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Original Article

Bioethanol Production as Renewable Biofuel from Rhodopyhtes Feedstock

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Abstract

Today the transportation sectors and Indian government have been forced to give attention on the climate change threat along with the increasing oil prices and to consider the alternative fuels in order to eliminate the vulnerability of energy sector on their sustainability. Biofuels presents itself as a suitable replacement and has received much attention over recent years. Macroalgae-based second-generation bioethanol (a liquid fuel replacement for petrol) provides a possible solution for this energy issue. The present study was, bioethanol recovery from seaweed biomass using integrated biomass fractionation using hydrolysis which converts 'toughest' biomass to 'soft' biomass. In this study we used common species of *Hypnea* and *Eucheuma* to compare the amount of bioethanol production. Algal polysaccharides and bioethanol production was higher in *Eucheuma* than *Hypnea* sp. However, biomass (after ethanol extraction) was higher in *Hypnea* than *Eucheuma* sp. These results indicate that bioethanol can be produced from both species and *Eucheuma* is better source than *Hypnea* sp.

Keywords: Biofuels, Bioethanol, Biomass, *Eucheuma, Hypnea*

Introduction

Back in the 1970s, when oil prices first rose, the principle of growing crops for energy was encouraged without challenge. When biofuels first came along, they were heralded as "green gold'' but, all too quickly, they became ''a crime against humanity'' and ''food versus fuel'' is a commonly heard phrase these days. Two points remain clear: The challenges ahead are formidable and energy crops have the potential to provide a source of renewable energy that can reduce GHG emissions and help combat climate change the use of dedicated biomass crops and crop wastes and the scale of land conversion was still relatively small (N. El Bassam,2009). ''Biorenewables'' is an all embracing term that covers the production of heat, power, transport fuel and other products from organic matter of recent (as distinct from fossil) origin. These converge in the ''biorefinery'' concept, which can be defined as the sustainable coproduction of a spectrum of biobased products (food, feed, materials, chemicals) and energy (fuels, power, heat) from biomass. However, not all biorenewable products are derived from biorefining per se. The need for more sustainable products with reduced carbon footprints has led to the development of many new supply chains from nonfood crops and wastes (Al Zuhair, 2007).

An upward trend is shown in global liquid biofuel production from 4.8billion gallons in 2000 to about 16.0 billion in 2007 (Jegannathan *et al.,* 2009). Technologies is then peppered with the exploitation of crops which have high energetic values such as edible oil and sugarcane to produce biodiesel and bioethanol, respectively (Luo *et al.,*2009). While development of fuels from biomass continues apace, first generation biofuel based on edible crops has raised morality and ethics issues as there are millions of people around the world still suffer from malnutrition and hunger. In order to overcome this issue, bioethanol refined from macro algal biomass, namely Third generation bioethanol (TGB) offers a great option which is compatible with economic growth and morality issues (Beer *et al.,* 2009).

ABB (Algae Based Biofuel) has some unique features that can greatly reduce some of the sustainability problems faced by many terrestrial biofuel crops, for example little or no competition for agricultural land, or even positive effects, such as fertilizer production instead of consumption. However, integrating the full potential of these benefits influences other choices within an ABB concept. Algae species starch contents over 50 percent have been reported. Algae have some beneficial characteristics compared to woody biomass, the traditional target for this technology. Most notable is the absence of lignin in algae, making its removal needed for woody material redundant. Furthermore, algae composition is generally much more uniform and consistent than biomass from terrestrial plants, because algae lack specific functional parts such as roots and leaves (S jors van, 2008). Algal cell walls are largely made up of polysaccharides, which can be hydrolyzed to sugar. Another algae-specific technology for ethanol production is being developed, in which green algae are genetically modified to produce ethanol from sunlight and CO_2 (Deng and Coleman,1999). Third-generation bioethanol (TGB) represents fuel ethanol produced from algal biomass. Certain species of algae have the ability to produce high levels of carbohydrates instead of lipids as reserve polymers. Macroalgae can be grown on ropes. As the photosynthetic efficiency of algae (6-8% on average) is higher than terrestrial plants (1.8-2.2% on average) they are able to accumulate biomass at faster rates. These species are ideal candidates for the production of bioethanol as carbohydrates from algae can be extracted to produce fermentable sugars. Seam biotic, in collaboration with inventure chemicals. Successfully demonstrate the production of bioethanol by fermentation of the algal polysaccharides. Fermentation and distillation to produce ethanol from sugar have been at the heart of the brewing and of the wine and spirit industries for a long time in human history (Ellis A and Jacquier JC.2009). No literatures are found regarding present comparing research such as bioethanol from macro algae having species *Hypnea* and *Eucheuma*. Hence the work is aimed to know the proper fermentation and the amount of bioethanol production from algae.

