia angophorae* for conversion of glucose and mannitol derived from a brown macroalgae and got ethanol yield of 0.43 g/g sugar. Accordingly, if higher yield of ethanol is desired, mannitol-fermenting microorganisms can improve the yield (Ota et al., 2013). Thus, a technoeconomical study is necessary to choice the best way between fermentation of mannitol and its purification.

3.7 Biogas production from untreated and pretreated macroalgal biomass

Biogas is a suitable and widely applied form of energy. Simplicity of production and separation as well as low processing cost are among the advantages of biogas production.

In this study, production of biogas from the macroalgae was investigated via an anaerobic digestion under mesophilic conditions, and the results, as methane yields at standard

conditions, are summarized in Fig. 4. Pretreatment with hot water for 30 min, with the highest biomass recovery among different pretreatments, was chosen for the biogas enhancement. No significant improvement of biogas production by the pretreatment was observed during the first four days of digestion; however, considerable enhancement was observed after 10 days. The biogas production continued until 28 days. Then, methane production declined slowly and no significant gas production was observed after 28 days of digestion. The ultimate methane yields from the untreated and pretreated macroalgae biomass were 117 and 143 ml/g VS, respectively, which were obtained after 40 days with respective methane concentrations of 50 and 52% v/v. Considering the concentration of volatile solids in the substrate, the biogas yield was 170 and 200 ml/g for untreated and pretreated biomass, respectively. The highest yield of 143 ml/g VS of the algal biomass, is in line of the results presented by Biswas et al. (2009) in which methane yield of 110-230 ml/g VS was achieved from *Polysiphonia.*

In this study, hot water pretreatment for 30 min increased the methane yield and purity by 22 and 2%, respectively. Biswas et al. (2009) also reported increasing the biogas and methane yield by 28% and 19%, respectively, and decreasing the methane purity after hot water pretreatment for 30 min.

3.8 Suggested biorefinery

In this study, a biorefinery concept was suggested for production of different value-added products from biomass of *N. zanardini* (Fig. 5). The macroalgal biomass was either pretreated with hot water or hot dilute sulfuric acid to extract mannitol from the biomass

and improve the biological conversion of the residual solids. The solid residue of the pretreated biomass was then subjected to separate saccharification and fermentation process to produce ethanol and an alginate rich solid residue. The alternative route in this biorefinery was direct subjection of the mixture of the pretreated biomass and liquid (hot water extracts) to the anaerobic digestion process for production of biogas. Under the best conditions tested in this study, from each kg of dry biomass, 200 l biogas or 100 g mannitol, 150 g alginic acid, and 34.6 g ethanol was obtained.

4. Conclusions

The biomass of *N. zanardini* contains different valuable chemicals that can be applied for production of ethanol, biogas, seaweed extract (rich in mannitol), and alginic acid. Dilute sulfuric acid and hot water pretreatments were promising processes to improve the digestibility of the initial biomass and increased the yield of ethanol and biogas.

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Figure Captions

Fig. 1. *N. zanardini* biomass composition (numbers in parentheses are presented in g per kg of dried macroalgae biomass).

Fig. 2. (a) Composition of untreated (Black line) and pretreated macroalgae biomass, (b) seaweed extract compositions after pretreatment with sulfuric acid (7% for 45 min, Gray line) and hot water (60 min, White line).

Fig. 3. Glucose yield from 24 hour enzymatic hydrolysis of untreated and pretreated macroalgae biomass. The white bars indicate hot water pretreatment and the black bars indicate the dilute acid pretreatments (7% for 45 min).

Fig. 4. Accumulative methane yields from untreated and hot water pretreated macroalgae biomass, (a) during 40 days digestion (b) zoom-in to the 0-10 days period. The symbols represent: (Diamond) untreated and (Square) hot water treated biomass**.**

Fig. 5. A scheme of a biorefinery suggested for production of ethanol, alginic acid, biogas, and seaweed extract from *N. zanardini* biomass

Tables

Table 1: Furfural and HMF yields by pretreatment with 7% sulfuric acid and hot water for 45 min.

Table 1

Pretreatment conditions		Furfural yield	HMF yield
Solid loading (%)	Sulfuric acid (%)	(g/kg)	(g/kg)
5	0.0	<0.1	<0.1
	7.0	0.6±0.1	<0.1
10	0.0	<0.1	<0.1
	7.0	0.6±0.1	0.4±0.1

Table 2: Acetic acid and monosaccharide yields (g/kg) after pretreatment of *N. zanardini* biomass with hot water and 0.5, 3.5, and 7% sulfuric acid solution for 30, 45, and 60 min.

