A review on the biomass pretreatment and inhibitor removal methods as key-steps towards efficient macroalgae-based biohydrogen production

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Abstract

(Red, green and brown) macroalgal biomass is a propitious candidate towards covenant alternative energy resources to be converted into biofuels i.e. hydrogen. The application of macroalgae for hydrogen fermentation (promising route in advancing the biohydrogen generation process) could be accomplished by the transformation of carbohydrates, which is a topic receiving broad attention in recent years. This article overviews the variety of marine algal biomass available in the coastal system, followed by the analyses of their pretreatment methods, inhibitor formation and possible detoxification, which are key-aspects to achieve subsequent H_2 fermentation in a proper way.

Keywords: Macroalgae biomass; Pretreatment; Detoxification; Biohydrogen; Fermentation

1. Introduction

Biohydrogen has become a noteworthy renewable energy carrier because of its beneficial properties including high gravimetric energy density (Rahman et al., 2015) and clean combustion (Xia et al., 2015; Bahadar and Khan, 2013; Cai et al., 2011). Therefore, it could have the potential to reduce environmental and ecological concerns (Fan et al., 2006; Jeong et al., 2011; Khambhaty et al., 2012; Guo et al., 2008; Ren et al., 2008). The technologies for H_2 gas production can rely on the use of certain sustainable resources (Elliott et al., 2014; Hargreaves et al., 2013; Venkata Mohan, 2010) but presently, large-scale methods depend mostly on the conversion of natural gas, heavy oils, naphtha and coal and only limited quantities are delivered in alternative ways e.g. electrolysis and biomass processing (Zhao and Yu, 2008).

Among the various biomass sources as starting materials for bioH_2 production, algae have attracted particular attention due to their features such as relatively lower land requirement for cultivation and remarkable organic matter content (Vardon et al., 2012; Zhou et al., 2017). The macroalgae species productivity ranges from 150 to 600 t fresh weight/hectare on annual grounds, the entire world production is estimated as 12 million tones dry matter/year (FAO Statistics, 2010). As for the current, global algae farming, the notable dominance of Asian countries is observed with an estimated 96 % contribution (Kawai and Murata, 2016).

Despite such definitive advantages of algae biotechnology, improvements are still encouraged in aspects such as the design of cost-efficient photo-bioreactors, flocculation and harvesting techniques in order to further promote the scale-up and commercialization of algae-based bioenergy production (Kim et al., 2011; Kumar et al., 2013; Mazumdar et al., 2013). Biofuels – for instance hydrogen – derived from (macro)algae (referred also as seaweed or marine algae) are distinguished as third-generation ones, where this type of biomass, attributed to its effective growth rate, CO_2-fixing capability, lack of lignin as a cell wall constituent, etc. is considered as a promising raw material. (Azapagic and Stichnothe, 2011; Huesemann et al., 2012; John et al., 2011; Jung et al., 2013). In this regard, many papers of the recent literature have also emphasized the benefits in the application of algae feedstock for bioenergy production both in the academic and industrial sectors. (Kawai and Murata, 2016; Kumar et al., 2014a; 2015a; Roberts and Upham, 2012; Carlsson et al., 2007; Chisti, 2007). Just in the recent years, the potential of algae in bioelectrochemical systems has been realized too, opening a quite fresh avenue for biotechnological application (Saratale et al., 2017)

Macroalgae are multicellular, showing plant-like characteristics (Aitken et al., 2014; Borines et al., 2013; Maceiras et al., 2011) and accumulating carbohydrates in significant amounts. This latter feature makes them plausible feedstock candidates in the biohydrogen fermentation process, where sugars as substrates are preferred compounds. In fact, the lignin-free red, green and

brown marine algae (containing agar and fibre-based carbohydrate moieties in considerable quantities) have been successfully applied in the dark fermentative biohydrogen technology (Kumar et al., 2015a; Park et al., 2011).

Though algae are apparently suitable to generate H_2 via biological routes, an efficient process from such a complex feedstock should concern the pretreatment and successive detoxification of the biomass obtained. Hence, in the coming parts of this review, characteristics of macroalgal biomass will be discussed, followed by the analyses of recent achievement on the topics of (i) algal pretreatment and (ii) detoxification of pretreated fraction (called also as inhibitor removal).

2. Characteristics of macroalgae biomass

On historical grounds, the algal biorefinery has started in the 17th century towards industrial soda and alginate in France and Ireland (Chen et al., 2015). In 1980s, *Macrocystis* spp. was appointed for biofuel production in California (Jiang et al., 2016) and the blooming crude oil price in USD from 1990s has approached the peak in 2008. Basically, the gradually increasing and peaking oil prices have acted as strong inducers of biofuel research and as a result macroalgal biorefinery has been remarkably developed in the last decades, as well (Jiang et al., 2016).