Materials and Methods

Chemical composition of seaweeds

Different species from *Hypnea* found growing abundantly in Moroccan Atlantic coast contains mainly carbohydrates, accounting for more than 50% of the dry weight (Mouradi *et al.,* 2008). Eucheumata found growing abundantly in Singapore water contains mainly carbohydrates, accounting for over 70% of the dry weight (Tong *et al.,* 2007). Carrageenan is a linear, sulphated polysaccharide, the primary structure being made up of alternating α(1-3)-D-galactose-4-sulphate and β(1,4)-3,6-anhydro-D-galactose residues (Jacquier, 2009).Both the *Eucheuma* and *Hypnea sp* containing the polysaccharides, largely in the form of carrageenan as cell wall component. The carrageenan content could reach up to 55% of the dry matter galactose and 3,6-anhydrogalactose were the main components of the carrageenan, varied respectively between 53.79 and 63.79 mole % for galactose and 31.69 and 41.41 mole % for 3,6-anhydrogalactose. Other than carrageenan, small amount of sulphates also oscillated between 15 and 23% of carrageenan dry matter present in *Hypnea sp*. *Eucheuma sp* contains 56.2% of D-galactose and 43.8% of 3, 6-anhydro-galactose (Chopin and Whalen, 1993; Lin *et al.,* 2000)

Study area

The study area was located at Mandapam coast, Tamil Nadu (Lat. 78º11' to 79º15' E; Lat. 8º49' to 9º15' N) in the Gulf of Mannar (Janaurary to June, 2010), also from 10 km stretch of the Palk Bay side towards Mandapam Ramanathapuram District,Tamil Nadu, South India.

Sample collection

Specimens of Rhodophyceae (*Hypnae* and *Eucheuma)* collected at low tide from Mandapam coastal regions, Southeast coast of India. The collection of seaweeds can be done along the sea roads. To overcome the difficulties in transportation of hygroscopic ethanol, dry seaweeds are transported to a suitable location for ethanol production and refining. The handpicked seaweeds immediately washed with seawater to remove the extraneous foreign particles, sand particles and epiphytes. Then it was kept in an ice box containing slush ice and immediately transported to the laboratory and washed thoroughly using tap water to remove the salt on the surface of the sample. Then the seaweeds were spread on blotting paper to remove excess water and air dried in shade at 65ºC. The tissue samples were stored in precleaned polythene containers and the species were identified by the method described by Umamaheshwara Rao, (1987).

Extraction and purification of bioethanol

Mechanical pre-treatment

After collecting the seaweeds, drying can be done under the sun shade. Desalination has to be carried out because salinity would cause problem during purification. 20g of dried seaweeds will then be chopped into desired sizes (vary from a few centimeters to 1-3mm), smallest particle sizes can make up one third of the power requirements of the entire process. The samples are then cooked with hot water and alkali to extract the polysaccharides (Chandel, 2007). A good yield of carrageenan may range between 23% and 36%. The extract will be purified through filtration and centrifugation. The extraction process of carrageenan is very little publicized. These processes are where the principal costs lie within. Currently, there are two options for water removal after purification: spray drying on steam-heated drums or precipitation with alcohol (Arjunan *et al.,* 2009).

Dilute Acid Hydrolysis

Compared with another method such as enzymatic hydrolysis, the dilute acid hydrolysis had an advantage of nonspecific action towards carbohydrates in the feedstock, thus leading to the higher yield In mild acid hydrolysis, the resulted polysaccharides can be converted to fermentable sugar, in which sulphuric acid will be added at 80˚C and heated at 100˚C for 3 h. Neutralization can be carried out withBaCO3 (Del Campo *et al.,*2006). Currently, there are still no publicized technologies to hydrolyze carrageenan using enzymes. However, it is believed that advancing genetic engineering is capable to modify the available enzymes such as amylase to carry out the hydrolysis process (Azhar and Hamdy, 1980).

