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Research paper

Great potency of seaweed waste biomass from the carrageenan industry for bioethanol production by peracetic acid–ionic liquid pretreatment

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ABSTRACT

Seaweed waste biomass from the carrageenan industry (SWBC) is a potential biomass feedstock for producing sustainable biofuel because it increases the product value and reduces the pollutant risk. Peracetic acid (PAA) followed by ionic liquid (IL) pretreatment has been used to increase the enzymatic saccharification of pretreated SWBC. The SWBC cellulose content was comparable with that of terrestrial biomasses. PAA + 1-hexylpyridinium chloride ([Hpy][Cl]), and PAA + 1-ethyl-3-methylimidazolium diethylphosphate ([Emim][DEP]) pretreatments produced saccharide and unknown oligosaccharide fractions in regenerated water (~4–6% SWBC cellulose content). For 48 h of saccharification, the untreated SWBC and the SWBC pretreated using PAA followed by [Hpy][Cl], [Emim][DEP] or 1-ethyl-3-methylimidazole acetate ([Emim][OAc]) produced cellulose conversions of 77, 91, 84 and 62%, respectively. The untreated SWBC had a high cellulose conversion, which may be caused by the low lignin and hemicellulose contents of the SWBC. PAA + IL pretreatment could yield pretreated SWBCs with more amorphous cellulose structures, which lead to an almost-complete cellulose conversion.

1. Introduction

Bioethanol is a promising biofuel candidate to substitute or replace liquid fossil fuels such as gasoline. Unfortunately, most commercial bioethanol production (79.3%) is obtained from staple food resources, such as coarse grains, sugar crops and wheat [1]. The high utilization of food material for biofuel feedstock has been blamed for rising food prices; with the growth in world population, this may threaten food security. The sustainability of these feedstocks has been debated considerably [2]. Because of these limitations, a second generation of bioethanol production based on lignocellulosic biomass from agricultural and forest residues and dedicated energy crop feedstock has been developed. However, native lignocellulosic biomass is difficult to process economically because of its lignin–carbohydrate structural complexity, its low yields and high production costs, and it has therefore not been produced commercially [3]. An exploration of new sustainable bioethanol feedstocks, which could lower the production costs of second generation biofuels, is therefore essential.

Seaweed or macroalga is a multicellular photosynthetic organism and a potential biomass resource for the full or partial substitution and displacement of terrestrial biomass to produce sustainable biofuels and biochemical products [4]. This aquatic plant is evolutionarily diverse and abundant in both shore and offshore areas. Compared with terrestrial plants, seaweed is three to four times more able to grow and convert solar energy to chemical energy. Seaweed therefore has the potential to generate and store sufficient carbon resources for biorefinery products [4]. Adams et al. compared the yield and dry weight of hydrolysable carbohydrates among seaweed and terrestrial plants such as wheat, maize, sugar beet and sugar cane. Compared with terrestrial plants, seaweed measured higher than 11- and 4.5-fold on yield and dry weight, respectively. Seaweed also has a potential bioethanol

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volume of 23,400 L/ha/year, whereas that of terrestrial plants is less than 1010 L/ha/year [5].

Some articles have reported on the use of seaweed as bioethanol feedstock, as reviewed by Jung et al. [4] and Daroch et al. [6]. However, to the best of our knowledge, there is still limited utilization of seaweed waste biomass from the carrageenan industry (SWBC). The advantages of SWBC feedstock are that it does not compete with food staples, it reduces or minimizes pollutant loading and it increases the added value of the main product from the carrageenan industry. Carrageenan has been reported to be the highest seaweed hydrocolloid (phycocolloid) marketed, and it is used mostly in the food, pharmaceutical and cosmetic industries. For instance, the volumetric sales of carrageenan reached 50,000 tons in 2009 [7].

To produce refined carrageenan, seaweed as a raw material, should be treated with 1–10% alkali solution such as NaOH or KOH at 80 °C for 0.5–5 h [8]. Alkaline treatment converts the precursor moieties in the galactan backbone, L/D-galactose-6-sulphate, to 3,6-anhydrogalactose, which can increase the gel strength of the seaweed extract [9]. Seaweed extraction without alkali therefore produces a weak carrageenan gel strength [8,10]. After extraction, the slurry extraction is screened using a filter press to separate the solid and liquid fractions. The liquid fraction, which contains mostly carrageenan, is precipitated with excess ethanol. In this step, the range of carrageenan yields is 31.8–43.2% [10]. Approximately 60–70% will therefore be the resultant solid fraction that is known as SWBC. SWBC is presumed to contain high concentrations of carbohydrates, which have high potential for use as biofuel feedstock or other biochemical products.

