POTENTIAL VALUE OF RED AND BROWN SEAWEED FOR SUSTAINABLE BIOETHANOL PRODUCTION

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Abstract

Algae are renewable sources of feedstock for bioethanol that can be grown on non arable lands, non productive water sources and inexpensive culture systems. Red seaweed *Laurencia obtusa* and brown seaweeds *Cystoseira compressa*, *Colpomenia sinuosa* were analysed by determining sugar content by HPLC and converted into suitable fermentable feedstock by NaOH, H_2SO_4, HCl and H_3PO_4 at concentrations 1, 2, 3, 4 and 5% at 21°C of 20 minutes. The efficiency of hydrolysis significantly improved by 5% HCl for *Laurencia obtusa* at 42.84 g sugar/100 g dry biomass. Pretreatment of *Cystoseira compressa* and *Colpomenia sinuosa* with 3 and 5% H_3PO_4 gave higher sugar content of 30.51 and 41.34 g/100 g dry biomass, respectively. A relatively high level ethanol of 0.146 g/g dry biomass of *Laurencia obtusa* was produced*.* Results indicate that *Cystoseira compressa* and *Laurencia obtusa* can be good feedstocks for bioethanol production.

Introduction

Population outburst together with increased motorization has led to an overwhelming increase in the demand for fuel (John *et al.* 2011). Either microalgae or seaweeds could be used for solar energy conversion and biofuel production (Ross *et al*. 2008). Algae contain complex long-chain sugars (polysaccharides) in their cell walls. The cell walls account for a large production of the carbon contained in these organisms (Singh *et al*. 2010). Kelp as a rule contains approximately 60 % carbohydrates of the dry weight. Laminaran (a glucose polymer) and mannitol are energy storage compounds, resembling starch of inland plants, while alginates are structural compounds and correspond to cellulose and lignin in land plants (Kraan 2010). The amount of bioenergy produced by the biomass of red algae is greater than any other source of biomass (Wi *et al*. 2009). Carbohydrate contents of red algae *Pachymeniopsis lanceolata* and *Gelidium elegans* were 60.1 and 51.3% (w/w), respectively (Kang *et al*. 2010). Algae offer a unique alternative biomass feedstock as it lacks lignin that make the release of the fermentable sugars easier and does not compete with agricultural food production (Roesijadi *et al*. 2010). Brown seaweeds may have a high content of easily degradable carbohydrates, making them a potential substrate for the production of liquid fuels (Horn *et al*. 2000a). Production of bioethanol requires three steps; a saccharification of raw material, a fermentation, and a distillation. Pretreatments such as chemical treatment, thermal treatment, and enzyme treatment are used in the saccharification process (Truus *et al*. 2001). One of the most common chemical pretreatments is adding sulfuric acids, that resolve hemicelluloses as well as low cost and high reaction, and no acid-recovery system is required

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(Esteghlalian *et al*. 1997). Treatment of biomass with sodium hydroxide causes the disruption of H-bonding in cellulose and hemicelluloses (Simpson *et al*. 2003). The liquid stream obtained during acid and alkaline pretreatment likely contained fermentable sugar and inhibitory substances released from the breakdown of hemicelluloses (Ohgren *et al*. 2006). It is, therefore, necessary to develop saccharification processes of seaweeds feedstocks for bioethanol production. The objective of this study was to investigate the possibility of conversion of three seaweeds to ethanol via acid and base hydrolysis and fermentation.

Materials and Methods

Laurencia obtusa and *Cystoseira compressa* were collected from Red Sea coast at Safaga, while *Colpomenia sinuosa* from Mediterranean Sea coast at Abo Quar in May, 2012. After collection, algae were thoroughly washed with fresh seawater, air-dried under shade and oven dried at 60ºC for 5 hrs and ground to fine dried powder.

The fine-dried powder algae were hydrolysed in 1, 2, 3, 4 and 5% of NaOH, H_2SO_4, HCl and H_3PO_4 by ratio of 1:10 (w/v). The hydrolysates were autoclaved at 121°C for 20 min., and pressed through cheesecloth. Reducing sugar concentration was estimated by the phenol-sulphuric acid method (Krishnaveni *et al*. 1984).