The macroalgal photoauxotrophic organisms aid the biodiversity in marine eco-systems by contributing to the prevention of eutrophication and pollution (Sambusiti et al., 2015; Rajkumar et al., 2013; Shi et al., 2011). Based on their pigmentation progression, they are categorized into *Rhodophytae*, *Chlorophytae* and *Phaeophytae* (Lobban et al., 1985; Schultz-Jensen et al., 2013; Scullin et al., 2015; Trivedi et al., 2013). The most important constituents of the macroalgae include reserve as well as structural carbohydrate portions (Yoza and Masutani, 2013; Laurens et al., 2012), the amount of which varies between species (Luning, 1990; Ross et al., 2008; Renaud and Luong-Van, 2006). For example, red, green and brown algae are to be characterized with carbohydrate quantities such as 25–60 %, 30–60 % and 30–50 % of dry weight, respectively. Further main components of the species include proteins (7–15 % of dry weight), lipids (1–5 % of dry weight), etc. (Sambusiti et al., 2015; Yanagisawa et al., 2013; Shi et al., 2011; Jensen, 1993).

In general, red algae comprise of heterosidefloridoside [*α-D*-galactopyranosyl-(1–2)-glycerol], a floridean starch as major component. Besides, red algae contain carbohydrates in the form of agar (agarose and agaropectin), carrageenan and glucans and certain species restrain some other carbohydrates, for instance digeneaside (*Ceramiales*), mannitol (*Caloglossa*, *Ceramiales*), sorbitol, and *D*- and *L*-isofloridoside (*Porphyridiales*), which are the isomeric forms of floridoside (Karsten et al., 1999, 1993). As for brown algal species, they possess alginate, mannitol, glucose chains (M- and G-

chains, respectively) and laminarin, a *β*-1,3-linked glucan (Davis et al., 2003; Mauseth, 2003). In comparison, green algae contain polymerized glucose (i.e. cellulose and starch), sucrose as well as sulfated polysaccharides (ulvan) (Bruhn et al., 2011; Jiang et al., 2016; Kawai and Murata, 2016; Suutari et al., 2015; Van der Wal et al., 2013).

Commercially significant genera such as *Gelidium* and *Gracilaria* consist of agarose and agaropectin (building blocks of agar). The former polysaccharide substance, agarose, is composed of repeating disaccharide units involving *β-D*- galactose and 3,6-anhydro-*α-L*-galactose (AHG). Some of the *L*-galactose can be replaced with either sulfated galactose or with 4,6-*o*-(1-carboxyethylidene)-*D*-galactose in agaropectin though it has the same repeating units as agarose. In addition, *Gigartina*, *Chondruscrispus*, *Eucheuma* and *Hypnea* species yield *μ*- / *ν*- / *λ*-carrageenans, which all chiefly comprised of the repeating disaccharide units containing *β-D*-galactose and *α-D*-galactose. Moreover, κ- / *i*- /*θ*-carrageenans are mainly built-up by disaccharide units made of *β-D*-galactose and 3,6-anhydro-*α-D*-galactose (Kawai and Murata, 2016).

Nevertheless, it is worth noting that composition (i.e. relative ratio of constituents) of different sort of macroalgae can be dependent on the place of origin and seasons of the year (due to various stages of algal development) (Kumar, 1993). For instance, the literature reveals that the highest carbohydrate profile is found during summer and autumn (Kerjean et al., 2007; Renaud and

153 Luong-Van, 2006; Kumar, 1993; Meng and Srivastava, 1993), however, in
154 particular cases (i.e. red alga *Acanthophora muscoides* and brown alga *Dictyota*
155 *ciliolate*) the higher percentages of carbohydrates are observed in winter time
156 (Meng and Srivastava, 1993). Furthermore, Meng and Srivastava (1993) have
157 pointed to the increase of carbohydrate content with day temperature.

158 As commented briefly above, the carbohydrate part of lignin-free
159 macroalgal biomass plays a crucial role in biohydrogen production, which
160 requires the hydrolysis of polysaccharides for subsequent fermentation of the
161 monomeric sugars i.e. glucose and galactose molecules released. In addition to
162 the importance of carbohydrates, the production of gaseous energy carriers
163 under anaerobic conditions and achievable yields are markedly determined by
164 other factors such as the C/N ratio (Hughes et al., 2012).

165 Macroalgae can grow faster than land/terrestrial plants and can be
166 cultivated on vast tracts of sea under ambient conditions without the need of any
167 fertilizer. The advantageous cellular composition algal biomass – as they
168 normally do not contain lignin and sugars can be liberated via milder
169 pretreatment and hydrolysis compared to second-generation lignocelluloses
170 (Kumar et al., 2015b) – has made it a promising feedstock for biorefineries.
171 Examples of carbohydrate profiles for a range of macroalgae are listed in Table
172 1.