In this work, the potential for use of SWBC as a bioethanol feedstock has been explored experimentally based on chemical compositions, structural characteristics, recovery yield and cellulose conversion produced. In our previous research, the combination pretreatments of peracetic acid (PAA) followed by 1-ethyl-3-methylimidazole acetate ([Emim][OAc]) on the Pine wood was carried out to increase enzymatic saccharification, [11]. In this paper, we extended the new PAA-ionic liquid (IL) combined pretreatment technique by using different types of ILs: 1-hexylpyridinium chloride ([Hpy][Cl]), 1-ethyl-3- methylimidazolium diethylphosphate ([Emim][DEP]) and ([Emim][OAc]) as a positive control.

2. Materials and methods

2.1. Materials

[Hpy][Cl] (>90% purity) was purchased from IoLiTec (Ionic Liquids Technologies GmbH, Heibiomassronn, Germany). Peracetic acid 40% (w/w) was supplied by Mitsubishi Gas Chemical Company, Inc., Japan and SWBC (particle size ~300 mesh) was obtained from CV Ocean Fresh, the refined carrageenan industry (Bogor, Indonesia). [Emim][OAc], [Emim][DEP] (purity 90%) and cellulase from *Trichoderma reesei* ATCC 26921 were purchased from Sigma–Aldrich (St. Louis, MO, USA). All reagents were used as received.

2.2. Chemical composition of SWBC

Fig. 1 shows a schematic of the experimental procedure. The hemicellulose, cellulose and lignin contents were determined according to the Goering and Van Soest protocol [12] by applying the concept of the use of neutral and acid detergents for carbohydrate and tannin removal. The hemicellulose content was estimated as the difference between neutral and acid detergent fiber, and the cellulose content was calculated based on the difference between the acid detergent fiber and lignin.

2.3. SWBC pretreatments

Dried SWBC was ground and screened using 300-mesh screen. The two-step pretreatment method was used as referred to in Uju et al. [11], in which temperature and time were modified. In the first pretreatment step, 50 mg of SWBC was heated in a 1.9% (v/v) PAA solution at 80 °C, stirred at 250 rpm for 3 h, and then the pretreated SWBC was frozen rapidly in liquid nitrogen, then freeze dried by a lyophilizer (FDU-1110, EYELA, Tokyo, Japan) at −45 °C for 16 h. In the second pretreatment step, the SWBC pretreated with PAA was heated in 300 mL of IL ([Hpy][Cl], [Emim][DEP] or [Emim][OAc]) at 100 °C for 30 min. The hot IL-SWBC solution was cooled to room temperature. To obtain the regenerated cellulose, the solution was added to 5 mL of deionized water, followed by centrifugation at 5800 × *g* for 10 min. To remove residual IL from the regenerated cellulose, the material was washed with 5 mL of deionized water three times. The process involved vigorous shaking and centrifugation.

The yield of regenerated biomass (YRB) was expressed as the ratio of the mass of dried regenerated SWBC to the mass of untreated SWBC. The YRB was calculated based on the mass of dried regenerated biomass using the following equation:

$$\mathrm{YRB}(\%) = \frac{\text{Mass of dried regenerated SWBC}}{\text{Mass of dried untreated SWBC}} \times 100 \quad (1)$$

2.4. Enzymatic saccharification

Enzymatic saccharification was carried out at 45 °C in 10 mM citrate buffer (pH 5.0) at a stirrer speed of 200 rpm. The untreated or regenerated SWBC obtained at each pretreatment was hydrolyzed in a 0.05 mg/mL cellulase solution. The biomass concentration was 5 g of untreated or regenerated SWBC per liter based on the initial biomass mass. Samples for saccharide content analysis were taken between 0 and 48 h.

2.5. Glucose and cellobiose analyses

The saccharide content in the reaction mixture was measured by high performance liquid chromatography (HPLC) using an LC-10AT VP (Shimadzu, Japan) and a Honenpak C18 column (J-Oil Mills Inc., Japan). The mobile phase was deionized water containing 0.02% trifluoroacetate (v/v) at 1 mL/min, and the column temperature was 40 °C. A 20 μL sample volume was injected. Refer to the *p*-aminobenzoic ethyl ester derivation described by Yasuno et al. [13] for a quantification of the saccharide concentration. Cellulose conversion to glucose or cellobiose was estimated by:

$$\text{Conversion of cellulose to glucose}(\%) = \frac{\text{glucose produced}(\mathrm{mol})}{\text{glucose unit in cellulose}(\mathrm{mol})} \times 100 \quad (2)$$

$$\text{Conversion of cellulose to cellobiose}(\%) = \frac{\text{cellobiose produced}(\mathrm{mol})}{\text{cellobiose unit in cellulose}(\mathrm{mol})} \times 100 \quad (3)$$

2.6. Scanning electron microscope (SEM) analysis

Before analysis, the sample was spread on a metal-cylinder plate with carbon tape and coated with platinum under vacuum. SEM analysis was performed using a JEOL JSM 5310LV operated at a