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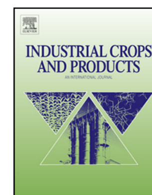

Bioethanol production from individual and mixed agricultural biomass residues

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ABSTRACT

Cellulosic bioethanol production has been fraught with challenges, including fluctuations in feedstock supply, handling costs, pretreatment, enzymes, and other logistical problems. Most studies of lignocellulosic ethanol production have focused on a single type of biomass; however, full utilization of various lignocellulosic biomass sources might enhance bioethanol production and the economic feasibility of the biorefinery. The goal of this study was to evaluate the effectiveness of popping pretreatment on saccharification and fermentation for individual and mixed biomass. We then compared separate hydrolysis and fermentation (SHF) with simultaneous saccharification and fermentation (SSF) processing, with the aim of optimizing production of bioethanol from biomass. Saccharification efficiencies were increased significantly in all the popping-pretreated compared to the non-pretreated individual and mixed biomass. The SSF was superior compared to SHF processing. Our results indicated that the saccharification efficiencies of both individual and mixed biomass were improved after popping pretreatment; in particular, the production of bioethanol from mixed biomass was identified as a suitable approach for more extensive application.

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1. Introduction

The energy industry is constantly looking for renewable and environmentally friendly energy resources. Among the suggested solutions, bioethanol has recently emerged as an effective solution to address the concerns arising from limited fossil fuels and the effects of greenhouse gas emissions. The industrial-scale production of lignocellulosic bioethanol from lignocellulosic biomass has been investigated for many years (Energy, 2015). Biomass available from agricultural residue and waste is generated from harvesting and processing cultivated crops. It is cheaper than starch and does not compete with food sources, making it is attractive for utilization in bioconversion.

In tropical countries such as Vietnam, coffee (*Coffea canephora*), cassava (*Manihot esculenta*), and coconut (*Cocos nucifera*) are common crops. Huge amount of residues is generated after harvesting of coffee, cassava, and coconut, but only small amounts of their residue yields are used for handicrafts or fertilizer production, while the remainder is mostly burned or considered waste, becoming a source of pollution (Prata and Oliveira, 2007; Mussatto et al., 2011; Esquivel and Jiménez, 2012; Ferraz et al., 2012; Nuwamanya et al., 2012; Wi et al., 2015). According to the Food and Agriculture Organization (FAO) of the United Nations (FAO, statistics, updated to 2013), in each year, 1.5, 9.8, and 1.3 million tons of coffee bean, cassava starch, and coconut, respectively, are produced in Vietnam. For every kg of coffee bean, cassava starch, and coconut produced, approximately 1.0, 0.4, and 0.4 kg of CH, CS, and CC, respectively, are generated (Esquivel and Jiménez, 2012; Nuwamanya et al., 2012).

The operational costs of producing ethanol from biomass are increased due to the necessary addition of a pretreatment step. Pretreatment destroys recalcitrant structures consisting of cellulose, hemicelluloses, and lignin to improve accessibility of poly-carbohydrate components to enzymes (Hendriks and Zeeman, 2009; Sarkar et al., 2012). One promising pretreatment method involves popping (Choi et al., 2012; Wi et al., 2011, 2015), which is based on a non-chemical physical pretreatment principle and allows a simple system to be used to achieve greater saccharification efficiency, with lower environmental impact compared with conventional methods (Wi et al., 2013).

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A consistent, stable supply of sustainable feedstock from a variety of sources is required to support large-scale lignocellulosic bioethanol production. Unfortunately, mixed biomass feedstock has rarely been the subject of experimental studies due to differences in composition and density among different types of feedstock (Sokhansanj and Hess, 2009; Tumuluru et al., 2011). One approach that has been studied extensively involves the formation of a pellet after grinding and densification (Panwar et al., 2010), which is then pretreated under harsh conditions to change the distribution of the lignin and reduce biomass recalcitrance (Rijal et al., 2012). This process consumes a large amount of energy. In addition to providing diversified sources of biomass, the use of mixed biomass might be an important alternative when constructing a lignocellulosic bioethanol production facility. In this study, we examined a popping pretreatment method for individual and mixed biomass samples prepared from leftover solid CH, CS, and CC wastes under identical conditions, and performed bioethanol conversion.

2. Materials and methods

2.1. Biomass materials and popping pretreatment

We collected CH, CS, and CC from Daklak province, Vietnam. The biomass was dried in an incubator (Labtech Co.) at 60 °C for 1 week, ground with a milling machine, and then put through a sieve that allowed particles of less than 60 μm in size to pass. This step helps to ensure homologous characteristic related to the size of each biomass that used in single or mixture, which is important for chemical compositions analysis and further experiments. To produce biomass mixtures, the selected biomass sources were combined in equal quantities (w/w) in each mixture: ([CH+CS, 50/50], [CH+CC, 50/50], [CS+CC, 50/50], [CH+CS+CC, 33.4/33.3/33.3). A total of 300 g of each biomass sample was treated with popping equipment at 1.47 MPa pressure and 150 °C temperature, as described by Choi et al. (Choi et al., 2012). The popping pretreatment instrument included a laboratory-scale cast iron cylindrical reactor with an inner horizontal cylinder of 300 mm and a total volume of 3 L, a gas heater, a hatch, and a mechanical rotator (Wi et al., 2011). Before the popping pretreatment, the biomass samples were mixed with water at a biomass/water ratio of 1:5 (w/v), and then transferred to a cylindrical reactor. The reactor was heated by a gas heater at a rate of 15–20 °C min^{-1}; a rotator ensured complete heat and pressure treatment of the biomass samples. During the popping process, the cylinder was tightly sealed by a hatch attached to the reactor, and temperature and pressure increases were observed by an automatic controller via a pressure gauge and temperature probe in the cylinder. The gas heater stopped at 1.47 MPa and 150 °C. At the end of the reaction, the hatch was quickly loosened to reduce the internal pressure and rapidly expose the sample to normal atmospheric pressure. Non-pretreated and popping-pretreated biomass samples were lyophilized at a temperature of −50 °C (Lyophilizer/Lyoph-Pride XP50, IlShin BioBase Co. Ltd, Korea) to reduce moisture content to 3 ± 0.2%.