Table 2

Pretreatment conditions	Time (min)	Acetic acid	Glucose	Mannitol	Xylose	Galactose	Arabinose	Mannose
Hot water pretreatment	30	4.8±0.1	<0.1	92.5±6	<0.1	12.8±1.0	1.5±0.1	0.1
	45	5.1±0.1	<0.1	75±4.5	<0.1	12.3±1.0	3.8±0.1	0.1
	60	5.8±0.1	0.8±0.1	100±6.5	0.9±0.1	12.1±1.0	3.5±0.1	0.1
Sulfuric acid (%)	30	24.8±1.5	1.2±0.1	105.7±6.5	5.9±0.3	12.9±1.0	1.8±0.1	0.1
0.5	45	25.6±1.5	3.1±0.2	83.7±5.0	6.5±0.3	12.5±1.0	4.6±0.3	0.1
	60	26.1±1.5	4.3±0.2	111.9±7.0	6.2±0.3	12.0±1.0	3.3±0.2	0.1
	30	25.8±1.5	1.8±0.1	119.7±7.0	6.6±0.3	13.1±1.0	8.3±0.5	0.1
3.5	45	27.3±1.7	3.7±0.2	117.5±7.0	8.8±0.5	11.9±1.0	15.2±1.0	0.1
	60	30.1±2.0	5.2±0.3	122.8±8.0	8±0.5	11.9±1.0	11.2±0.7	0.1
	30	28.8±1.5	4.1±0.2	117.3±7.0	7.5±0.5	12.4±1.0	12.4±1.0	5.0±0.3
7	45	31.8±2.0	6.3±0.4	117±7.0	8.2±0.5	11.6±1.0	12.8±1.0	3.2±0.1
	60	31.9±2.0	10.5±0.6	109±6.5	19.2±1.1	10.6±1.0	9.6±0.5	2.9±0.1

1

2 Table 3: Ethanol yields from hydrolysates and mannitol by *S. cerevisiae*.

3

Substrate	Pretreatments	Fermentation conditions	Ethanol Yield (g/kg)*
Enzymatic hydrolysate of pretreated algae	Dilute acid**	Anaerobic	30.5±1.1
	Hot water***		34.6±1.5
Mannitol solution	-	Aerobic	<0.1
		Anaerobic	<0.1

4 *The yield was calculated based on the initial macroalgae biomass dry weight.

