A brief review on bioethanol production using marine biomass, marine microorganism and seawater

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Abstract

This review introduces a new approach of completely marine based bioethanol production by analyzing and evaluating the recent trends in bioethanol fermentations using algae, marine microorganisms and the replacement of freshwater with seawater. Both macroalgae and microalgae have been successfully used for bioethanol production. Marine yeasts showed excellent tolerance to salt and inhibitors, and fit for seawater fermentation. The combination of marine biomass, marine microorganism and seawater has a potential for a greener bioethanol production.

Keywords

Seaweed; pretreatment and enzymatic hydrolysis; bioenergy; yeast

1. Introduction

Increasing concerns over energy shortages and environmental pollution has led to a growing focus on the development of renewable energy sources, such as solar, wind, bioenergy and geothermal energy. When compared with other renewable energy sources, biofuels especially bioethanol, have several unique advantages, such as its use as a liquid fuel, which can be directly used in existing vehicle engines, it can be distributed via the existing fossil fuel system and encourages rural economy. The increasing demand for bioethanol has led to the excessive usage of food material and arable land for production. This has resulted in food price rises and has restricted the growth of the bioethanol industry.

A promising alternative choice of bioethanol production is the development of a marine resource based bioethanol production process, as shown in Figure 1. Marine biomass, specifically microalgae and macroalgae, are fast growing photosynthetic species which contain little or no lignin content, and require no arable land and minimum nutrients for their cultivation. They are considered as the 3rd generation of bioethanol feedstock [1]. In the past decade, there has been an increase in research focus on bioethanol production from marine biomass. Besides marine biomass, marine-derived microorganisms have unique properties, such as high osmotic tolerance, utilization of particular sugars and production of special enzymes [2]; these properties provide extra benefits for bioethanol production, especially when using marine biomass. Seawater is an abundant under estimated resource.

The use of seawater as a substitute for freshwater in bioethanol production was suggested to reduce the water footprint of bioethanol production.

This paper reviews the latest progress in bioethanol production using marine biomass, marine microorganisms and seawater. It also discusses future trends in marine resources based bioethanol production.

2 Bioethanol production using marine biomass

2.1 Macroalgae (seaweed)

Macroalgae can be divided into three types, brown (*Phaeophyta*), red (*Rhodophyta*) and green (*Chlorophyta*). In order to evaluate the bioethanol production potential, the composition and carbohydrate profile of various seaweed species have been determined (Table 1). Although the results did not always concur, in general, seaweed contains 23.8-67% carbohydrate, 4.8-23% protein, 0.53-4.8% lipid and 14-42% ash content (w/w dry weight basis, (dw), based on 90% of the values listed in Table 1). When comparing sugar composition, brown seaweed typically contains alginate, mannitol, laminarin, fucoidin and cellulose; red seaweed typically contains carrageenan, agar, cellulose and lignin and green seaweed typically contains mannan, ulvan, starch and cellulose, though there is considerable variation [9]. Similar to lignocellulosic bioethanol production, pretreatment and saccharification are required to hydrolyze the seaweed into a fermentable sugar solution. Dilute acid pretreatment using sulfuric acid and moderately high temperatures (100-150°C) is a typical pretreatment method for converting seaweed into a hydrolysate suitable for conversion into bioethanol [10, 11]. Other pretreatment methods developed for lignocellulosic bioethanol production process, such as alkali [12] and microwave [13] pretreatments have also been successfully applied to seaweed hydrolysis processes. A subsequent enzymatic saccharification step is normally required after pretreatment. Using a cocktail of cellulosic enzyme solution, an overall hydrolysis yield over 90% has been achieved [11]. Utilization of seaweed specific enzymes, such as alginate lysase [14] and laminarinase [15] have also been reported, which effectively hydrolyzed brown seaweeds.