Ethanol Fermentation by Yeast

Fermentation, followed by distillation, is the biological conversion process used for converting sugars to ethanol or, depending on the microbial strain, other low molecular weight alcohols. Currently, large - scale galactose fermentation to ethanol is still not available. In fact, research on galactose fermentation is still not sufficient. Similar to glucose, galactose is a hexose sugar found in disaccharide lactose. Galactose has similar structure as glucose with just one difference in the stereochemistry of C_4 carbon. All carrageenans are high-molecular-weight polysaccharides made up of repeating galactose units and 3, 6 anhydrogalactose (3, 6-AG), both sulfated and nonsulfated. The units are joined by alternating α 1-3 and β 1 - 4 glycosidic linkages (Chandel *et al.,* 2007). However, from a GB-Analysts report, it was claimed that most of the microbes found to be effective in the fermentative oxidation of glucose substrate are also effective for the fermentative oxidation of galactose substrate (Keating *et al.,* 2004).From the literature, a strain of *S. cerevisiae* was found to exhibit exceptional fermentative performance on galactose (Sonderegger and Sauer, 2003). The solid fraction of the pretreated algal biomass was removed by centrifugation (4,000 ×g, 15min). The pH of the liquid fraction was adjusted from 4.5 to 5.0 with $CaCO_3$, a pH range suitable for yeast for ethanol fermentation. Conventional yeast fermentation produces 0.51kg of ethanol from 1kg of any of the C_6 sugars. However, this feed stock contain high-molecular-weight polysaccharides made up of repeating galactose units and 3,6 anhydrogalactose and hence they required hydrolysis step first to get break down into simple sugars for fermentation. It is able to completely exhaust the sugar in significantly less time (6 h) than that typically required by other strains tested (10–24h). Yeast pre-grown in 100 ml YEPD to OD 500-550 Klett unit (8-9g dry wt/L) and than transfer into 100ml hydrolysate supplemented with 10 ml 10 % yeast extract. Fermentation performed in a bioreactor containing YP-sugar liquid media in an orbital shaker for 24–48 h at 30˚C and 125rpm. Aliquots were centrifuged (14,000 rpm) for 4 min at 4˚C to yield cell-free supernatants, which were then decanted. The concentration of the produced ethanol was analyzed by high pressure liquid chromatography (HPLC). By using this method, maximum ethanol product per unit substrate can be as high as 39% (Chun Sheng Goh and Keat Teong Lee, 2010).

$$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2 \quad (1)$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (2)$$

Product recovery

The product stream from fermentation, also called ‘‘beer’’, is a mixture of ethanol, cell mass and water. The first step is to recover the ethanol in a distillation or beer column, where most of the water remains with the solids part (Niessen and DeLaat, 2000). The product (37% ethanol) is then concentrated in a rectifying column to a concentration just below the azeotrope (95%). Hydrated ethanol can be employed in E95 ICEVs, or in FCVs (requires onboard reforming), but for mixtures with gasoline ater-free (anhydrous) ethanol is required (Niven,2005). One can further distillate in the presence of an entrainer (e.g. benzene), dry by desiccants (e.g. corn grits), or use pervaporation or membranes. By recycling between distillation and dehydration, eventually 99.9% of the ethanol in the beer is retained in the dry product.

Results and Discussion

As a result, the percent dry weight of algae (before ethanol extraction) was higher in *Eucheuma* than in *Hypnea sp* (Table-1). *Hypnea*

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showed higher biomass than *Eucheuma* after fermentation (Fig.2,3&4). It was found by HPLC analysis that the liquid fraction of the pretreated algal biomass contained two forms of monomeric galactose sugars vise 3,6 anhydro D-galactose 44% galactose and D-Galactose 55% with Sulphate element 20 % and traces of Glucose 1% at 63˚C.

Table-1: Measurement of Initial Dry weight and ending biomass in Fermentation

Treatments	Fresh weight	Dry weight		Biomass	
	(g)	(g)	%	(g)	%
Hypnea sp	20	11.38	56.9	8.36	41.8
Eucheuma sp	20	13.94	69.2	6.73	33.65

Table- 2: Ethanol productivity from hydrolyzed Galactose of Algal biomass

Treatments	Galactose (g)	Ethanol production/ galactose (g/g)	HPLC Result of Ethanol amount (%)
Hypnea	4.48	20.1	20%
Eucheuma	5.44	51.2	59%

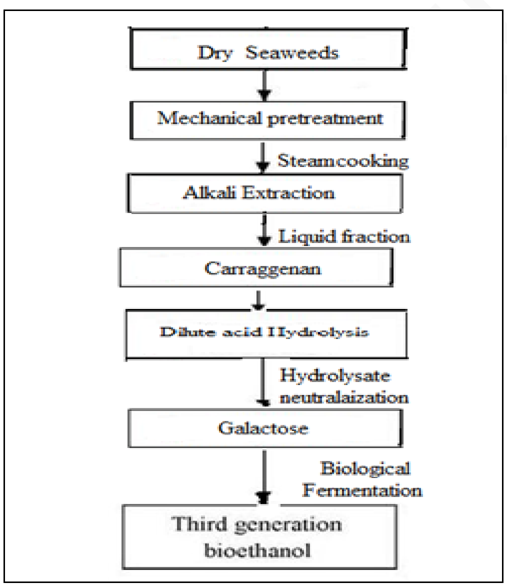

Fig.1: Flowchart for Bioethanol Production from seaweed

Potential ethanol yield from *Eucheuma* reached 51.2g/g galactose, was higher than that from the *Hypnea sp* 20.1g/g galactose. This result also suggested that the pretreated algal biomass that served as feedstock suitable for yeast fermentation to produce high yields of ethanol (Table-2).

