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Saccharification of Seaweed by Acid Hydrolysis with Gamma-Irradiation

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Abstract: Gamma irradiation was applied to the saccharification process of seaweed. Seaweed biomass was gamma-irradiated at doses of 0, 50, 100, 200, 500 kGy and then the irradiated biomass was hydrolyzed using sulfuric acid. The concentration of reducing sugar of hydrolysates significantly increased by gamma irradiation. Microscopic images show that gamma irradiation causes structure breakage of the seaweed cell wall. These results indicate that the combined method of gamma irradiation with acid hydrolysis can significantly improve the saccharification process for bioenergy production from seaweed biomass.

Key words: Gamma irradiation, Saccharificaiton, Seaweed, hydrolysates, bioenergy production

INTRODUCTION

Consumption of fossil fuel increased rapidly during the age of global industrialization. High energy consumption caused environmental problems such as global warming and it is also causing the exhaustion of fossil fuel. A potential solution to these world-wide problems is the development of alternative energy from renewable biomass.

Bioenergy is a renewable alternative fuel that could replace petroleum-based fuel like gasoline and diesel. Although land plant biomass is easily fermented for bioenergy, bioenergy production from land plants causes rising of food prices through competition with food sources (Adams *et al*., 2010).

Marine algae are an abundant and year-round source of carbohydrates. Approximately 20-30% of marine algae biomass is made up of cellulose or starch and marine algae also contain various mono-saccharides such as glucose, xylose and galactose. Brown algae also known as marine macroalgae, contain up to 67% carbohydrates by dry weight including materials such as alginate, laminarin and mannitol (Horn *et al*., 2000).

Moreover, algal cells can be harvested within a short span of time as compared to other land feedstocks and hence they can meet the increasing demand of feedstocks for bioenergy production (Harun *et al*., 2009).

These marine algal sugars can be extracted using saccharification method and these extracts can be converted into bioethanol and biodiesel (Saha *et al*., 2005). However, the saccharification yield from algae is not sufficient for economical production of bioethanol.

Lignocellulosic biomass which is composed of cellulose, hemicellulose and lignin has long been recognized as a potential sustainable source of sugars for biofuels and value-added biomaterials. However, many physicochemical structural and compositional factors inhibit the enzymatic digestibility of the lignocellulosic biomass that liberates the sugars necessary for fermentation. Therefore, pretreatment is an essential processing step to break the lignin and to expose cellulose and hemicelluloses for enzymatic digestion. Several pretreatment methods of improving saccharification in cellulosic raw materials have been investigated (Silverstein *et al*., 2007).

Various pretreatment methods which include physical, chemical and biological pretreatments have been developed to enhance the efficiency of enzymatic hydrolysis and to lower the cost of biofuel production using lignocellulosic biomass. Although, there are a number of reports on various pretreatment options, none of them can be considered as the ideal option because the choice of optimal pretreatment method depends on the type of biomass and its economic and environmental impact. Ionizing radiation such as gamma rays and electron beam can modify and disrupt the structure of lignocellulose by penetrating photons into the lignocellulosic structure and producing free radicals. Irradiation reduced the crystallinity of lignocellulose biomass, leading to a decrease in the degree of polymerization and increase in the surface area which in turn increase the enzyme accessibility and enzymatic digestibility. For this reason, irradiation pretreatment has been investigated for decades as a physical pretreatment

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method of biomass. Even though irradiation could be simply applied to all type of lignocellulose biomass with large volume, the glucose saccharification yield after a simple irradiation was relatively lower than that obtained by chemical methods. Therefore, more research on irradiation pretreatment in combination with other pretreatment methods should be conducted to make this option economically viable. Radiation degradation for increasing sugar yield has been reported in regards to various lignocellulosic materials such as bagasse (Han *et al*., 1981), wheat straw (Yang *et al*., 2008) and sawdust (Kumakura and Kaetsu, 1984; Miller, 1959).

Different biomass from various sources like agricultural, forestry and aquatic have been taken into consideration as the feedstocks for the production of several biofuels such as biodiesel. However, the environmental impact raised from burning of fuels has a great impact on carbon cycle (carbon balance) which is related to the combustion of fossil fuels. Besides, exhaustion of different existing biomass without appropriate compensation resulted in huge biomass scarcity, emerging environmental problems such as deforestation and loss of biodiversity. Recently, researchers and entrepreneurs have focused their interest, especially on the algal biomass as the alternative feedstock for the production of biofuels. Moreover, algal biomass has no competition with agricultural food and feed production. Interestingly, the low content of hemicelluloses and about zero content of lignin in algal biomass results in an increased hydrolysis and/or fermentation efficiency. Other than biofuels, algae have applications in human nutrition, animal feed, pollution control, biofertilizer and waste water treatment . Therefore in this work, the effect of gamma irradiation on the saccharification of seaweed was investigated.

MATERIALS AND METHODS

The brown seaweed *Undaria* sp. was harvested in the region near Wando Island, South Korea. Before the experiment, samples were washed with distilled water and then stored at 4°C until they were used.