Subsequent to pre-treatment and saccharification, seaweed hydrolysates have been evaluated in various fermentation models for bioethanol production. Figure 2 plots bioethanol concentration and overall bioethanol yield, the two crucial economic indicators in seaweed to bioethanol fermentations. In general, relatively low bioethanol concentration of less than 30 g/L was observed (Figure 2). When the hydrolysate was concentrated, e.g. by rotary evaporation, the initial sugar content in the hydrolysate was enhanced and a bioethanol concentration of 65 g/L has been reported [16]. Bioethanol yields of 28% (w/w) have been reported, which is decent comparing to the theoretical maximum overall bioethanol yield of 38% (w/w) (Figure 2).

Saccharomyces cerevisiae is the most commonly used microorganism due to its high glucose fermentation capacity. However, existing *S. cerevisiae* strains are inefficient in fermenting algae specific sugar monomers, such as mannitol and laminaran. Therefore, non-*S. cerevisiae* strains, such as *Pichia angophorae*

[17] and *Defluviitalea phaphyphila* [18] have been investigated to promote conversion of mannitol, laminaran and alginate contained in seaweed hydrolysates. Another promising strategy is the construction of macroalgae sugar utilization pathways in high ethanol producing strains. Enquist-Newman et al., (2013) constructed an alginate transportation and metabolism system in *S. cerevisiae,* which efficiently converted 4-deoxy-L-erythro-5-hexoseulose uronate (DEHU) and mannitol into bioethanol [19]. In a novel process, a genetically modified *Escherichia coli* strain (*E coli* KO11) was developed, which hydrolyzed, transported and converted alginate into bioethanol simultaneously [20]. A bioethanol concentration of 4.7% (v/v) was obtained with a yield of 0.281 g bioethanol per g dry weight macroalgae.

2.2 Microalgae

Microalgae have attracted great attention for biodiesel production due to their fast growing character and their high lipid content in certain species, such as *Chlorella sp.* [21]. Apart from lipid, some microalgae species, e.g. *Synechococcus sp.* accumulated 60% carbohydrate content in favorable culture conditions [22]. In a recent paper, a microalgae, designated SP2-3 containing 70% (w/w, dw) carbohydrate content was identified, indicating it could be a promising marine feedstock for bioethanol production [23]. When compared with macroalgae or terrestrial biomass, microalgal cell wall is relatively easy to break down following a lysozyme, dilute acid or a combination of both pre-treatment [23]. Early research on the hydrolysis of a green microalgae *Chlamydonoas reinhardtii* with 3% (w/w) H_2SO_4 at 110^oC

for 30 minutes led to a hydrolysate with a glucose concentration of 28.5 g/L [24]. Subsequent fermentation of the hydrolysates by *S. cerevisiae* resulted in a bioethanol production of 14.6 g/L, which corresponds to 0.292 g bioethanol per g biomass (dw) [24]. Since then, various microalgae, such as *Cyanobacterium synechococcus* sp. [22] *Chlorella sp.* [25], have been explored for bioethanol production. These results have been summarized in Table 2 and recent articles [1, 23]. Normally, a microalgae hydrolysate contains around 10-30 g/L sugars, and 3.6-14.6 g/L bioethanol was obtained with a typical bioethanol to biomass yield of 0.2-0.3 (w/w, dw). When the hydrolysate was concentrated, the sugar content can reach 137 g/L and produce a bioethanol titre of up to 61.2 g/L [23].

3 Marine microorganisms in bioethanol production

The majority of microorganisms that are used for bioethanol synthesis have been isolated from terrestrial environments. Hydrolysates derived from marine biomass typically contain a different spectrum of sugar monomers from hydrolysates from terrestrial plants [9] and as a result terrestrial microorganisms struggle to utilize these sugars efficiently. An alternative approach other than genetically modifying a microorganism is to screen for new microorganisms which could utilize sugars present in the marine biomass-derived hydrolysates. Isolation of marine-derived yeast was first reported in 1894, since then, hundreds of marine yeasts had been isolated, and some of these have been successfully used for bioethanol, pharmaceutical and industrial enzyme production [2, 32]. Recently, Zaky et al., (2014) compared various marine yeast isolation methods and developed an efficient three-step protocol for marine yeast isolation [2]. Applying this method to 14 geographically different marine samples, over 100 marine yeasts were isolated, of which 17 displayed efficient sugar utilization strains and were subsequently identified [33]. Fermentations using *S. cerevisiae* AZ65, one of the isolates in the above study produced 97.41 g/L bioethanol from a glucose based medium in 15 L fermenters [34]. Obara et al. (2012) reported fermentations of a concentrated paper shredder scrap hydrolysate using marine-derived *S. cerevisiae* which achieved 122.5 g/L of bioethanol [35]. When this strain was used to ferment a mixture of seaweed hydrolysate (*Undaria pinnatifida*) and paper shredder, 87.7 g/L bioethanol was produced [36]. Besides *S. cerevisiae*, marine-derived microorganisms, such as *Pichia sp., Candida sp. Yarrowia sp*. and *Wickerhamomyces sp*. have also been investigated for their suitability for bioethanol production [2].