Saccharomyces cerevisiae was used for ethanol production. The strain was stored in potato agar slants at 4°C. For the preparation of inoculum, a loopful of *S. cerevisiae* was transfered from agar slants into 250 ml Erlenmeyer flasks containing 100 ml of sterile culture medium. The flasks were incubated in a rotary shaker at 30°C for 48 hrs at 150 rpm.

The identification and quantification of the sugars were done by high-performance liquid chromatography (HPLC). The algal extraction were performed with HPLC (Agilent 1100 HPLC system) using an Hypersil ASP-2 column (4.6 × 250 mm) with a mobile phase of acetonitrile-water (80 : 20) at a flow rate of 0.4 ml/min. The temperature of column and optical unit were set at 35 and 40°C, respectively. The injected volume of mixed monosaccharide standards and sample hydrolyzates was 10 µl. Identification of the monosaccharides in the extracts was carried out by comparing their retention times obtained for the authentic standards for seven monosaccharides (rhamnose, galactose, glucose, arabinose, xylose, mannose and glucuronic acid) in mobile phase at a concentration of 10 mg/ml under the same HPLC conditions.

The samples from 5% sulfuric acid pretreatment were taken out for fermentation experiments. Yeast fermentations were carried out in 250 ml Erlenmeyer flasks at pH 4.6 and 30°C consisting of, supplementary nutrients 0.9 g/l $(NH_4)_2SO_4$ and 0.375 g/l yeast extract and the yeast inocula $(1.3 \times 10^8/ $ml). The flasks were sealed with rubber stoppers through which hypodermic needles had been inserted for removal of CO_2 produced during experimentation. The flasks were incubated for 48 hrs. Samples were withdrawn after 24 and 48 hrs of fermentation and the ethanol content and residual sugars were analyzed. Ethanol was measured following Caputi *et al*. (1968). Conversion rate of ethanol was calculated according to the ratio of ethanol produced and the initial sugar content in the fermentation medium using following equations.

Ethanol efficiency (%) = [(Gram ethanol produced) / (Gram sugar used)] (0.511) (100)

Ethanol yield (%) = (Gram ethanol produced/gram sugar used) (100)

The constant 0.511 is the theoretical yield of ethanol produced from glucose

Experiments were conducted in triplicates. Results are expressed as mean with SE (±).

Results and Discussion

In the *Laurencia obtusa* the concentration of ribose, galactose and arabinose were 16.91, 52.26 and 3.18 mg/g dry weight, respectively. *Cystoseira compressa* was found to contain fucose, xylose and mannose with concentration of 11.08, 46.83 and 31.66 mg/g dry weight, respectively. Fucose, ribose and glucose at a concentration of 48, 87.81 and 19.67 mg/g dry weight, respectively were present in the *Colpomenia sinuosa*. (Table1). The results indicate that the seaweeds are capable of production of bioethanol by hydrolysis and fermentation of sugars. Horn *et al*. (2000a) reported that fresh brown seaweed harvest contains about 15 - 20 % carbohydrates of the total wet weight, which appear to be an appropriate substrate concentration for microbial conversion processes.

Table 1. Composition of polysaccharides isolated from extracts of seaweeds.

Monosaccharides	As amount (mg/g sample)		
	Laurencia obtusa	*Cystoseira compressa*	*Colpomenia sinuosa*
Fucose	–	11.08	48
Ribose	16.91	–	87.81
Glucose	–	–	19.67
Galactose	52.26	–	–
Arabinose	3.18	–	–
Xylose	–	46.83	–
Mannose	–	31.66	–

Higher sugar content 42.84 g sugar /100 g dry biomass was extracted from *Laurencia obtusa* using 5% HCl at 121°C for 20 minutes rather than the other different concentrations of acid and base (Fig.1a). The higher sugar concentration was associated with high acid concentration that was applicable to the acid, catalyzed the hydrolysis process. The catalyst activity was proportional to H concentration. The more hydrogen ions formed in the solution the more rapid the hydrolysis process occurred (Mosier *et al*. 2002)**.**