The wet samples were irradiated in a cobalt-60 gamma-irradiator (IR-221, Nordion International, Ltd., Ontario, Canada) equipped with 11.1 PBq strength at 22±0.5°C and operated at a dose rate 10 kGy/hr. The applied dose levels were 50, 100, 200 and 500 kGy. Dosimetry was performed with 5 mm-diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany) and after irradiation the sample was stored at 4°C for further experiments. Non-irradiated samples were also prepared for control studies.

The samples were re-suspended in 1% sulfuric acid (H_2SO_4) and incubated at 121°C for 0-240 mins. The hydrolysates were cooled down to room temperature and then neutralized with Calcium Carbonate ($CaCO_3$). After neutralization, Calcium Sulfate ($CaSO_4$) a product of the chemical reaction was removed using a centrifuge operated at 8,000 rpm for 15 mins.

The concentrations of reducing sugar after the saccharification process were determined by the 3, 5 Dinitrosalicylic Acid (DNSA) method. Specifically, 1 mL pretreated samples were transferred into 15 mL glass tubes and 2 mL of the modified DNSA reagent (0.5 g dinitrosalicylic Acid, 8 g sodium hydrate and 150 g Rochelle salt in 500 mL distilled water) was added to the tube. The mixture was vortexed for 5 sec, boiled at 90°C for 10 mins and then cooled in ice. The absorbance of the samples was measured at 550 nm using a spectrophotometer (UV-1601 PC, Shimadzu Co., Tokyo, Japan) and the OD values were converted to concentration of reducing sugar using a standard curve prepared with different concentrations (0.625, 1.25, 2.5, 5 and 10 mg/mL) of glucose.

RESULTS AND DISCUSSION

Before The brown seaweed Undaria biomass was saccharified at 121°C with 1% sulfuric acid concentrations for different residence times (0-180 mins). Table 1 shows the changes in the concentration of reducing sugar resulting from acid hydrolysis. The concentration of reducing sugar increased with increasing residence time. In sulfuric saccharification, a low acid concentration (e.g., 0.1-1% sulfuric acid) at high temperature is commonly used because a high acid concentration is extremely corrosive and dangerous (Fig. 1). The positive effects of conversion of cellulosic biomass into

Table 1: Concentration of reducing sugar on seaweed biomass after hydrolysis (1% H_2SO_4)

Absorbed dose (kGy)	Reducing sugar concentration (mg/L)
0	16.9
15	18.6
30	19.3
60	23.2
120	29.8
180	38.3

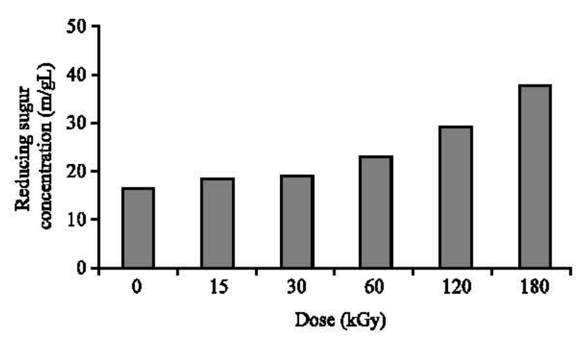

Fig. 1: Reducing sugar concentration after acid hydrolysis

Table 2: Concentration of reducing sugar on seaweed biomass after gamma irradiation

Absorbed dose (kGy)	Reducing sugar concentration (mg/L)
0	16.7
50	21.9
100	27.3
200	38.0
500	49.3

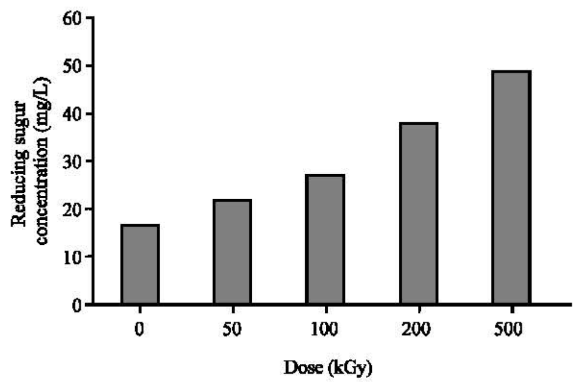

Fig. 2: Reducing sugar concentration only with irradiation

Table 3: Concentration of reducing sugar on seaweed biomass after acid hydrolysis with gamma irradiation

Absorbed dose (kGy)	Reducing sugar concentration (mg/L)
0	36
50	68
100	87
200	136
500	235

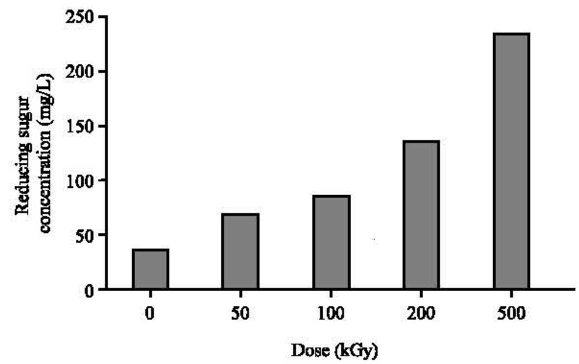

Fig. 3: Reducing sugar concentration after combined treatment

fermentable sugars for bioethanol production through the use of low acid concentration have been reported however, inhibitors that form at the low pH usually inhibit the microbial growth and fermentation and this results in lower ethanol yield. Moreover, this process requires acid recovery for economic reasons.