The utilization of marine microorganisms in marine biomass hydrolysate was recently explored. Khambhaty et al. (2013) reported fermentations of red seaweed *Kappaphycus sp.* hydrolysate which contained 5.5% sugar and 11.25% salt by a marine-derived *Candida* sp. and 12.3 g/L bioethanol was observed [37]. A thermophilic marine bacterium *Defluviitalea phaphyphila* was isolated, which converted un-hydrolyzed brown seaweed powder (*S. japonica*) to bioethanol with a yield of 0.25 g/g seaweed (dw) [18].

Marine microorganisms have also been used in enzymatic hydrolysis processes and used as gene donors for the construction of novel bioethanol producing strains. Trivedi et al., (2015) demonstrated the enzyme solution obtained from a marine fungus *Cladosporium sphaerospermum* hydrolyzed green seaweed *Ulva fasciata* [38]. The enzyme solution maintained 74-94% of its activities in ionic liquid (IL), indicating it could be used together with IL for biomass hydrolysis. Parab et al., (2017) successfully used an enzyme solution produced from a marine bacterium *Bacillus sp.* BT21 for the hydrolysis of red, green and brown seaweeds (*Ahnfeltia plicata, Ulva lactuca* and *Padina tetrastromatica*) [39]. Sugar yields of 0.23, 0.10 and 0.073 g/g biomass (dw) respectively were observed. Inulinase genes originated from marine-derived yeasts *Pichia guilliermondii* [40] and *Candida membranifaciens* [41] were successfully expressed in *Saccharomyces sp.* W0, respectively. The transformants *Saccharomyces sp.* Inu-66 and W14-3-INU-112 both produced over 12% (v/v) ethanol from Jerusalem artichoke derived inulin solution.

4 Use seawater in bioethanol fermentation

Seawater, which represents 97% of world's total water, is a potentially important marine resource for bioethanol industry. With the successful demonstration of using marine biomass and marine yeast for bioethanol production, the further replacement of freshwater with seawater would lead to a fully marine based process. The replacement of freshwater by seawater in bioethanol fermentation using marine yeast *S. cerevisiae* AZ65 showed no inhibitory effect. In 15 L batch fermentations using a sugarcane molasses derived medium prepared in seawater, marine yeast *S. cerevisiae* AZ65 produced 52.2 g/L of bioethanol after 48 hours of culture (unpublished data).

5 Challenges and opportunities

Marine biomass is a promising feedstock for bioethanol production. It is estimated that macroalgae has the potential of producing 23.4 m^3/ha/y bioethanol, which is 10.6 and 2.5 folds higher than those for corn and sugar cane, respectively [15]. However, currently marine biomass has an annual production of only 27 million tons (wet weight) [42], in comparison, sugar cane production was 1.68 billion tons in 2012 [43]. Unlike major terrestrial crops, which had been bred and screened for increasing productivity for thousands of years, marine biomass are under-investigated, especially in terms of breeding. This indicates that the potential for marine biomass productivity could be improved dramatically and this development will have a crucial impact on bioethanol production and growth of the industry. The near 90% (w/w) water content in both microalgae [21] and macroalgae [44] is a concern for industrial bioethanol production. A low cost, highly efficient dewatering technology has yet to be developed. A combination of new strain discovery, especially marine yeasts isolation, gene discovery and therefore strain development of novel microorganisms which have the capacity to use the full range of algae sugars would improve marine bioethanol production and perspectives. The replacement of freshwater by seawater in bioethanol industry could reduce the bioethanol production water footprint and possibly provide freshwater for other sectors, possibly achieving bioethanol production from sole marine resource. Integrating bioethanol production with the existing algae industry, CO_2 fixation or wastewater treatment would be an attractive approach [45, 46].

