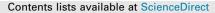
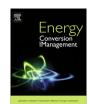
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Optimization of twin gear-based pretreatment of rice straw for bioethanol production

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ABSTRACT

A laboratory twin-gear reactor (TGR) was investigated as a new means for the pretreatment of high solid lignocelluloses. Response surface methodology based on Box Behnken Design was used to optimize the enzymatic digestibility with respect to the pretreatment process variables: temperature of 50–90 °C, NaOH concentration of 2–6% and no. of cycles of 30–60. The results revealed that the TGR-based pretreatment led to the significant structural alterations through increases in pore size, pore volume, cellulose crystallinity and surface area. SEM images also confirmed the surface modifications in the pretreated rice straw. A response surface quadratic model predicted 90% of the enzymatic digestibility, and it was confirmed experimentally and through the analysis of variance (ANOVA) as well. The TGR extrusion proved to be an effective means for exceedingly high solids lignocellulose.

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1. Introduction

Among potentially sustainable feedstock candidates for liquid fuel production, lignocellulose is only a practical option at the present time [1]. Due to the nature of any biofuel, the use of lignocellulosic biofuels, in comparison with conventional fuels, can reduce the emission of greenhouse gases even up to 86% [2]. Lignocellulose is a multicomponent composite comprised of fermentable polysaccharides surrounded by non-fermentable lignin.

In its native form, glucose-based cellulose and C5 sugar-based hemicellulose constitute major fractions of the composite which is structurally reinforced by lignin in an intertwined and complicated way [3]. It is this complex and strong structure that renders the plant a resistance not only in its natural settings but also in bioprocessing. Its crystalline feature makes it harder to process the already persistent material by way of interfering with enzyme adsorption and subsequent hydrolysis [4].

Therefore, pretreatment, a step to sufficiently weaken the feedstock, is unavoidable prior to the subsequent processing [5]. Thus far, various strategies employing alkaline, acidic, steam explosion, organasolve, hot water and ionic liquids have been investigated [6,7]. These commonly practiced pretreatment technologies often consist of two steps, size reduction and ensuing

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http://dx.doi.org/10.1016/j.enconman.2016.06.022 0196-8904/© 2016 Elsevier Ltd. All rights reserved. thermo-and/or- chemical treatment, as a single step only is less effective. These methods also suffer from inefficiency associated with small capacity, batch processing and low substrate loading [8].

Recently extrusion has received increasing attention as a promising means of pretreatment because it offers mechanical, chemical and thermal powers in a simultaneous manner [9]. The shear force, generated during extrusion, reduces the particle size and provides efficient heat transfer and better mixing thereby leading to enhanced sugar recovery [10,11]. Extrusion assisted physiochemical modification of lignocelluloses is possible even at mild conditions, ending in fewer inhibitory products [12]. This approach has been applied to various biomass types such as miscanthus, rapeseed straw, corn stover, soybean hulls, rice straw, prairie cord grass, switchgrass, sugarcane baggase, barley straw and wheat straw [13,14].

In all of the aforementioned works, the biomass slurry was passed through the extruder only once or thrice. So, in order to benefit from synergetic effects of alkali assisted extrusion of high solids through the reactor we developed a laboratory scale twin gear reactor. To make best use of its potential, desired high loading slurry was subjected time and again to the high mechanical shear that enables to overcome innate limitations that a thick biomass slurry causes, such as slow catalyst diffusion, energy intensive mixing and handling difficulty.

In this study, therefore, the clearly advantageous extrusion approach was explored using a laboratory TGR, with which

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exceedingly high solids loading of 25% [15,16], higher than a general value of 15% [17], was tested in terms of surface alterations and enzymatic digestibility of rice straw. Furthermore, simultaneous saccharification and fermentation also yielded considerable amounts of bioethanol from the pretreated biomass.

2. Methods

2.1. Sample preparation

Rice straw was collected from the Nonsan city in South Korea. It was air-dried, chopped and ground to a particle size of greater than 2 mm. Composition analysis revealed that the biomass had 35.3% glucan, 18.5% xylan, 10.8% ash and 16.9% lignin on dry weight basis.