173 Among them, the species with higher amounts of carbohydrates in the
174 cell (i.e. in terms of *D*-galactose, anhydrogalactose, cellular mannuronic and

175 guluronic acid blocks, etc.) are preferred and more appropriate for
176 bioconversions to yield biofuels (i.e. bio-methane, bio-hydrogen, bio-ethanol, *n*-
177 butanol, 2,3-butanediol, etc.) with improved efficiency (Sambusiti et al., 2015;
178 Mazumdar et al., 2013; Wei et al., 2013; Shi et al., 2011). In particular, certain
179 red macroalgae of genera *Gelidium*, *Gracilaria* and *Euchema* are reportedly
180 attractive resources because of the relatively high ratios of galactose and glucose
181 (Park et al, 2011), which are known to be sugars with high fermentability.
182 Galactose is an isomeric form of glucose sugar with an opposite hydroxyl group
183 (-OH) at C_4 carbon. This sugar, though complex metabolic pathways are needed
184 for its fermentation under anaerobic circumstances (Cheon and Kim, 2012),
185 appeared to fermentable feedstock for biogas (Vanegas and Bartlett, 2013) as
186 well as bioH_2 production. Actually, successful biohydrogen production tests
187 from both (i) galactose-glucose mixture and (ii) the hydrolysates of red algal
188 biomass were already communicated in the literature (Chen et al., 2015).
189 Although biotransformation of galactose and glucose take place different ways
190 in the biohydrogen fermenter, the two processes lead to comparable organic acid
191 (as secondary-product) profiles (Sivagurunathan et al., 2016; Mathews and
192 Wang, 2009).
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3. Pretreatment of macroalgae for hydrogen production

To get access to the carbohydrate regions, help fermentable sugar recovery from complex biomass i.e. algae and ensure the feasibility of gaseous biofuel fermentation, different pretreatment techniques can be suggested (Kumar et al., 2015b; Montingelli et al., 2015).

The pretreatment techniques available for the macroalgal substrates are divided up into four main categories, such as physical (mechanical, extrusion and pyrolysis), physicochemical (steam/ammonia/fiber/CO_2 explosion, liquid hot water, wet oxidation, sonication and microwave-irradiaton), chemical (ozonolysis, acidic/alkaline treatment, oxidative delignification, organosolv-process and ionic liquid-based treatment) and biological (enzymatic curing) ones (Fig. 1). Unfortunately, however, the phenomena so-called inhibitor formation is a general consequence in case of most pretreatment methods (Palmqvist and Hahn-Hägerdal, 2000). These compounds present a threat on the performance of the hydrogen fermenter and therefore, actions to detoxify pretreated-biomass fractions can be seen as a key-step.

Table 2 provides some examples about the pretreatment of various macroalgal biomass and their hydrogen production efficiencies. As it can be seen, all the studies referenced could realize the best hydrogen production after pretreatment, regardless of the type of seaweed used as feedstock. While some of the paper reported on single-step biomass treatment employing acid, alkali,

heat and electric field, others have demonstrated that a combined (two-stage) procedure may be even more advantageous from a hydrogen production point of view. In general, the trend to observe is the adoption of mixed anaerobic sludge for the conversion of marine algae (Table 2) as normally, pure cultures are not robust enough to degrade complex materials. A possible way ahead, as reviewed by Kumar et al. (2016) might be the reinforcement of mixed bacterial communities by particular strains in the concept of bioaugmentation, which has eventually led to significant enhancement of biohydrogen fermentation during the valorization of various biomass feedstocks. An additional note to make here is that literature results obtained with macroalgae (Table 2) is quite difficult, mostly due to the non-interconvertible units expressing the H_2 gas evolution yields and rates (Kumar et al., 2015b). Standardization of performance indicators would be very helpful for such analysis, which would also bring benefits to the readers for the rapid and easy catch-up with data.

3.1. Formation of toxic reaction inhibitors and effect of pretreatment techniques on macroalgae

In the course of pretreatment, hexoses i.e. glucose may be degraded via side-reactions and as a result toxic components such as 5-(Hydroxymethyl)furfural (5-HMF) are formed, taking a negative effect on the cellular growth and respiration (Kumar et al, 2014b). From kinetic studies, it

was revealed that quantities of 5-HMF increase with the rise of temperature and duration of pretreatment (Srikanth et al., 2010; Mussatto and Roberto, 2004). This inhibitory pathway mainly depends on both the reaction temperature and residence time (Arantes and Saddler, 2011).