2.2. Chemical composition analysis

Qualitative analysis for monosaccharide composition of the individual and mixed non-pretreated or popping-pretreated biomass samples was performed using gas chromatography (GC) (Choi et al., 2013). To release all monosaccharides from sugar polymers in the biomass, two-step acid hydrolysis was performed. The first hydrolysis step was performed by treating 30–50 mg of each sample with 0.25 mL of 72% sulfuric acid (H_2SO_4) for 45 min at 30 °C, followed by dilution with 67.9 mL of distilled water to produce 4% H_2SO_4. The second step was performed at 121 °C for 1 h in an autoclave machine. After cooling to room temperature, a known amount of internal standard (myo-inositol) was added, and then neutralized by ammonia solution. Then, 0.5 mL of the solution was transferred to a new glass tube and several steps were performed to analyze monosaccharide composition. A volume of 1 mL of sodium borohydride solution (dissolved in dimethyl sulfoxide [DMSO] at a concentration of 20 mg/mL) was added to the solution, which was then incubated at 70 °C for 1 h. After cooling, 0.1 mL of glacial acetic acid (18 M) was added to degrade the sodium tetrahydroborate. Next, 0.2 mL of methyl imidazole and 2.0 mL of anhydrous acetic acid were sequentially added. Finally, 5.0 mL of deionized water was added and extracted with 2.0 mL of dichloromethane. The samples were dried and analyzed using GC (GC-2010; Shimadzu, Otsu, Japan) with DB-225 capillary column (30 m × 25 mm i.d., 0.25 μm film thickness, J&W; Agilent, Folsom, CA, USA) containing helium. Detector temperature and injector temperature were set at 250 °C and 220 °C, respectively. Oven temperature was risen from 100 °C (1.5 min) to 220 °C at 5 °C min^{-1}. Compounds were measured by comparing retention times with those of standard compounds (Sigma).

2.3. Measure pore radius and volume of biomass samples by BET

Pore size and volume were measured using the Brunauer, Emmett, and Teller (BET) equation nitrogen adsorption-desorption isotherm at −196 °C in a surface-area analyzer (ASAP 2020, Micromerits Co., USA). Prior to the determination, the samples (~0.7 g) from individual and mixed, non-pretreated and pretreated samples were degassed for 1.5 h at 110 °C under vacuum to remove moisture and any other contaminants. The total pore volume of biomass samples was analyzed by converting the amount of nitrogen gas adsorbed to the volume (cm^3/g at STP – standard temperature & pressure = 0 °C and 760 mm Hg) of liquid adsorbate (Wi et al., 2015).

2.4. Selection of appropriate enzyme loading for enzymatic hydrolysis

Enzymes account for the major portion of the cost of lignocellulosic bioethanol production (Balat et al., 2008). To evaluate and measure appropriate enzyme loadings for enzymatic hydrolysis, various volumes of commercial cellulase (Celluclast 1.5 L) were loaded on 1% dry matter biomass (w/v), with addition of a determined amount of β-glucosidase (Novozyme 188). All of these enzymes were purchased from Novozymes A/S (Bagsvaerd, Denmark), and their enzymatic unit activity were determined by a National Renewable Energy Laboratory (NREL; 1996) method, with enzymatic unit activity of 0.122 filter paper units (FPU)/mg protein and 2.6 IU/mg protein for cellulase and β-glucosidase, respectively. Loading of 2.96, 5.91, 11.83, 17.74, and 23.65 mg/g biomass of cellulase (equal to 0.36, 0.72, 1.44, 2.16, and 2.89 FPU/g biomass, respectively), with a concentration of 2.1 mg/g biomass of β-glucosidase (5.46 IU/g biomass), were applied for enzymatic hydrolysis. For measurement, reducing sugars from all performed samples were first measured by a 3,5-dinitrosalicylic acid (DNS) reagent, and then selected samples which achieved high enzymatic hydrolysis yield would be measured via high-performance liquid chromatography (HPLC) by refractive index detector (2414; Water, Milford, MA, USA), REZEX RPM (Phenomenex, Torrance, CA, USA) column (300 × 7.8 mm) with program: 85 °C, flow rate of 0.6 mL/min. For dinitrosalicylic acid (DNS) method, enzymatic conversion yield (%) was calculated according to the rate of the total fermentable sugar (reducing sugar but nonspecific for each monosugar) after enzymatic hydrolysis to initial compositions of total sugar in each sample. With results from HPLC method, enzymatic

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