



Comparison of red, brown and green seaweeds on enzymatic saccharification process



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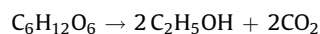
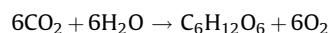
ABSTRACT

The production of bioethanol from seaweeds using acid hydrolysis and the enzymatic saccharification was studied. Red seaweed (*Gelidium amansii*), brown seaweed (*Laminaria japonica*), and green seaweed (*Codium fragile*) were selected, and the characteristics of their conversion to bioethanol were analyzed. The optimum conditions of the dilute acid hydrolysis preprocessing for bioethanol production from the seaweed were a reaction temperature of 150 °C, sulfuric acid content of 5.0 wt.%, and reaction time of 60 min. The seaweeds listed in order of bioethanol conversion performance are red seaweed > brown seaweed > green seaweed. The optimum dosage of enzyme was 2.0 mL per 10 g of seaweed. The optimal fermentation conditions for bioethanol production using seaweed included a commercial yeast dosage of 30 wt.% and a fermentation time of 3 days.

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1. Introduction

The consumption of fossil fuels has increased sharply since the industrial revolution, and current global energy consumption is running at about 86%. These fossil fuels are associated with problems regarding global warming, limited reserves, and unstable supply due to regional bias. Solar energy and wind energy are two mainstream alternative energies that could replace fossil fuels. Alternative energy is being actively developed using biomass, geothermal energy, waves, and tidal energy. Bioethanol can be utilized as an oxygenation of gasoline to elevate its oxygen content, allowing for better oxidation of hydrocarbons and reducing the amounts of greenhouse gas emissions into the atmosphere [1,2]. The production of bioethanol using biomass is known as an important alternative energy resource to replace gasoline, and is being commercially produced in Brazil and the United States [1]. The use of bioethanol does not elevate carbon dioxide levels in the air. The following reaction mechanisms show the circulation system of carbon dioxide emitted by using bioethanol as a fuel [4].



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Bioethanol can be produced using plants containing sugar, starch, and lignocelluloses [2,3]. Bioethanol produced from materials containing sugar and starch is regarded as the first generation of bioethanol. However, many problems have arisen with increasing demand, including food security, and land substitution. The second generation of bioethanol uses cellulosic wastes, which are relatively abundant and low-cost [4]. However, the lignin contained in cellulosic wastes interferes with the ethanol production. Thus, a pretreatment process for removing lignin must be added. However, the use of lignin-free seaweed as a raw material is arising as a third generation of bioethanol production [5]. Seaweed includes brown algae (kelp, sea mustard), red algae (laver, agar), and green algae (green laver, sea staghorn). 414 kinds of seaweed (95 kinds of brown algae, 61 kinds of green algae, 247 kinds of red algae, and 11 kinds of blue-green algae) can be found in the waters around the Korea peninsula, which indicates great potential for the development of seaweed as a resource. Table 1 shows the typical contents of seaweed [6,7].

Seaweed is classified into macroalgae and microalgae, and the macroalgae is classified into red algae, brown algae, and green algae. The red algae cell walls consist of polysaccharides (agar, cellulose, xylene, mannan, and carrageenan), and most of the carbohydrates are contained in agar, and less cellulose [8]. Agar is a viscid polysaccharide that composes the outsides of cell walls and exists in between cells. The polysaccharides in the cell walls of brown algae are cellulose, alginate, and fucoïdan, and its cellulose content is the same as that of red algae. The polysaccharides in the cell walls of green algae are cellulose, mannose, and xylene, which make the cell walls' frames. Cellulose represents a large ratio of the carbohydrate content in these algae. The cellulose could be used as

Table 1
Composition of typical seaweed [6,7].

	Species	Carbohydrate	Protein	Lipid	Ash
Green seaweed	<i>Codium fragile</i>	58.7	15.3	0.9	25.1
	<i>Capsosiphon fulvescens</i>	48.1	24.4	0.6	26.9
	<i>Enteromorpha prolifera</i>	53.3	22.9	0.8	22.9
	<i>Ulva lactuca</i>	50.4	26.8	0.6	22.2
	<i>Caulerpa lentillifera</i>	45.5	11.7	1.2	41.6
	Average	53.2 ± 1.8	19.8 ± 2.5	0.8 ± 0.1	26.1 ± 3.1
Red seaweed	<i>Gelidium amansii</i>	66.0	20.5	0.2	13.3
	<i>Porphyra Sp.</i>	45.5	43.6	1.9	9.0
	<i>Gigartina tenella</i>	42.2	27.4	0.9	29.5
	<i>Hypnea charoides</i>	57.3	18.4	1.5	22.8
	<i>Carpopeltis cornea</i>	60.7	23.4	0.4	15.6
	Average	55.2 ± 2.8	23.1 ± 2.4	0.9 ± 0.2	20.6 ± 1.6
Brown seaweed	<i>Laminaria japonica</i>	51.5	8.4	1.3	38.8
	<i>Undaria pinnatifida</i>	43.2	23.8	3.5	29.5
	<i>Hijikia fusiforme</i>	47.5	9.8	1.2	41.5
	<i>Eisenia bicyclis</i>	72.7	8.2	0.2	18.8
	<i>Ecklonia stolonifera</i>	65.0	15.3	1.5	18.1
	Average	57.5 ± 4.2	12.1 ± 2.0	1.8 ± 0.6	28.6 ± 4.4