Pretreated biomass fractions can contain aliphatic acids, namely formic and levulinic acids from 5-HMF *via* acid-catalyzed thermochemical degradation of polysaccharides. The concentration of the formed acids depends strongly on the traits of feedstock (i.e. its composition), pretreatment (experimental) conditions i.e. their harshness. Liposoluble, inhibitory organic acids such as undissociated form of levulinic and formic acids once present in the fermentation medium can diffuse into the cells at under acidic conditions ($pKa_{levulinic}$= 4.49 and pKa_{formic} = 3.75). Thereafter, inside the cell, near neutral pH, the dissociation of acids causes severe pH reduction in the intracellular environment and can deteriorate the biocatalyst activity. It is noteworthy that the actual inhibitory action is influenced by (i) the toxicity of the particular compound, (ii) the fermentation circumstances and (iii) the individual tolerance of the particular microorganisms.

According to findings in the literature, the formation of organic acids is side-reaction that can never be suppressed or avoided completely. Nonetheless, some strategies may help to reduce their negative impact, such as neutralization prior to subjecting the pretreated biomass to the next stages i.e. hydrolysis and fermentation (Harmsen et al., 2010; Almeida et al., 2007). In addition, some

other less toxic inhibitory extracts – derived from the cellular organisms – were found in the fermentative medium, inlcuding tannic and terpenic acids, etc. (Ran et al., 2014; Jonsson et al., 2013; Arantes and Saddler, 2011).

Besides the already mentioned components, certain ions of heavy metals (Cr, Ni, Fe and Cu) should also be concerned, which may originate from corrosion of reaction vessel and their toxicity may slow down the metabolism of microorganisms involved in the fermentation (Ran et al., 2014; Jonsson et al., 2013;Harmsen et al., 2010; Almeida et al., 2007).

3.2. Example regarding the effect of pretreatment methods on macroalgae structural composition

In accordance with literature reports, structural compositions of the raw macroalgae can undergo a significant alteration, caused by the pretreatment. For instance, it was shown via techniques i.e. FT-IR spectroscopy and X-ray Diffraction (XRD) that the pretreatment of a particular seaweed (*Saccharina japonica*) resulted in the removal of non-cellulosic components such as alginate, mannitol, etc. (Lee et al., 2013).The FT-IR spectrum of the raw and pretreated macroalgae in Fig. 2 illustrates a number of strong peaks at different wave numbers. The broad peak at 3355 cm^{-1} is ascribed to the ν(–OH) stretch of alcohols, phenols, and δ(–NH) stretch of primary and secondary amines in the raw seaweed and these peaks disappeared in the pretreated seaweed (Fig.

2). The –NH bend vibrations of primary amines were established at 1632 cm^{-1} and this peak underwent a slight modification following the pretreatment process. Raw seaweeds exhibit the appearance of –C–C– stretch of aromatics and –C=O– stretch of esters as well as carboxylic acids at 1459, 1428, and 1236 cm^{-1}, respectively, meanwhile in the pretreated biomass, there were some distinguished modifications The steep peak observed at 1000 cm^{-1} was responsible to the –C–O– stretch of ethers and the =CH bend vibrations of alkenes was also appeared in both samples. An intense peak at 878 cm^{-1} appears owing to the presence of –NH swing of primary and secondary amines. A stretch of alkyl halides at 517 cm^{-1} represents the presence of impurities in the samples and were symbolized through the C–Br. Both of these peaks are missed in the pretreated biomass.

The raw seaweed biomass showed a characteristic diffraction peak at 30.5º along with inter planar spacing (d-spacing) of 2.92775 and the crystallainity index of about 37.84 (Fig. 2), which is the feature for determining the sugar availability all the way through the hydrolysis of cellulosic materials (El-Sakhawy and Hassan, 2007), while there was no any well-defined peaks for the pretreated biomass and negative value of the crystallainity index indicates the amorphous nature of the sample (El-Sakhawy and Hassan, 2007)

4. Detoxification methods for inhibitor removal from pretreated algal biomass

To conduct detoxification after macroalgal biomass pretreatment, there is a variety of chemical, biological and physical techniques (Pienkos and Zhang, 2009), as presented in Table 3. Though, various methodologies are promising, among the cost-effective detoxification, over-liming using calcium hydroxide and subsequent adsorption using charcoal have come forward as proficient ones (Jonsson et al., 2013; Cantarella et al., 2004). It is indicated in the literature that the detoxification effect by over-liming and consecutive removal employing charcoals is associated with (i) the precipitation and (ii) chemisorption processes of inhibitory compounds present after pretreating macroalgal biomass, respectively (Cantarella et al., 2004; Van Zyl et al., 1988). In relation with the adsorption of 5-HMF, Gonzales et al. (2016) suggested the use of granular activated carbon (GAC), which can be a beneficial material as well to achieve this purpose using algal biomass.