The utilization of macroalgae and microalgae for bioethanol production has been reviewed in this paper. Significant improvement has been achievement recently both in fermentation process optimisation and strain development. Marine microorganisms and seawater have been demonstrated to be able to used in algal biofuel fermentation. The development of an algae-based biorefinery, extracting or producing value-added chemicals together with completely marine based bioethanol fermentation would improve the overall economic feasibility of algal biofuel production [47].

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Figure 1. Schematic diagram of marine resource based bioethanol production processes in comparison with the 1st and 2nd generation bioethanol production processes.

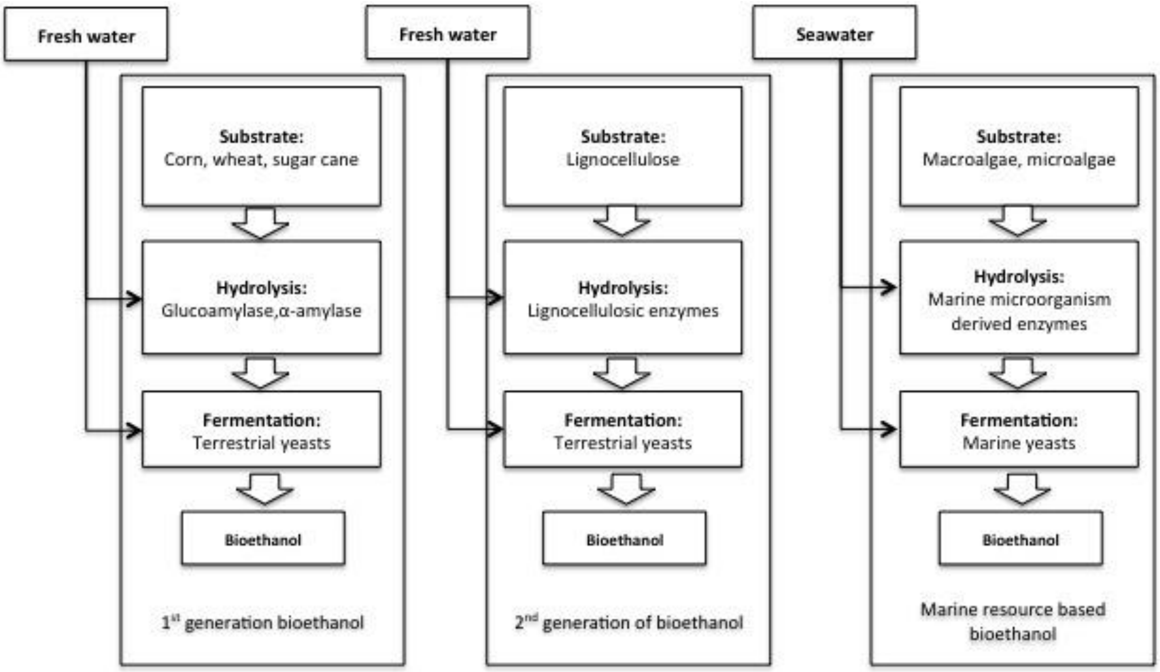

Figure 2 Comparison of bioethanol concentration (g/L) and overall bioethanol yield (g bioethanol per g dry weigh seaweed) in fermentations using seaweed hydrolysates [5,6,11]. The theoretical maximum overall bioethanol yield of 38% (w/w) was calculated based on the carbohydrate content in seaweed (67% w/w) and bioethanol to sucrose yield of 0.568 g/g.