5 **Dilute acid pretreatment with 7% for 45 min

6 ***Hot water pretreatment at 120°C for 60 min

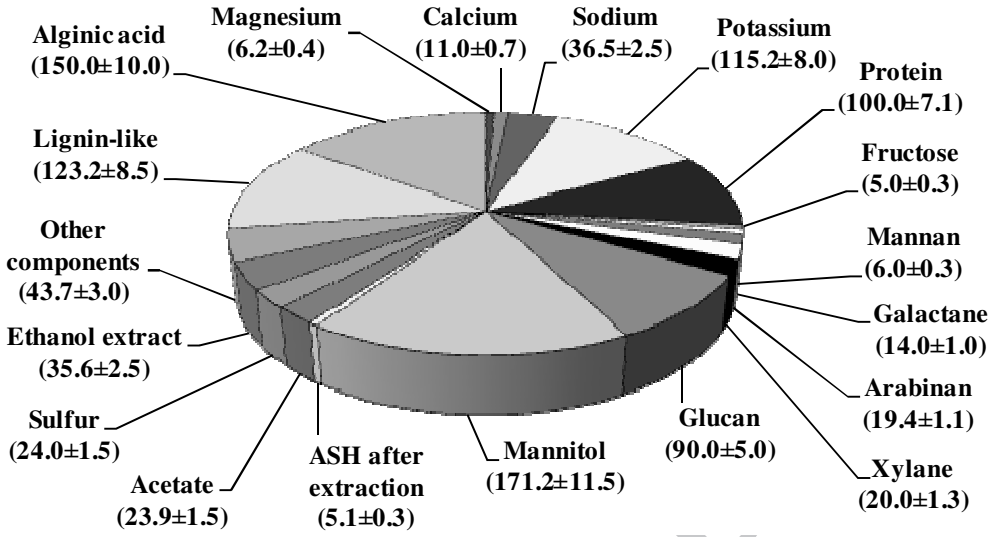

Fig. 1

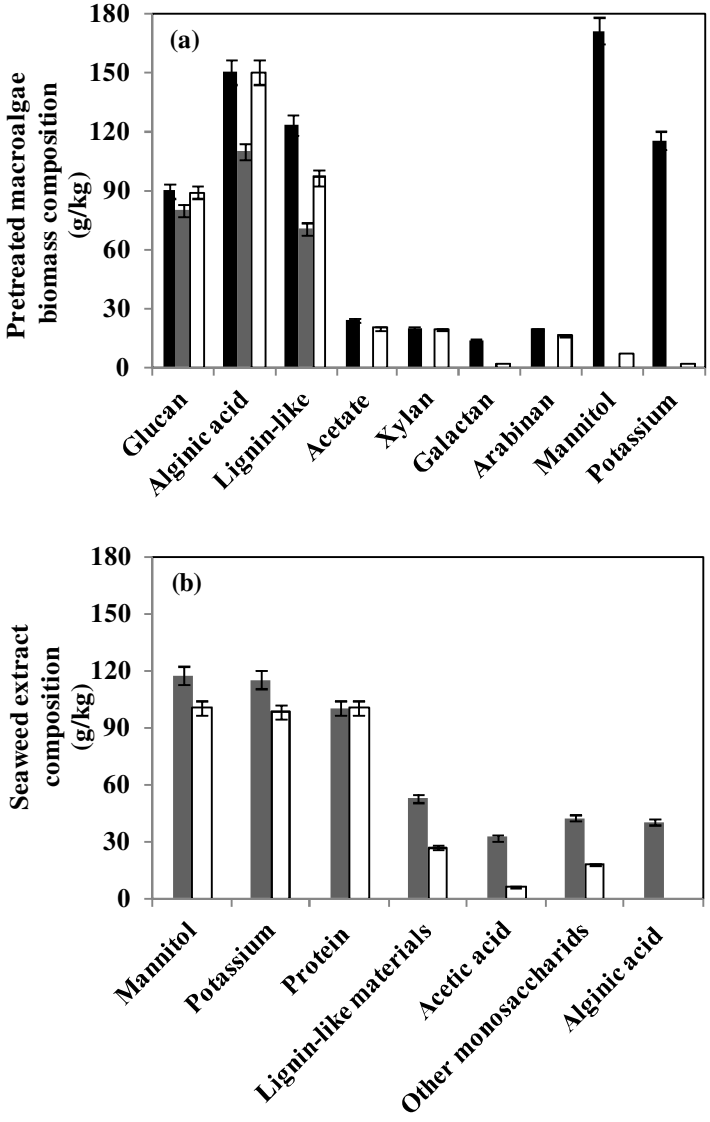

Fig. 2

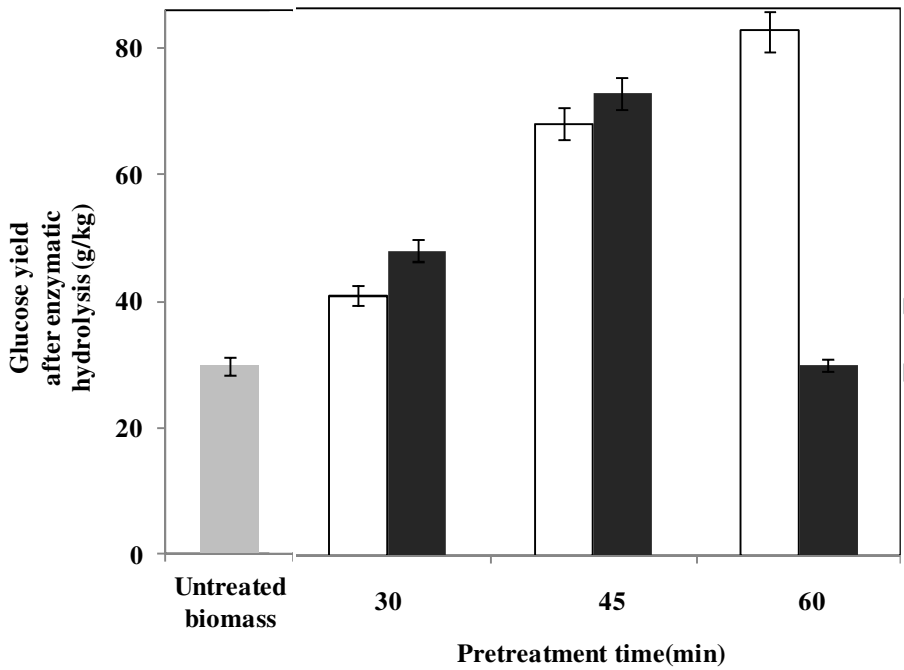

Fig. 3

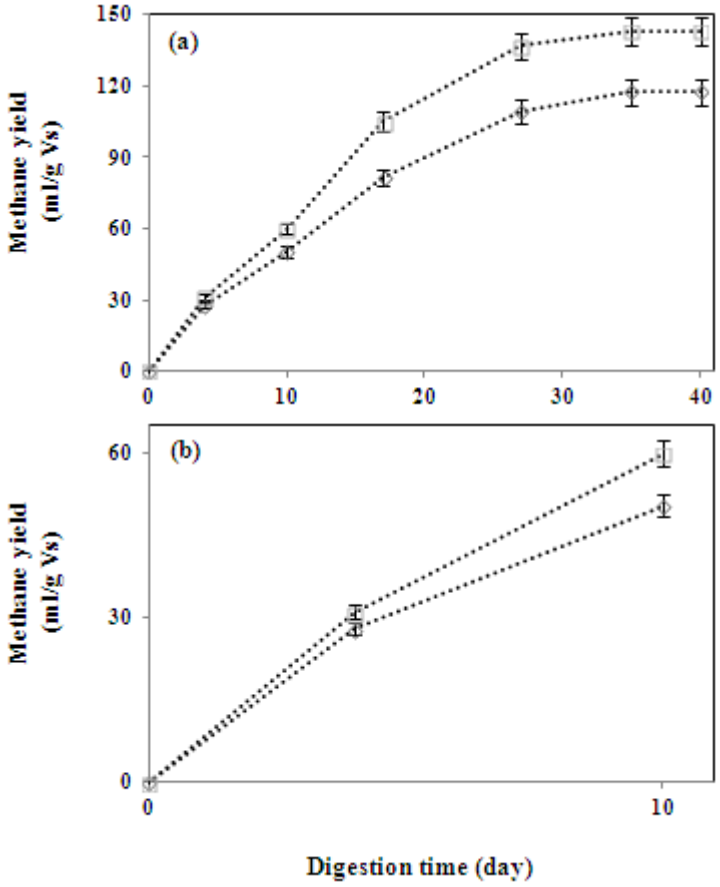

Fig. 4

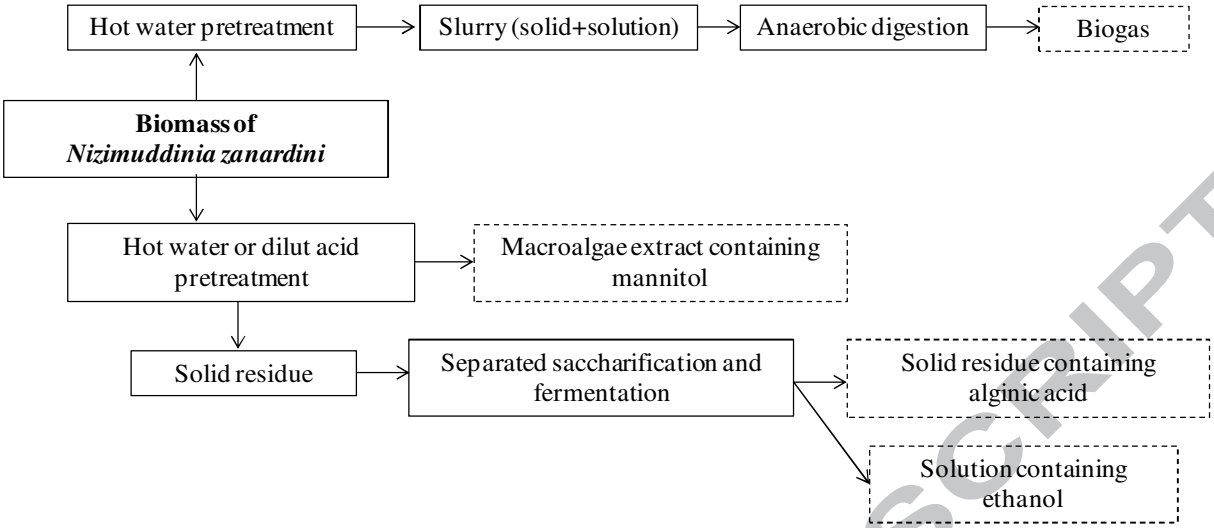

Fig. 5

Biorefinery of biomass of the macroalgae, *Nizimuddinia zanardini,* was investigated.

The biomass mainly contained mannitol, alginic acid, potassium, proteins, and glucan.

A valuable seaweed extract and also alginic acid were separated from the biomass.

The rest of biomass was converted to ethanol or biogas through fermentation process.

The biofuel yields were enhanced by hot water or dilute acid pretreatments.