Improvement Enzymatic Hydrolysis of Wheat Straw for Bioethanol Production by Combined Treatment of Radiation and Acid

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1. Introduction

The cost of ethanol production from starch and sucrose for use as a vehicle fuel is ultimately high. Consequently, it has been suggested that the large-scale use of ethanol as a fuel will require the utilization of cellulosic feedstock. Lignocellulosic biomass has the potential to serve as a low cost and renewable feedstock for bioconversion into fermentable sugars, which can be further utilized for biofuel production. It is estimated that there is over one billion tons of biomass available for conversion into biofuels on a renewable basis to displace a substantial portion of the fossil fuels currently consumed within the transportation sector [1].

Among different pretreatment methods such as biological, physical, chemical, and physic-chemical pretreatments, chemical pretreatment using dilute acid as catalyst, which has been extensively evaluated for treating a variety of lignocellulosic feedstocks, is reported as one of the leading pretreatment technologies [2].

Ionizing radiation can easily penetrate lignocellulosic structure and undoubtedly produce free radicals useful in modification of lignin structure as well as breakdown cellulose crystal regions. Phenoxy radicals appeared to be important radical intermediates that ultimately transformed into o-quinonoid structures in lignin [3]. Therefore, ionizing radiation such as gamma ray and electron beam can be a great alternative.

In this study, the effect of ionizing irradiation of wheat straw prior to dilute sulfuric acid treatment is investigated. The combined pretreatment for wheat straw was performed to evaluate the efficiency of enzymatic hydrolysis and compared with that of the effect of enzymatic hydrolysis by individual pretreatment.

2. Methods and Results

2.1 Pretreatment

The wheat straw (50 g) with 3% (w/w) of sulfuric acid was autocleaved at 121oC for 60 min and exposed to gamma radiation before and after dilute acid pretreatment from 25-1000 kGy which was generated by a gamma irradiator (60Co, ca. 111 TBq of MDS Nordion, Ottawa, Canada).

2.2 Observation by scanning electron microscope (SEM)

The observation of morphological changes after pretreatments, especially surface of leaf, was carried out with a scanning electron microscope. The pretreated wheat straws were fixed on stubs by double-side tape after general steps of fixation and dehydration. The stubs were dried over P2O5 in a vacuum oven overnight at 40^{o}C. The stubs were sputter-coated with Pt and observed with a JEOL JSM-6390 SEM (Tokyo, Japan) using an accelerating voltage of 10 kV.

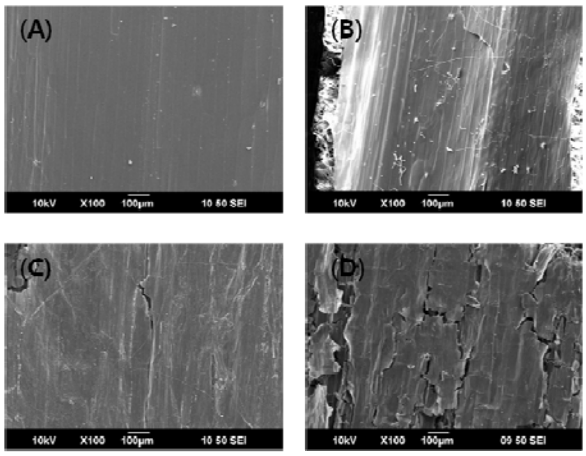

Fig. 1. Scanning electron micrographs of pretreated wheat straw. (A) untreated, (B) 1000 kGy of gamma irradiation, (C) 3% sulfuric acid, (D) combination of 3% sulfuric acid and 1000 kGy of gamma irradiation.

SEM revealed morphological changes in wheat straw following various pretreatments. When compared to the surface structure of control sample, which has a continuous, even and smooth flat surface, the gamma irradiated wheat straw has a rugged, unsmooth and little broken face. Wheat straw treated dilute acid was dimly visible that cells would be tangled, resulting from collapsed their shape. The pretreating wheat straw with combined use of dilute acid and gamma ray was clearly showed that severely rugged on surface and leaf split up many pieces (Fig. 1). This would be due to bombardment with reactive oxygen species (ROS) produced by gamma irradiation.