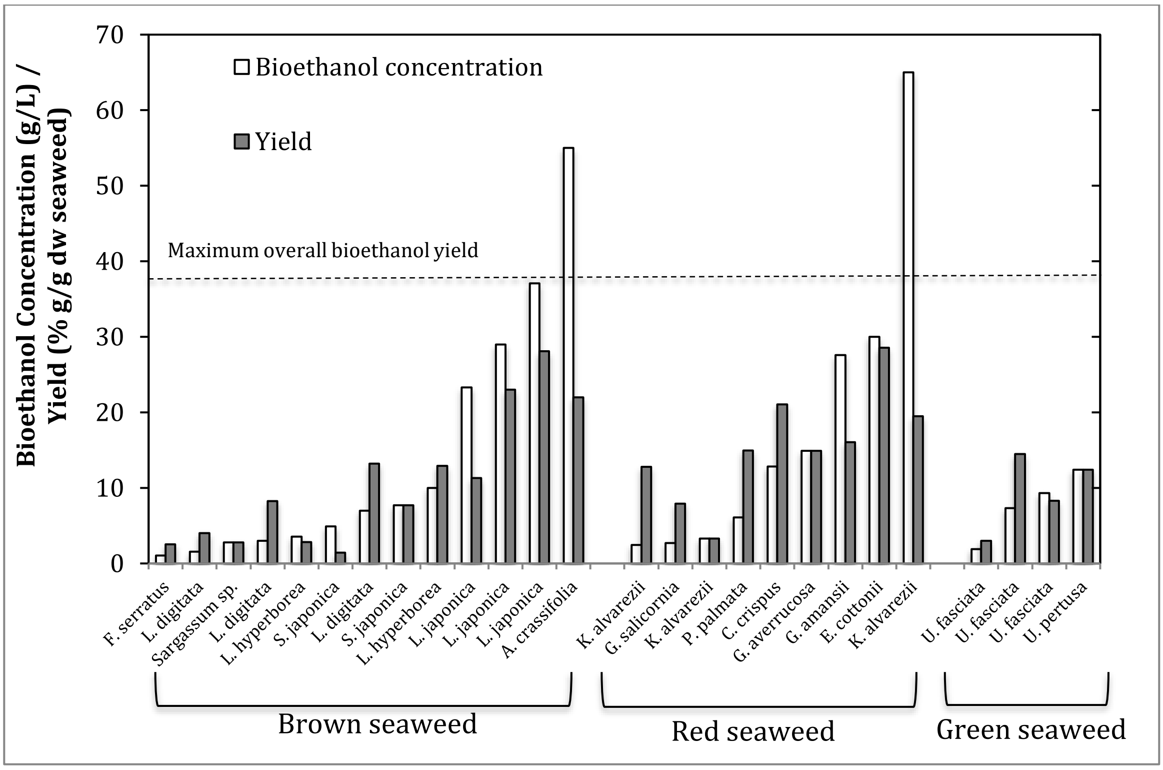

Table 1 The carbohydrate, protein, lipid and ash composition of macroalgae, (dry weight basis, %).