Recently, Sambusiti et al. (2015) reviewed the algal biorefinery approach for fermentative biohydrogen production and encouraged more extensive research to examine the impact of by-products such as 5-HMF (released i.e. during thermo-chemical pretreatments of algae) on different hydrogen producing bacteria. As for the inhibition caused by 5-HMF during the biohydrogen fermentation process, Kumar et al. (2014b) reported that 5-HMF

326 can act as a non-competitive inhibitor (with 1.37 g/L of IC_{50}) using galactose (a
327 component to be derived from algal biomass) substrate. Moreover, as for other
328 inhibitors, it was found that the negative impact associated with levulinic (1.33
329 g/L) and formic acids (2.99 g/L) resulted in 50% drop of the biohydrogen
330 production rate. Besides, it was observed that – unlike in case of glucose –
331 galactose utilization was reserved by formic acid while the concentration was
332 below 5 g/L. Furthermore, experiments demonstrated the possibility of
333 simultaneous (i) 5-HMF removal and (ii) hydrogen gas production from
334 H_2SO_4-pretreated, red-algal hydrolysate (AH) (Kumar et al., 2015a). Under
335 batch conditions, peak hydrogen production was achieved at AH content of 50
336 % (v/v) with 1.6 g/L 5-HMF concentration. Nevertheless, it is worth further
337 investigating the inhibition phenomena applying various types of inoculum (i.e.
338 pure or mixed cultures), and the possible interactive (i.e. synergetic) effects
339 between different by-products in the course of the dark fermentation process.
340 This avenue would help to select microorganisms that exhibit appropriate
341 resistivity towards inhibitors and besides, the employment of genetic
342 engineering to acquire the transformed hyper resistant microbes may be also
343 possible (Jonsson et al., 2013).
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5. On the economic assessment of biohydrogen production considering pretreatment and detoxification methods

The economies of macroalgal bioenergy technologies are dependent on the biomass processing knowledge and fundamental research, in the midst of a numerous ecological and communal issues (Ingle et al., 2011; Jiang et al., 2016). The macroalgae assure high yield of biomass and photosynthetic efficiency compared to terrestrial crops but use of the macroalgae for biohydrogen production as feedstock represents certain challenges which are attributable to high moisture, ash and alkali contents (Saqib et al. 2013). The adopted pre-treatment methods for the macroalgal biomass to produce biohydrogen appear promising but upgrading in these technologies is preferred. Additionally, technologies for the maximal sugar recovery and detoxifications are still in developing stage, however, growing concern and advancements would eventually lead to the cost-effective ways, helping the implementation at realistic scale.

6. Outlook and challenges

Macroalgal biomass is a candidate of one of the promisingalternative energy resources to alternate fossil fuels (Maity et al., 2014). The application of marinealgae for hydrogen fermentation is accomplished by the conversion of

carbohydrates specifically galactose into biohydrogen. Besides some additional challenges exist for the organization of a practical system in the dark fermentative hydrogen production from macroalgae (which include cultivation, collection), the saccharification of some of the existing carbohydrates like alginate, agar, carrageenan, etc. Challenges in the successful dark fermentation procedure are related with the production of high and low quantities of fermentable sugars and inhibitors, respectively. Optimization of saccharification protocols to for efficient sugar recovery i.e. galactose (the major monomer sugar among the other fermentable sugars in the macroalgal biomass) should be of primary objective. Accordingly there are various troubles to overpower to achieve realistic employment of macroalgae. Nevertheless, macroalgae are emerging alternative biomass and taking their advantages over terrestrial biomass into account and with the further efforts the developmentsof biotechnologies relying in macroalgae are anticipated. Integrating with biorefinery scheme for the production of valuable chemicals along with the energy production from the residues would increase the benefits and also opens windows for various industrial activities.

7. Conclusions

This review has provided an insight to the macroalgae-based biohydrogen fermentation with primary scope on seaweed characteristics, biomass pretreatment and issues related to inhibitor formation/removal. Further outlook and challenges have also been documented towards sustainable biohydrogen technologies using macroalgae biomass. As a result, it could be concluded that fermentation efficiency and process economics are both dependent on the biomass processing techniques and their conditions, which also influence the fate of scale-up and the future of this biotechnological avenue.

Acknowledgements

Gopalakrishnan Kumar highly acknowledges the financial assistance from the Faculty of Environment and Labour Safety, Ton Duc Thang University. Péter Bakonyi acknowledges the support received from National Research, Development and Innovation Office (Hungary) under grant number PD 115640.

