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Anaerobic digestion as a pretreatment to enhance ethanol yield from lignocelluloses

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ABSTRACT

Biogas production process was used as a biological pretreatment for efficient ethanol production. Three types of lignocelluloses, including rice straw, hardwood sycamore, and softwood pine, were pretreated with anaerobic digestion (AD) process. The effects of ultrasonication on AD-treated and untreated lignocelluloses were studied prior to ethanol production by separate hydrolysis and fermentation (SHF). The pretreated and untreated samples were compared using compositional analysis, Fourier transform infrared spectroscopy, and scanning electron microscopy. The biological pretreatment significantly reduced the hemicellulose content of samples. Rice straw had the highest conversion of carbohydrate to biogas. Additionally, the highest ethanol yield after SHF of the straw was 69.5% with respect to the theoretical yield. This value was obtained after the pretreatment with AD process followed by 1 h ultrasonication at 60 °C. As a pretreatment, the application of AD process increased the overall production of biofuels, expressed as gasoline equivalent volume, by 3.6–4.6 fold. The gasoline equivalent volumes were improved from 75, 51 and 29 mL for untreated straw, sycamore and pine, respectively, to 260, 204 and 130 mL for the pretreated materials. About 35% of these volumes are attributed to methane.

1. Introduction

Biofuels produced from lignocellulosic biomass are viable alternatives to fossil fuels to reduce greenhouse emissions and improve energy security. Lignocelluloses are abundant and low-cost feedstocks, and unlike starch- and sugar-based raw materials, their use in ethanol production does not reduce the human food supply [1–3]. The main drawback of these materials is their recalcitrant structure, which resists microbial attacks and biological conversion [4,5]. In order to open up the structure of lignocelluloses and improve their biological conversion in subsequent steps, i.e., enzymatic hydrolysis, a pretreatment process is required. Pretreatment methods are classified into physical, chemical and biological techniques [6]. Physical pretreatment methods have high-energy demands, whereas chemical methods require expensive chemicals. Pretreatments with ionic liquids, NMMO, NaOH, and phosphoric acid are among the most efficient methods but require expensive chemicals [7]. Furthermore, chemical processes generate waste, for which waste disposal costs are imposed. Production of inhibitors such as furans is another disadvantage of chemical pretreatment methods [8–11].

Biological pretreatment methods are safer and more environmentally friendly processes compared to the first two treatments. Biological methods require less energy to enhance the structure of lignocellulosic biomass [8,10,12]. These methods have been studied using different types of microorganisms, mainly white-rot fungi, with the aim of lignin removal [13–18]. However, low ethanol yield and significant carbohydrate loss during fungal pretreatment reduces the overall efficiency of the process [8,12].

In this study, anaerobic digestion (AD) was applied as a pretreatment process. The main advantages of AD as a pretreatment are enhancement of subsequent enzymatic hydrolysis, production of biogas, i.e., a biofuel, and no need for an additional process for pentose fermentation. Hemicellulose removal has been proven to enhance the enzymatic hydrolysis of lignocellulosic feedstocks [11,19–21]. AD consists of 4 main stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Unlike ordinary AD, in which methanogenesis is the rate-limiting stage, in AD of lignocelluloses, hydrolysis reactions are the slowest [22]. Furthermore, hydrolysis of cellulose is slower than that of hemicelluloses due to its more compact and recalcitrant structure [22]. Based on prelimi-

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nary experiments in this study, complete AD until no more biogas is produced, i.e., for more than 40 days, results in partial conversion of cellulose to methane, as well as nearly complete conversion of hemicelluloses. Thus, incomplete AD is preferred to maintain higher amounts of cellulose, while subsequent enzymatic hydrolysis is also enhanced. Another advantage of AD pretreatment is the production of methane from pentoses. Pentoses are not fermentable by industrially available yeast cells [3,6]. The amount of biofuel produced by the integrated process, i.e., AD as pretreatment and ethanol fermentation, is greater than that from only one process for ethanol production. To our knowledge, there is no previous report on incomplete AD as a pretreatment step for ethanol production.

This study focuses on partial AD as a biological pretreatment method of 3 types of lignocellulosic material, namely, rice straw, sycamorewood and pinewood. After biological treatment and ultrasonication of feedstocks, enzymatic hydrolysis and fermentation were performed to investigate the effects of pretreatments.

2. Materials and methods

2.1. Raw materials and microorganisms

Rice straw was received from the Babolsar paddy fields (Mazandaran Province, Iran). Pinewood was obtained from the Isfahan University of Technology forest conservation area (Isfahan, Iran), and sycamorewood was received from Delijan (Markazi Province, Iran). Pine and sycamore woods were cut into chips. Then, all three raw materials were milled and screened to achieve particle sizes of less than 1 mm. The inoculum was obtained from a 7000 m^3 anaerobic digester of a municipal wastewater treatment plant operating at mesophilic conditions (Isfahan, Iran). Dry weight of the lignocelluloses and the inoculum was measured by oven drying at 105 °C.

