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Research paper

Evaluation of fuel ethanol production from aqueous ammonia-treated rice straw via simultaneous saccharification and fermentation

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ABSTRACT

Rice straw (RS) has been considered a promising feedstock for ethanol production in Asia. However, the recalcitrance of biomass, particularly the presence of lignin, hinders the enzymatic saccharification of polysaccharides in RS and consequently decreases the ethanol yield. Here, we used aqueous ammonia pretreatment to remove lignin from RS (aRS). The reaction conditions were a solid:liquid ratio of 1:12, an ammonia concentration of 27% (w w^{-1}), room temperature, and a 2-week incubation. We evaluated enzymatic digestibility and the ethanol production yield. A 42% reduction in lignin content increased the glucan conversion of aRS to glucose from 20 to 71% using a combination of Cellic Ctec2 cellulases and Cellic Htec2 xylanases at enzyme loads of 15 FPU +100 XU g^{-1} solid. Scanning electron microscopy highlighted the extensive removal of external fibres and increased porosity of aRS, which aided the accessibility of cellulose for enzymes. Using the same enzyme dosage and a solid load of 100 g L^{-1}, simultaneous saccharification and fermentation using a monoculture of *Saccharomyces cerevisiae* and co-culture with *Candida tropicalis* yielded ethanol concentrations of 22 and 25 g L^{-1}, corresponding to fermentation efficiencies of 96 and 86% fermentation, respectively. The volumetric ethanol productivities for these systems were 0.45 and 0.52 g L^{-1} h^{-1}. However, the ethanol yield based on the theoretical glucose and xylose concentrations was lower for the co-culture (0.44 g g^{-1}) than the monoculture (0.49 g g^{-1}) due to the low xylose consumption. Further research should optimise fermentation variables or select/improve microbial strains capable of fermenting xylose to increase the overall ethanol production yield.

1. Introduction

Ethanol production from lignocellulosic materials has been evaluated as a clean, renewable energy source that can reduce the dependency on fossil fuels and replace the use of petroleum-based fuels, particularly in the transportation sector. Potential sources of lignocellulose includes waste from the food industry, municipal waste, dedicated energy crops and agricultural by-products [1].

Rice straw (RS) is a lignocellulosic waste derived from rice grain collection. In Thailand, more than 37.8 million tons of RS are generated annually. Because of its low value, including its low protein content, high mineral content, high bulk density, and slow degradation in soil, farmers usually remove RS by fire before the next cultivation season [2,3]. The burning of RS is a poor agricultural practice because it generates soot and smoke, which leads to air pollution and negatively impacts human health. In addition, straw burning can reduce the nutrients and microbiota present in the soil [2]. Therefore, RS disposal needs to be improved to reduce the impact on the environment and human health.

To increase the value of RS and protect the environment, RS can be used as a feedstock for ethanol production because straw is a non-food, under-utilised and abundant resource, especially in Thailand and other Asian countries. According to its chemical composition, up to 70% of the dry biomass consists of polysaccharides, which represent a rich source of fermentable sugars [3]. The straw generally consists of 32–47% of cellulose and 13–27%

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of hemicellulose [4,5]. However, these polysaccharides are sealed with lignin, which provides resistance to microbial attack and enzymatic degradation [6]. Therefore, lignin needs to be removed to expose polysaccharides to saccharifying enzymes in order to yield sugars.

Ethanol production from plant biomass includes the enzymatic degradation of cellulose in biomass to glucose (saccharification) and glucose conversion to ethanol using common baker's yeast (ethanol fermentation). Thus, the final ethanol yield heavily depends on the availability of glucose in the fermentation tank. In practice, this availability is reduced by the recalcitrance of plant biomass (i.e., cellulose crystallinity, low accessibility of the surface area, structural heterogeneity and complexity of the cell wall composites, and degree of lignification) to enzymatic digestion [7,8]. In addition, lignin constitutes a significant physical barrier that hinders cellulose accessibility to cellulases and causes enzyme-lignin adsorption, which leads to high enzyme loads [6,9]. This high load results in inferior cellulose hydrolysis by enzymes, producing low sugar yields. Thus, this hydrolysis is the rate-limiting step for ethanol production. Therefore, the properties of feedstock should be modified prior to the subsequent saccharification and ethanol fermentation process.

Pretreatment is now considered a necessary step in lignocellulosic ethanol production. This approach aims to improve cellulose digestibility by removing non-cellulosic components and disorganising the crystalline structure at the macro- or micro-fibril level to create new polymer chains of cellulose. This approach also increases the porosity of the plant materials to allow enzymes to penetrate the cellulose fibres in order to aid enzymatic degradation [9,10]. From a technical and economic standpoint, a low-energy-input and inexpensive pretreatment approach that preserves carbohydrates (cellulose and hemicellulose) but not lignin is required [11]. Aqueous ammonia is an abundant and inexpensive chemical. Earlier studies show that, as a weak base, it selectively removes lignin from corn stover [12], barley hull [13], wheat straw [14], sugarcane bagasse [15], palm residue [16], switchgrass [17], miscanthus [18], and bamboo [19]. It is less corrosive to equipment than acids, such as sulphuric acid. One mechanism that explains the lignin removal by aqueous ammonia is the cleavage of C–O–C bonds in lignin as well as ether and ester bonds in the lignin–carbohydrate complex [12].