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Table Legends

Table 1.

Carbohydrate profile of red, green and brown macroalgal species

Table 2.

Insights to literature studies on macroalgae based $BioH_2$ fermentation

Table 3.

Techniques available for detoxification of pretreated algal biomass

Table 1733

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Macro algal species	Carbohydrate profile (% of dry weight)	Season & Collection location
Red macroalgae		
Acanthophoramuscoides	29.5 [a]; 32.6 [a]	Summer and Winter & Northern Territory and Australia
Ahnfeltiopsisconcinna	31.2; 33.4 [b]	February and October & Hawaii and USA
Asparagopsistaxiformis	9.2 [b]; 13.2 [b]	April & Hawaii and USA
Bostrychiatenella	31.2 [a]	Winter & Northern Territory and Australia
Botrycladialeptopoda	23.1 [a]	Summer & Northern Territory and Australia
*Ceramium*sp.	0.23 [a]	May & The Sea of Marmara and Turkey
*Champia*sp.	23.4 [a]	Winter & Northern Territory and Australia
Chondrusocellatus	30.6 [b]	January & Hawaii and USA
Eucheumadenticulatum	30.6 [a] ; 28 [b]	Summer & Northern Territory and Australia; February & Hawaii and USA
Eucheumaisiforme	25.9 [c]	Spring & Yucatán peninsula and Mexico
Halymeniaformosa	16.9 [b]	March & Hawaii and USA
*Hypnea*sp.	33.0 [a] ; 31.7 [a]	Summer and Winter & Northern Territory and Australia; Winter & Northern Territory and Australia
Gracilaria cornea	36.3 [c]	Spring & Yucatán peninsula and Mexico
Gracilariacoronopifolia	15.2 [b]	November & Hawaii and USA
Gracilariacrassa	18.7 [a]	Winter & Northern Territory and Australia
Gracilariaparvispora	22.9 [b]	March & Hawaii and USA
Gracilariasalicornia	24.4 [a] ; 20.0 [b]	Summer & Northern Territory and Australia; October & Hawaii and USA
*Gracilaria*sp.	21.6 [a]	Summer & Northern Territory and Australia
Gracilariaverrucosa	4.31 [a]	June & The Sea of Marmara and Turkey
Laurenciadotyi	17.1 [b]	June & Hawaii and USA
Laurenciamajuscula	18.8 [a]	Summer & Northern Territory and Australia
Laurenciamcdermidiae	16.5 [b]	June & Hawaii and USA
Laurencianidifica	16.0 [b]	
Portieriahornemannii	21.8 [a]	Summer & Northern Territory and Australia
*Polysiphonia*sp.	1.94 [c]	May & The Sea of Marmara and Turkey
Porphyravietnamensis	30.5 [b]	February & Hawaii and USA
Solierarobusta	22.5 [a]	Summer & Northern Territory and Australia

*Spiridia*sp.	39.2 [a]	Winter & Northern Territory and Australia
Tolypiocladiacalodictyon	26.7 [a]	
Wrangelia plumose	22.3 [a]	Summer & Northern Territory and Australia
Green macroalgae		
Anadyomenebrownii	25.8 [a]	Summer & Northern Territory and Australia
Caulerpalentillifera	12.8 [a] ; 11.8 [b]	Winter & Northern Territory and Australia; October & Hawaii and USA
Caulerparacemosa	3.60 [b] ;16.6 [a] ; 14.8 [a]	Spring & Yucatán peninsula and Mexico; Summer and Winter & Northern Territory and Australia
Codiumisthmocladum	16.77 [c]	Spring & Yucatán peninsula
Codiumreediae	4.50–8.20 [b]	March& Hawaii and USA
*Codium*sp.	0.65 [a]	June & The Sea of Marmara and Turkey
Codiumtomentosum	3.30–4.40 [a]	May & The Sea of Marmara and Turkey
Enteromorphaclathrata	1.00 [a]	June & The Sea of Marmara and Turkey
Enteromorphacompressa	1.60 [a]	
Enteromorphaflexuosa	39.9 [b]	January & Hawaii and USA
Enteromorpha intestinalis	1.9 [a] ; 18.7 [a] ; 22.2 [b]	June & The Sea of Marmara and Turkey; Winter & Northern Territory and Australia; October & Hawaii and USA
Enteromorphalinza	2.42 [a]	June & The Sea of Marmara &Turkey
Halimedamacroloba	4.70 [a] ; 2.70 [a]	Summer and Winter & Northern Territory and Australia
Halimedaopuntia	2.70 [a] ; 2.50 [a]	
Monostromaoxyspermum	31.8 [b]	October & Hawaii and USA
Neomeris van-bosseae	15.2 [a] ; 8.30 [a]	Summer and Winter & Northern Territory and Australia
Ulva fasciata	20.6 [b] ; 17.1 [b]	January and March & Hawaii and USA
Ulva lactuca	2.9–1.6 [a]	June & The Sea of Marmara and Turkey
Ulva rigida	4.19–6.30 [a] ; 1.5–2.6 [a]	May and June & The Sea of Marmara and Turkey
Brown macroalgae		
Cystoseirabarbata	0.90–0.91 [a]	May & The Sea of Marmara and Turkey
Dictyotaacutiloba	5.9 [b]	January & Hawaii and USA
Dictyotaciliolata	15.2; 20.3 [a]	Summer and Winter & Northern Territory and Australia; January & Hawaii and USA
Dictyotasandvicensis	6.70 [b]	January & Hawaii and USA