Therefore, the pretreatments at low pH should be selected properly in order to reduce the formation of these inhibitors and economic efficiency should be taken into consideration. The marine algae samples were irradiated at doses of 0, 50, 100, 200 and 500 kGy. Table 2 shows the changes in the concentration of reducing sugar in marine algae resulting from the gamma irradiation. The concentration of reducing sugar increased with increasing gamma radiation dose. The reducing sugar concentration of the non-irradiated sample was about 17 mg L^{-1} whereas the concentration in the sample irradiated at a dose of 500 kGy increased to about 49 mg L^{-1}. In general, irradiation leads to the degradation of polysaccharides such as starch and cellulose because it breaks the glycosidic bonds in the presence of water. Saccharification of cellulosic biomass from plants for bioethanol production has been enhanced by the use of high-energy gamma irradiation. The effects of gamma irradiation on de-polymerization were consistent with those reported in other studies in regards to algal polysaccharides such as starch, agar, alginate and carrageenan (Fig. 2). Treatment involving a combination of irradiation and other methods such as acid treatment and mechanical crushing can further accelerate the saccharification process. Irradiation can enhance cellulosic degradation of cellulose into glucose.

In this study, the effects of irradiation used for pretreatment of marine algae prior to acid hydrolysis (1% sulfuric acid at 121°C for 180 mins) were studied (Table 3). The reducing sugar concentration of gamma-irradiated marine algae at the dose of 500 kGy increased from about 17 mg/L to about 48 mg/L while the reducing sugar concentration significantly increased to about 235 mg/L through combined treatment involving gamma irradiation and acid hydrolysis.

There was report on the application of gamma-ray irradiation for the hydrolysis of rice straw. when compared with the composition of the untreated rice straw, alkali pretreatment alone could increase the cellulose content by 40.5% while the xylan, lignin, extractives and water contents were reduced by 42.9, 23.4, 10.8 and 44.3%, respectively. But, the combined pretreatment with gamma-ray irradiation and alkali showed further increase in the cellulose content up to 60% depending on the dose of irradiation. In contrast, the levels of other components were decreased depending on the radiation dose. The combined pretreatment increased the removal percentage of xylan, lignin, extractives and water up to 63.8, 40.2, 32.3 and 58.7%, respectively. There were no detectable sugar degradation products such as furfural and hydroxymethylfurfural which are known to inhibit cell growth. Lignin limits enzyme access to carbohydrates by imposing a physical barrier and by causing unproductive binding of enzymes and hemicellulose is thought to restrict the access of enzyme to cellulose in pretreated biomass. Therefore, the increase of cellulose content and reduction of xylan and lignin can promote the process of enzymatic hydrolysis. Since, a large amount of lignin and xylan content was removed after the combined pretreatment, the results clearly indicated that the combined pretreatment could enhance the enzymatic digestibility of rice straw.

Also, when the rice straw hydrolysis was used as a sole carbon source, the cell concentration reached 6.5 g/L after cultivation for 8 days. When 10 g/L of purified glucose was added to the medium, the maximum cell concentration was about 8.7 g/L. The difference of final cell concentration between rice straw hydrolysate and purified glucose could be caused by the impurities in the rice straw hydrolysate. Growth of *C. protothecoides* in the rice straw hydrolysate medium was also carried out under the mixotrophic conditions. In this mixotrophic condition, glucose source was also added to the medium under light. The final cell concentration was 4 g/L in the mixotrophic culture which was lower than the final cell concentration of 6.5 g/L in the heterotrophic culture. However, the substrate conversion to cell mass was about 92% in the mixotrophic culture which was higher than 65% in the heterotrophic culture. The cell concentration was also higher with the use of purified glucose than with the use of rice straw hydrolysate in the mixotrophic culture. The lower microalgal growth in the mixotrophic culture was demonstrated in the lipid production by *Chlorella vulgaris.* Under mixotrophic growth conditions, addition of 5 and 10% glucose exerted inhibitory effect on the growth. This phenomenon was also observed for *C. protothecoides* on feeding with glucose in the range between 1.5 and 6%. However, the effects of glucose on microalgal growth have not been intensively explored.

CONCLUSION

These results demonstrate that the combined pretreatment with gamma irradiation was highly effective in preparing hydrolysate and seaweed hydrolysate could be used as an alternative carbon source for biofuel production.

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