an important ingredient for bioethanol production from seaweed. The lack of lignin negates the need of processes for removal of the lignin, and can facilitate saccharification process using cellulose. Additionally, hypotonic polysaccharide starch can be used as a glycocomponent. Thus, it is expected to be a better raw material for bioethanol production than lignocellulosic biomass. These high-molecular-weight polysaccharides can be converted into mono-saccharide through enzymatic hydrolysis [9–11] and chemo-physical hydrolysis [12–15]. Therefore, in this study, bioethanol was produced from seaweed by using acid hydrolysis and enzymatic saccharification. Depending on the type of seaweed, the bioethanol conversion characteristics was examined, and the optimum concentration of sulfuric acid and the amount of enzyme were determined by using a dilute acid hydrolysis process as a preconditioning process and enzymatic saccharification process as a bioethanol production process. Also, an optimum fermentation process is suggested to increase bioethanol output.

2. Experimental

2.1. Raw materials

Raw materials such as red seaweed (*Gelidium amansii*), brown seaweed (*Laminaria japonica*), and green seaweed (*Codium fragile*), were milled and screened through 14/18 mesh (1.00 ~ 1.16 mm) and dried at 40 °C prior to pretreatment. The milled raw material was stored in desiccators at room temperature.

2.2. Dilute acid hydrolysis and enzymatic saccharification

10 g of dried seaweed was hydrated by 200 mL of water at 50 °C, and acid hydrolysis process was conducted at 2.5–12.5 wt.% sulfuric acid of dried seaweed and 150 °C for 30–90 min. After the acid hydrolysis, the seaweed was neutralized using sodium hydroxide. For enzymatic saccharification, 10 g of seaweed was

Table 2
Optimum condition of enzymatic saccharification.

Enzyme	Temperature, °C	pH
<i>Lactozym</i>	37	6.5
<i>Spirizyme</i>	60 ~ 63	4.2 ~ 4.5
<i>Viscozyme</i>	25 ~ 55	3.3 ~ 5.5
AMG	60	4.5

put into 500 mL of water after acid hydrolysis, and enzymatic saccharification was conducted using *Lactozym*, *Spirizyme*, *Viscozyme*, and AMG (*glucoamylase*). The optimum saccharification temperature and pH of each enzyme are shown in Table 2, and the enzymatic saccharification process was conducted while changing the amount of injecting enzyme (1.0–4.0 mL) for 30 min to decide the optimum amount. Using 30 wt.% of commercial yeast, bioethanol was made by fermentation for 3 days at 28 °C in an incubator.

2.3. Chemical analysis

The fermented bioethanol was subjected to analysis out to calculate the yield by GC-FID (Gas Chromatography). The temperature of the sample injection port and detection part was kept constant at 200 °C using an HP-5 column with a column length of 30 m and diameter of 0.32 mm, and the oven temperature was elevated from 60 to 110 °C. The bioethanol yield (BE yield) was calculated using the following equation and the peak area measured from chromatograms.

$$\text{BE Yield} = \frac{A_i}{A_{\text{EtOH}}} \times \frac{V \rho}{m} \times 100(\%) \quad (3)$$

A_i is the peak area of bioethanol in the chromatogram and A_{EtOH} is the peak area of 99.9% purity ethanol. V is the volume of bioethanol (mL), ρ is the density of ethanol (g/mL), and m is the weight of seaweed (g) used in the experiment.

3. Results and discussion

3.1. Dilute acid hydrolysis

Acid catalyzed processes can be divided into two general approaches: based on concentrated-acid and low-temperature, and the other using dilute-acid and high-temperature hydrolysis. Sulfuric acid is a common acid employed, although other mineral acids such as hydrochloric and nitric acids have also been assayed [16]. Dilute-acid processes have been viewed primarily as a means of pre-treatment for the hydrolysis of hemicelluloses to render the cellulose fraction more amenable for a further enzymatic treatment. Both cellulose and hemicellulose components can also be hydrolyzed using dilute-acid catalyzed processes. Typical sulfuric acid concentrations for hemicellulose hydrolysis are in the range of 0.5 ~ 1.5 wt.%, with temperatures above 120 ~ 160 °C, but cellulose hydrolysis requires high temperatures of

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