Feldmanniaindica	18.7 [a]	Winter & Northern Territory and Australia
Hydroclathrusclathratus	18.3 [a]	
Sargassumdecurrens	22.2 [a]	
Sargassumechinocarpum	10.50 [b]	March & Hawaii and USA
Sargassumfilifolium	21.4 [a]	Winter & Northern Territory and Australia
Sargassumfilipendula	3.73 [c]	Spring & Yucatán peninsula and Mexico
Sargassumobtusifolium	12.3 [b]	March & Hawaii and USA
Padinaboryana	19.3 [a] ; 18.4 [a]	Summer and Winter & Northern Territory and Australia
Padinagymnospora	1.86 [c]	Spring & Yucatán peninsula and Mexico
Rosenvingeanhatrangensis	12.6 [a] ; 8.40 [a]	Summer and Winter & Northern Territory and Australia
Turbinariaconoides	19.7 [a]	Winter & Northern Territory and Australia

Modified Refs. (Jiang et al., 2016; Kawai and Murata, 2016; Suutari et al., 2015)

[a]Samples were washed with distilled water.

[b] Samples were washed with filtered seawater.

[c] Samples were brushed under filtered seawater and rinsed with deionized water.

Table 2.

Macroalgae species	**Inoculum**	**Pretreatment studied**	**Maximal hydrogen production index**	**Reference**
Laminaria japonica	Anaerobic mixed culture	Heat, acid, alkaline and ultrasound	83.45 ± 6.96 mL/g heat-pretreated biomass	Liu and Wang (2014)
Laminaria japorica	Anaerobic mixed culture	Electric field	102.7 mL H_2/g dry cell weight	Jeong et al. (2015)
Laminaria japonica	Anaerobic mixed culture	Thermal	109.6 mL H_2/g COD_{added}	Jung et al. (2011)
Laminaria japorica	Anaerobic mixed culture	Combined mechanical and thermal	70 mL H_2/L-h, 28 mL H_2/g dry algae	Park et al. (2009)
Padina tetrastromatica	Isolates from sewage sludge	Chemical (acid and alkaline)	78 ± 2.9 mL/0.05 g VS (after dilute H_2SO_4 pretreatment)	Radha and Murugesan (2017)
Gelidium amansii	Anaerobic mixed culture	Heat (+ detoxification)	518 mL H_2/g VSS-d, 53.5 mL H_2/g dry algae	Park et al. (2011)
Gelidium amansii	Anaerobic mixed culture	Combined thermal and acid	510 mL H_2/L-h, 37.0 mL H_2/g dry biomass	Park et al. (2013)

Table 3.

Procedure	**Pretreatment techniques agents / path**
Chemical additives	Alkalis: $Ca(OH)_2$, NaOH, NH_4OH
	Reducing agents: dithionite, dithiothreitol, sulfite
Enzymatic treatment	*Laccase*
	Peroxidase
Heating and vaporization	Evaporation
	Heat treatment
Liquid-liquid extraction	Ethyl acetate
	Supercritical fluid extraction: Supercritical CO_2
	Trialkylamine
Liquid-solid extraction	Activated carbon
	Ion exchange
Microbial treatment	*Coniochaetaligniaria*
	Trichoderma reesei
	Ureibacillusthermosphaericus