Because RS has been identified as an excellent feedstock for ethanol production in Asia and worldwide, numerous studies have examined this material, and several pretreatment strategies and process designs have been developed and proposed in recent years [5,20–24]. Ko et al. recently reported the successful aqueous ammonia pretreatment of RS [25]; specifically, 71.1% enzymatic digestibility and 83.1% ethanol yield was achieved by treating RS for 10 h at 69 °C with 21% ammonia (w w^{-1}). However, the enzymatic digestibility, overall ethanol yield and productivity likely depend on the biomass characteristics after pretreatment, which varies by method and pretreatment variables, such as the temperature, time, concentration of reagents/catalysts and biomass. In this study, we used commercial grade aqueous ammonia (27%, w w^{-1}), which is readily available for purchase, to remove lignin at room temperature. The pretreated RS was then subjected simultaneous saccharification and fermentation (SSF) to enzymatically digest cellulose (and xylan) and produce ethanol using mono- and co-fermentation systems. The results of these two systems were compared to previously reported results. Because aqueous ammonia pretreatment and second-generation lignocellulosic ethanol production are new technologies, this process needs to be characterised. This work demonstrates the utility of aqueous ammonia pretreatment under low severe conditions for ethanol production from RS using a simple methodology.

2. Materials and methods

2.1. Feedstock

RS (*Oryza sativa*) was collected from rice fields in the Ayutthaya Province of Thailand. The RS was air-dried and cut into small pieces (1–2 cm) using scissors prior to pretreatment.

2.1.1. Pretreatment

The RS was pretreated with aqueous ammonia. The pretreatment was carried out by soaking RS in 27% (w w^{-1}) ammonium hydroxide at a solid:liquid ratio of 1:12 at room temperature (25 ± 3 °C) for 14 days. After the pretreatment, the sample was filtrated to remove the liquid phase and the retrieved solid was washed with water to remove inhibitors/impurities. The biomass suspension was neutralised with 1 N HCl/NaOH and washed with water. The pretreated solid was dried at 60 °C and milled to a particle size of approximately 0.5 cm using a blender; this material was then used as a substrate for enzymatic hydrolysis and ethanol fermentation.

2.1.2. Composition analysis

The carbohydrate and lignin contents were determined according to the National Renewable Laboratory (NREL) Analytical Procedure [26]. A sugar-containing medium was subjected to acid hydrolysis, centrifuged and filtered through a 0.45 μm-syringe filter. This filtrate was then analysed using a high-performance liquid chromatograph (HPLC; Shimadzu, Japan) equipped with a Bio-Rad Aminex HPX-87P column (Bio-Rad Laboratories, CA) and a refractive index detector (Shimadzu RID-10A) to determine the carbohydrate content. The analytical column was operated at 85 °C using HPLC-grade water as a mobile phase at a flow rate of 0.6 mL min^{-1} and a run-time of 35 min. The lignin removal yield was calculated using the following equation [27]:

$$\text{Lignin removal yield } (\%) = 1 - \frac{\text{Lignin in the pretreated RS}}{\text{Lignin in the native RS}} \times 100\% \quad (1)$$

2.1.3. Scanning electron microscopy

The morphology of RS before and after pretreatment was observed using A JEOL JSM-6610LV (Tokyo, Japan) scanning electron microscope. The samples were gold-coated prior to imaging.

2.2. Enzymatic hydrolysis

The commercial cellulase (Cellic Ctec2) and xylanase (Cellic Htec2) preparations used in the present study were purchased from Novozyme (Bagsværd, Denmark). The enzyme activities were 148 filter paper unit (FPU) mL^{-1} and 1040 xylanase unit (XU) mL^{-1}, respectively. Enzymatic hydrolysis was conducted in a 50-mL falcon tube. Different doses of enzymes (FPU and/or XU g^{-1} solid) were loaded onto a 1.5 or 5.0% (w v^{-1}) solid at final volume of 10 mL. The hydrolysis reaction was performed at pH 4.8 using 0.05 M citrate buffer, 37 °C, and shaken at 200 rpm for 72 h. Sodium azide was added to the reaction at a final concentration of 0.2% to prevent microbial growth. Samples were removed at appropriate times to measure the released sugars. The glucose and xylose concentrations were determined using glucose and xylose assay kits (Megazyme), respectively.