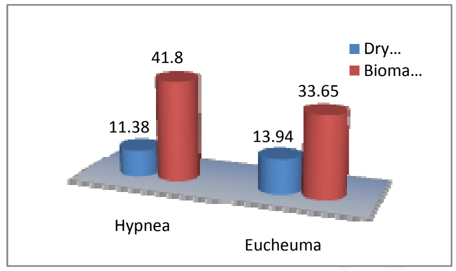

Fig. 2: Relative Fresh weight study with Algal Biomass

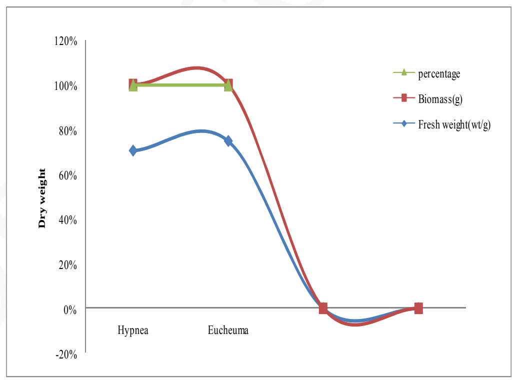

Fig.3: Mesurement of Algal Dryweight

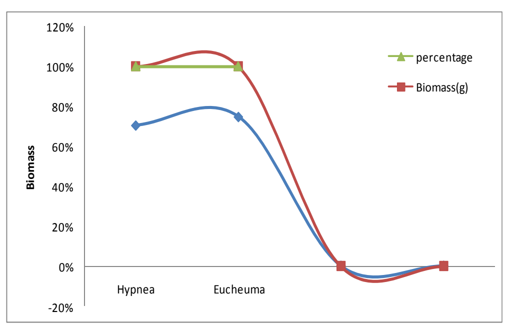

Fig. 4: Mesurement of Algal Biomass

Final result of HPLC analysis of fermented extract showing the presence of ethanol around 50% from *Euchema*, 30% from *Hypnea* and traces of D-Gulcose with Acetic acid as a byproduct of yeast fermentation.

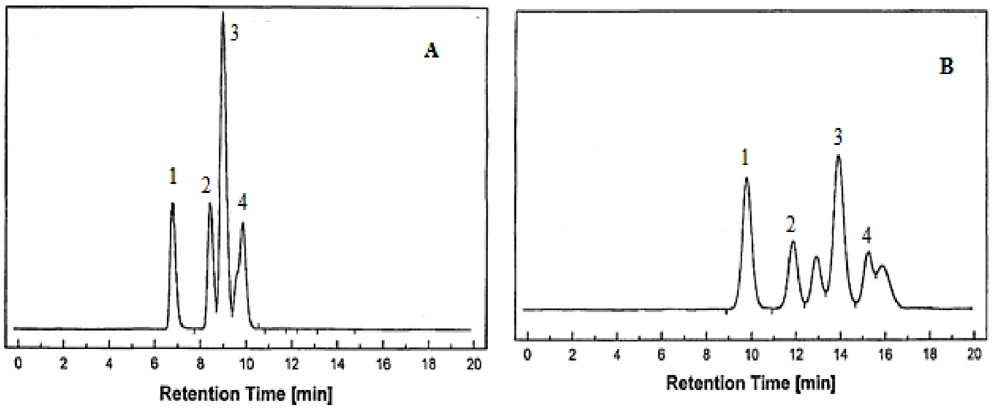

Fig.5: Authentic mixture of sugars as separated by HPLC system (HPX-87H column, 63˚C, 0.004 M H_2SO_4, 0.6 Ml/min), 1. 3,6 anhydro D-galactose (5mM); 2.Sulphate (4mM): 3,D-Galactose (6mM); 4. Glucose (0.01mM): A. *Eucheuma sp:* B. *Hypnea sp*

The report of this study revealed that nearly 200mg of ethanol can be produced from 1.0g of algal biomass from both *Hypnea* and *Eucheuma* Species through separate hydrolysis and fermentation. The main advantages of this comparative process and fermentation testing include the low cost of chemicals, short residence time, and a simple equipment system, all of which would promote its large-scale application.

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