The reducing sugars as by hydrolyzing *Cystoseira compressa* increased with increasing concentrations of H_2SO_4, HCl and NaOH at 121°C at 20 minutes whereas the highest amount of sugar was recorded in 3% H_3PO_4 (30.51 g sugars/100g dry biomass) (Fig. 1b). The results of present study agree with Saxena *et al*. (2009) the dilute acid hydrolysis process is used to hydrolyze the biomass to sugars and is one of the oldest, simplest and most efficient methods. Dilute sulphuric acid is most acidable, and gives high hydrolysis yields (Mosier *et al*. 2002). Hydrolyzing *Colpomenia sinuosa* with 5% H_2SO_4 and H_3PO_4 gave a higher reducing sugar production of 41.18 and 41.34 g sugar/100g dry biomass, respectively. Hydrolysis with HCl and NaOH showed different patterns, where 3% HCl and 4% NaOH concentrations produced the maximum amount of reducing sugars (26.41 and 19.76 g sugar/100g dry biomass, respectively) (Fig.1c). Jeihanipour and Taherzadeh (2009) reported that pretreatment with phosphoric acid results in a great improvement in the hydrolysis of the materials, but it is far from the results of alkali pretreatment by NaOH.

Lack of lignin in seaweeds implies that the harsh pretreatment applied for release of fermentable sugars from lignocellulosic biomass is not required (Horn *et al*. 2000).
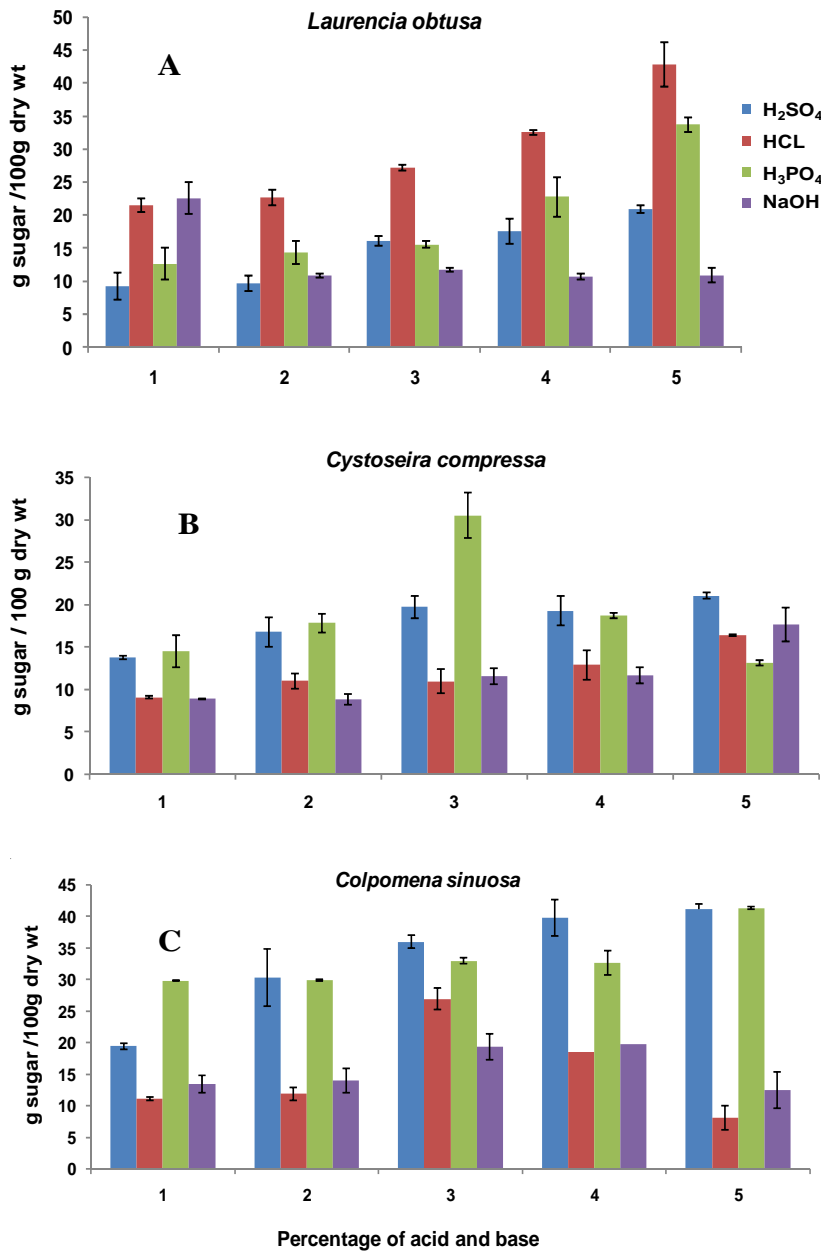
Figs 1A-C. Yield of reducing sugars by hydrolyzing seaweeds with acids and a base at 121ºC for 20 minutes from (A) *Laurencia obtusa,* (B) *Cystoseira compressa* and (C) *Colpomenia sinuosa.*