2.3 Enzymatic hydrolysis

Enzymatic hydrolysis was performed using commercial enzymes [R-10 cellulase (onozuka, Japan), β-glucosidase (sigma, USA) and xylanase (sigma, USA)] used in this study for hydrolysis. Enzymatic hydrolysis was performed at 37°C and 150 rpm with an enzyme loading of 3.2 mg cellulase g-1 biomass, 540 μg β-glucosidase g^{-1} biomass and 540 μg xylanase g^{-1} biomass for 96 hr. The contents of carbohydrates in the hydrolysate was determined by high-performance liquid chromatography (Waters, USA) and distilled water was used as an eluent at a flow rate of 0.6 mL min^{-1}. A refractive detector was used for carbohydrates [4].

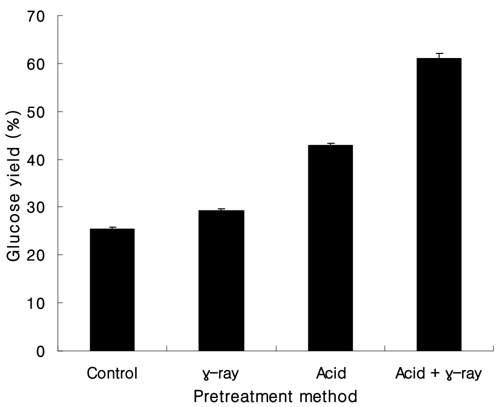

Fig. 2. Effect of various pretreatments on the enzymatic hydrolysis of wheat straw. Results are the means of three replicated experiments with S.D.

In contrast to cellulose, hemicelluloses have a random amorphous structure with little strength and are easily hydrolyzed by dilute acid [5]. Obviously, the glucose yield increased from 27-30% when wheat straw was untreated and pretreated with gamma irradiation only, to 40% following pretreatment with dilute acid (Fig. 2). Hydrolysis results clearly showed that soluble sugars were released faster and to a greater extent in dilute acid pretreated wheat straw than in gamma irradiation pretreated wheat straw. The loss of xylose indicates that most hemicelluloses would be removed by dilute acid and therefore has an increase in cellulose surface accessibility and would theoretically enable more efficient cellulose hydrolysis.

In addition, the modification of lignin after dilute acid pretreatment is remarkably different from gamma irradiated sample. Even though lignin contents are similar in all samples, absorption peaks of lignin in FTIR spectra and results of alkaline nitrobenzene oxidation are quite different. Thus, the modification of lignin should effect on enzymatic hydrolysis. The crystallinity of wheat cellulose exposed to gamma ray is a similar to dilute acid pretreated wheat straw (Fig. 3).

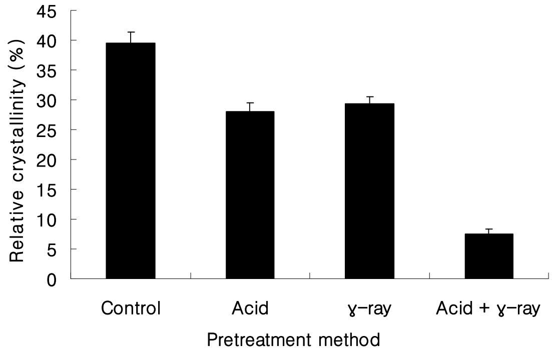

Fig. 3. Effect of gamma irradiation on the crystallinity index of wheat straw. Results are the means of three replicated experiments with S.D.

Based on this result, the crystallinity index does not critically affect the glucose yield released during enzymatic hydrolysis of wheat straw in individual pretreatment.

3. Conclusions

We have shown the possibility of increasing the convertibility of wheat straw following pretreatment by combined use of gamma ray and dilute sulfuric acid. Gamma irradiation after sulfuric acid pretreatment showed a great synergistic effect of enzymatic hydrolysis for bioethanol production (Fig. 2). Therefore, this combined pretreatment is one of the promising pretreatment method for making the lignocellulose accessible to enzymatic reactions, where crystallinity of cellulose, its accessible surface area, and removal or modification of lignin and hemicelluloses are main obstacles affecting the enzymatic hydrolysis.

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