Seaweed sp.	Carbohydrate (w/w)	Protein (w/w)	Lipid (w/w)	Ash (w/w)	REF
Brown seaweed					
Alaria esculenta		9.11	1.3	24.56	[3]
Ascophyllum nodosum	39.5-60.6	4.8–9.8	1.9–4.8	18–24	[4]
Fucus serratus	26.4	9.6	2.8	18.8	[5]
Fucus vesiculosus		6.11	3.51	20.92	[3]
Laminaria digitata		5.31	1.13	24.43	[3]
Laminaria digitata	21.7	26.8	1.9	24.3	[5]
Laminaria digitata	46.6	12.9	1	26	[6]
Laminaria digitata		4.63	0.53	26.5	[4]
Laminaria hyperborea		5.02	1.42	28.75	[3]
Laminaria japonica	51	8	1		[1]
Laminaria sp.	60	12	2	26	[4]
Macrocystis sp	41.7	17.3		41.1	[4]
Pelvetia canaliculata		5.72	5.81	21.24	[3]
Saccharina	40.8–67.0	8.4–14.8	1.3–2.4	14.3	[7]
Sargassum ilicifolium	32–33	8–9	2		[1]
Undaria	26.5–42.8	12.0–23.0	1.1–4.5	22.4	[7]
Undaria pinnitifida	43	24	3-4		[1]
Green seaweed					
Ulva sp.		13.6	2.7	30.2	[4]
Ulva lactuca	59	17	3-4		[1]
Ulva lactuca		8.65	2.62	29.31	[3]
Ulva lactuca	23.8	16.4	1	21.5	[5]
Enteromorpha intestinalis		11.33	1.03	55.29	[3]
Cladophora rupestris		3.42	0.63	77.8	[3]
Red seaweed					
Chondrus crispus	21.8	19.9	0.48	19	[5]
Eucheuma cottonii	26	09-10	1		[1]
Gelidium amansii	66	20	0.2		[1]
Gracilaria gigas	64.71	12.63	1.31	19.59	[8]
Gracilaria sp.		11.4		37.7	[4]
Gracilaria verrucosa	60.81	9.86	0.8	13.85	[8]
Palmaria palmata		12.26	1.33	42.23	[3]
Palmaria palmata	39.4	22.9	3.3	25.7	[5]
Vertebrata lanosa		11.56	1.3	28.78	[3]

Table 2 Comparison of bioethanol production using microalgae feedstock.

Microalgae species	Pretreatment		Fermentation		Bioethanol		REF
	Method	Sugar	Strain	Condition	Titre (g/L)	Yield (g/g)	
Chlamydomonas reinhardtii UTEX 90	3% H_2SO_4, 110°C, 30 min	0.58 g/g	*S. cerevisiae*	30°C, 24 h	14.6	0.292	[24]
Chlamydomonas reinhardtii UTEX 90,	0.005% a-amylase, 90°C, 30 min	N/A	*S. cerevisiae*	30°C, 40 h, 160 rpm	N/A	0.235	[26]
Chlorella vulgaris	240 IU/mg substrate pectinase, 50°C, 200 rpm, 72 h,	0.148 g/g	*S. cerevisiae*	30°C, 48 h	N/A	0.069	[25]
Chlorella vulgaris FSP-E	1% (w/v) H_2SO_4, 121°C, 20 min, pH 6.0	0.477 g/g	*Z. mobilis*	30°C, 24 h	11.7	0.233	[27]
Chlorella vulgaris FSP-E	2% (w/v) cellulase + amylase, 45°C, 200 rpm, pH 6.0	0.461 g/g	*Z. mobilis*	30°C, 24 h	4.3	0.214	[27]
Chlorococcum humicola	3% (w/v) H_2SO_4, 160°C, 15 min, pH 7.0	N/A	*S. cerevisiae*	30°C, 50 h, 200 rpm	7.2	0.520	[28]
Chlorococcum sp.	Supercritical CO_2 extraction of lipid, 60°C	N/A	*S. cerevisiae*	30°C, 60 h, 200 rpm	3.8	0.380	[29]
Cyanobacterium synechococcus sp	Sonication, lysozyme and a-glucanase	N/A	*S. cerevisiae*	34°C, 72 h, 160 rpm	30.0	0.270	[22]
Desmodesmus sp.	10% dry w/v, 2% (v/v) H_2SO_4, 120°C, 30 min, followed by lyophilization	137.2 g/L*	*S. cerevisiae*	28°C, 30 h, 120 rpm	61.2	0.310	[23]
Nannochloropsis oculata	0.75% (w/v) NaOH, room temperature, 10 min	1-2.4% (dw)	*S. cerevisiae*	30°C, 48 h 150 rpm	N/A	0.037	[30]
Scenedesmus obliquusCNW-N	0.5–5% (w/v) H_2SO_4, 121°C, 20 min, pH 6.0		*Z. mobilis*	30°C, 24h	N/A	0.213	[31]
Tetraselmis suecica	0.75% (w/v) NaOH, room temperature, 10 min	3.4-27% (dw)	*S. cerevisiae*	30°C, 48 h, 150 rpm	N/A	0.073	[30]

* After concentration by lyophilization.