2.2. Pretreatment

Biological pretreatment was performed in 118 mL digesters filled with 40 mL of inoculum, 0.5 g of the substrate and 10 mL of deionized water. Butyl rubber and aluminum caps were used to seal the digesters. In order to provide anaerobic conditions, the digesters were purged for about 2 min with a gas containing 99% nitrogen. The digestion process was performed at mesophilic conditions (37 °C) for 27 days (optimum digestion time obtained according to the preliminary experiments) [23]. In addition, control digesters containing inoculum and water were used in order to determine the biogas production of the inoculum. Gas samples were taken periodically from the generated biogas and analyzed by gas chromatography to determine the amount of produced methane and carbon dioxide. At the end of the pretreatment, the bottles' contents were filtered in order to recover the digested fibers and rinsed with water. Then the pretreated samples were dried at room temperature for 3 days and placed in sealed bags until use.

2.3. Enzymatic hydrolysis

Two commercial cellulases, Cellic® CTec2 (VCNI0013, Novozyme, Bagsværd, Denmark) and Cellic® HTec2 (VHN00002, Novozyme, Bagsværd, Denmark), kindly provided by Novozyme, were used for enzymatic hydrolysis. Cellulase activity of Cellic® CTec2 and Cellic® HTec2 were measured by the method presented by Adney and Baker [24] at 125 and 23 FPU/mL, respectively. A mixture of 90% v/v Cellic® CTec2 and 10% v/v Cellic® HTec2 was used in all hydrolysis experiments.

Bottles with total volume of 118 mL containing 0.3 g of untreated or biologically pretreated substrates in 30 mL of 50 mM sodium citrate buffer (pH = 4.8) were prepared. The batch reactors were autoclaved at 121 °C for 20 min and then 0.5 g/l sodium azide was added to each reactor to prevent bacterial contamination. Hydrolysis was conducted at 45 °C and 120 rpm for 72 h using 30 FPU/g cellulase and 50 IU/g β-glucosidase. Liquid samples were taken at 0, 24 and 72 h of hydrolysis and were analyzed to determine their sugar contents. A parallel set of hydrolysis was performed without addition of the antibacterial agent and without sampling. This set was then used for fermentation experiments [2].

2.4. Effect of ultrasonic irradiation

In a series of experiments, the effect of ultrasonic irradiation prior to enzymatic hydrolysis was evaluated to improve the hydrolysis of the pretreated and untreated substrates. The prepared 118 mL bottles containing 0.3 g of the substrates in 30 mL of 50 mM sodium citrate buffer (pH = 4.8) were subjected into an ultrasonic bath (SONICA 3200L ETH, Milan, Italy). The materials were treated at 0 and 60 °C for 0, 15 and 60 min. Input power and frequency of the ultrasonicator were 355 W and 45 kH, respectively. Then the bottles were subjected to enzymatic hydrolysis similar to the method for non-sonicated substrate (cf. Section 2.3).

2.5. Fermentation

Fermentation was performed using a flocculating strain of *Saccharomyces cerevisiae* (CCUG 53310, Culture Collection, University of Gothenburg, Sweden). The yeast maintenance and biomass production were conducted according to the method presented by Shafiei et al. [2].

An amount of 20 mL of hydrolysate with a culture medium containing 5 g/l yeast extract, 7.5 g/l $(NH_4)_2SO_4$, 3.5 g/l K_2HPO_4, 0.75 g/l $MgSO_4{\cdot}7H_2O$ and 1 g/l $CaCl_2{\cdot}2H_2O$ was mixed in glass bottles, according to method presented by Goshadrou et al. [25]. All mixtures were autoclaved and after cooling to room temperature, 1 g/L of yeast (based on dry weight) was added to each bottle. The fermenters were incubated at 37 °C and 130 rpm for 24 h. Liquid samples were periodically taken and stored frozen before being analyzed for sugar and metabolite contents.

2.6. Mass balance

The efficiency of pretreatment on the raw materials was determined by comparison of the amount of produced biofuels. Total amount of methane and ethanol produced during pretreatment and fermentation, respectively, were calculated. These values were converted to their equivalent gasoline volumes by considering the lower heating values of the fuels at 25 °C and 1 atm. The lower heating values for ethanol, methane and gasoline were 27.0 MJ/Kg, 32.8 MJ/m^3 and 31.7 MJ/L, respectively [26].

2.7. Analytical methods

Ash, total solids, volatile solids, carbohydrates and lignin contents of the untreated and biologically pretreated lignocelluloses were determined according to NREL analytical procedures [27,28].

A gas chromatograph (SP-3420A, thermal conductivity detector, Beijing Beifen Ruili Analytical Instrument Co., Beijing, China) equipped with a packed column (Porapack Q column, Chrompack, Engstingen, Germany) was used to analyze the methane and carbon dioxide generated in the pretreatment step. The carrier phase was nitrogen with a flow rate of 25 mL/min. Temperatures of the column, injector and detector were set to 50, 90 and 140 °C, respectively.

Sugar contents were analyzed by high performance liquid chromatography (HPLC) equipped with UV/vis and RI detectors (Jasco International Co., Tokyo, Japan) on an ion exchange Aminex HPX-87