Water soluble polysaccharides from red seaweed *Apophloea lyallii* were extracted with water at 100ºC for 3 hrs. Extraction of the dried seaweed four times yielded a total of 63.6% w/w of polysaccharides (Watt *et al*. 2002). Carbohydrate content of red algae *Pachymeniopsis lanceolata*

and *Gelidium elegans* were 60.15 and 51.3% (w/w), respectively and glucose contents were 45.9 and 15.3% (w/w), respectively (Wi *et al*. 2009).

Effects of fermentation time on the conversion of sugars to bioethanol in acid pretreated *Laurencia obtusa, Cystoseira compressa* and *Colpomenia sinuosa* are shown in Table 2 and Fig. 2. Ethanol yield increased with the time in all tested algae. Reducing sugar produced by saccharification can be totally fermented by the yeast *Saccharomyces cerevisiae*. Sugar fermentation in *Laurencia obtusa, Cystoseira compressa* and *Colpomenia sinuosa* showed ethanol efficiency 68.48, 80 and 51.33%, respectively. These results showed that not all sugars convert to bioethanol and this may be contributed to the reducing sugar released from cellulose that may convert further to hydroxymethyl furfural and soluble phenolic compounds which can inhibit the ethanol fermentation (Laser *et al*. 2002). High level ethanol of 0.146 g/g of dry biomass of *Laurencia obtusa* biomass followed by *Colpomenia sinuosa* and *Cystoseira compressa*, respectively were found in (Fig. 2). The maximum alcohol content of 9.4% (v/v) from red algae *Pachymeniopsis lanceolata* and 2.4% (v/v) from *Gelidium elegans,* and alcoholic production of 74 and 65%, respectively were recorded by Wi *et al.* (2009). The utilization of seaweeds for bioethanol production appears to be a sustainable and eco-friendly approach (John *et al.* 2011).
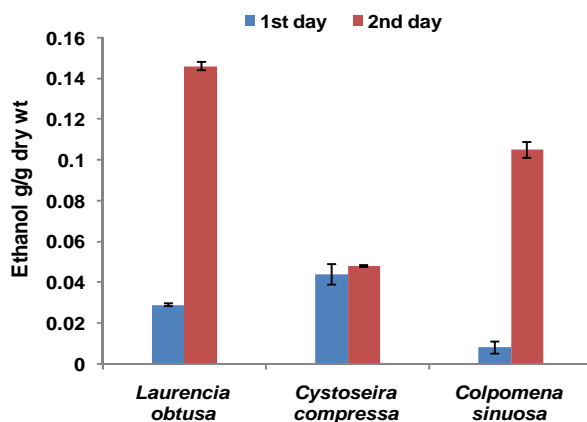

Fig. 2. Production of bioethanol by *Saccharomyces cerevisíae* at 30ºC using seaweed hydrolysates as the substrate.

Table 2. Ethanol yield after fermentation using seaweeds as feedstocks.

Seaweeds	Sugar in the hydrolyzate (g/ml)	Ethanol yield (%)		Efficiency (%)	
		24 h	48 h	24 h	48 h
Laurencia obtusa	0.0428	6.97	34.24	13.94	68.48
Cystoseira compressa	0.0210	21.09	40.04	42.18	80.00
Colpomenia sinuosa	0.0411	2.00	25.66	4.00	51.33

Red and brown seaweed biomass could be viewed as good economic feedstocks and ecofriendly for bioethanol production through a series of pretreatments either alkaline or acidic hydrolysis followed by fermentation via yeast (*S. cerevisiae*).

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