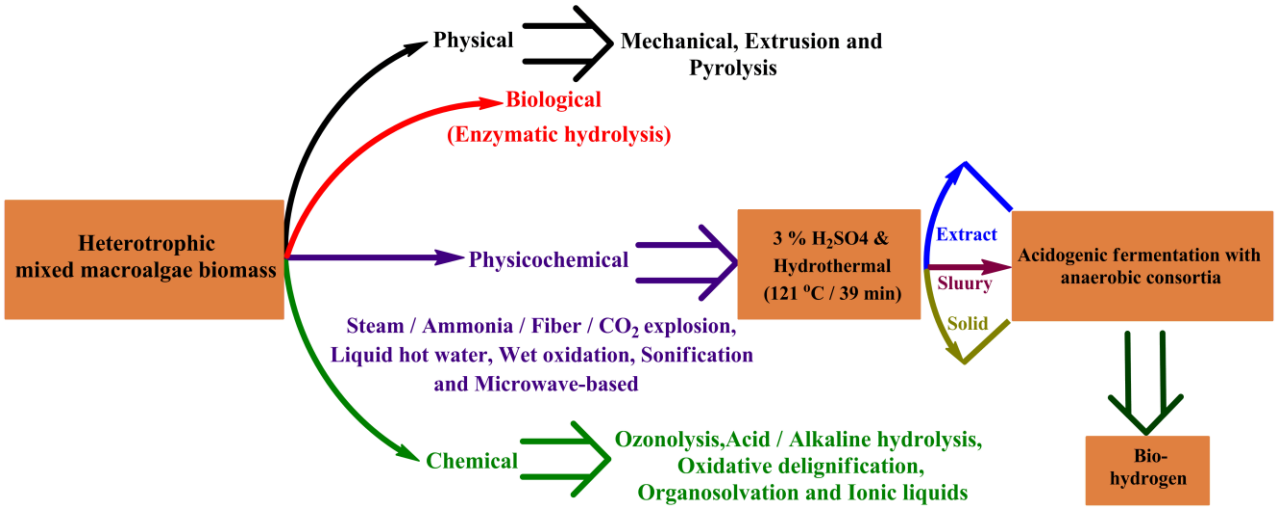
Adopted Refs.(Jonsson et al., 2013; Pienkos and Zhang, 2009; Cantarella et al., 2004)

Figure Legends

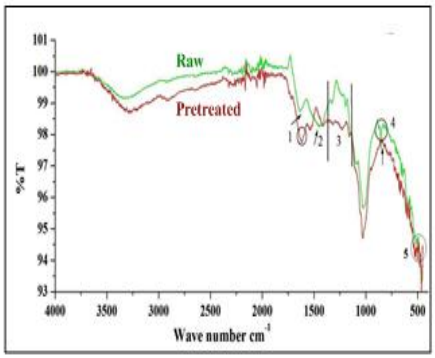
Fig. 1 –Pretreatment techniques for macro algal substrates

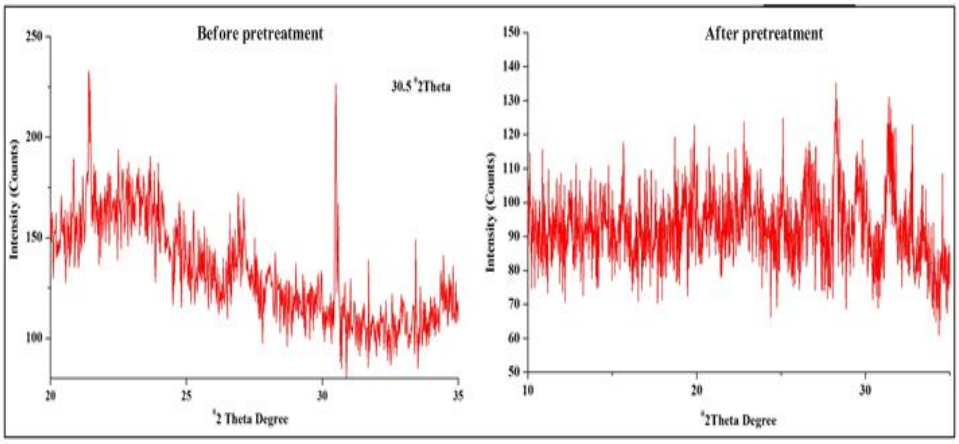
Fig. 2 FT-IR spectra and XRD pattern of raw and pretreated macroalgae

Fig. 1


Physical
Mechanical, Extrusion and Pyrolysis
Biological
(Enzymatic hydrolysis)
Heterotrophic mixed macroalgae biomass
Physicochemical
3 % H2SO4 & Hydrothermal (121 °C / 39 min)
Extract
Sluury
Solid
Acidogenic fermentation with anaerobic consortia
Steam / Ammonia / Fiber / CO2 explosion, Liquid hot water, Wet oxidation, Sonification and Microwave-based
Chemical
Ozonolysis,Acid / Alkaline hydrolysis, Oxidative delignification, Organosolvation and Ionic liquids
Bio-hydrogen

14 **Fig. 2**


Raw
Pretreated
%T
Wave number cm-1


Before pretreatment
30.5 °2Theta
Intensity (Counts)
°2 Theta Degree
After pretreatment
Intensity (Counts)
°2Theta Degree

15