2.2. Twin gear pretreatment

Extrusion was carried out with the help of a lab scale intermeshing co-rotating twin gear reactor (TGR). This TGR had a barrel length to screw diameter ratio of 10:2. It was connected with a 7.5 hp motor to drive its twin gears. Reaction temperature was controlled by an electric heating throughout the whole barrel. A biomass slurry, which was fed manually, was passed through the reactor with various cycles. The mean residence time for each cycle was approximately 2 s. After the intended pretreatment operation the biomass was washed and collected by filtration. The solid phase was then dried in an oven at 50 °C for overnight prior to the subsequent processing.

2.3. Enzymatic digestibility

An enzymatic hydrolysis experiment was performed in a 250 ml Erlenmeyer flask containing 50 mM sodium citrate buffer at 5% solids loading. A novel commercial enzyme blend Cellic C-Tec (Novozymes, Denmark) derived from *Trichoderma reesei* with an activity loading of 30 FPU/g cellulose was used for the enzymatic hydrolysis. The flask was incubated at 50 °C and 200 rpm for 72 h in a shaking incubator. All experiments were conducted in triplicate.

2.4. Ethanol production

Saccharomyces cerevisiae was used in fermentation experiments. Yeast malt agar plates prepared from a solution of peptone 5 g/L, agar 20 g/L, malt extract 3 g/L, glucose 10 g/L and yeast extract 3 g/L were used to grow the yeast cells. After 72 h, some colonies were transferred into an inoculation medium, consisting of peptone 5 g/L, glucose 30 g/L and yeast extract 3 g/L, and grown at 37 °C and 200 rpm. After 48 h of growth time, the actively growing cells were harvested and used as an inoculum in fermentation process. The fermentation medium was prepared with 5 g/L each of KH₂PO₄, peptone, yeast extract and 4 g/L MgSO₄. Sodium citrate and citric acid were used as a buffer to maintain pH in a range of 4.8–5.1. A small amount of trace elements in mg/L was also added, consisting of 270 mg ZnCl₂, 1.5 mg FeCl₂·4H₂O, 36 mg H₃BO₃, 100 mg MnCl₂·4H₂O, 2 mg CuCl₂·2H₂O, 190 mg CoCl₂·6H₂O, 36 mg Na₂MoO₄·2H₂O and 240 mg NiCl₂·6H₂O.

The simultaneous saccharification and fermentation experiment was performed in a 250-mL Erlenmeyer flask, provided with a cap and needle arrangement to remove CO_2 , in a shaking incubator at 30 °C and 170 rpm for 72 h. Rice straw was loaded into this flask at 100 g/L of solids loading along with 90% of fermentation medium and 10% of inoculum. For the sake of comparison, an untreated control was also subjected to the fermentation along

with the treated rice straw. The samples were withdrawn periodically and saved in refrigerator at 4 °C for analysis.

2.5. Composition analysis

For the chemical compositional analysis, National Renewable Energy Laboratory (NREL) protocol of two step acid hydrolysis was employed [18]. In the first step, 0.3 g of extruded biomass was treated with 72% H_2SO_4 and incubated at 30 °C in a shaking incubator for 2 h. In the second step, it was diluted to 4% H_2SO_4 and autoclaved at 121 °C for 1 h. A sample of aliquot was taken from this hydrolysis solution, filtered and analyzed by HPLC. The remaining solid was separated by filtration, dried and burned in a muffle furnace at 575 °C for lignin analysis. Monomeric sugars and ethanol concentrations were quantified by HPLC equipped with a reflective index detector (Agilent 1200 series, USA) and a HPX-87 H column (Bio-Rad, USA). Temperature during the analysis was maintained at 65 °C and the mobile phase containing 4 mM H_2SO_4 was flowed at a rate of 0.6 mL/min.

2.6. Surface features of rice straw

Physical characteristics of raw and pretreated biomass were examined after drying solid residue parts at 50 °C for overnight. Pore size and specific surface area were measured by a BET-N₂ surface analyzer (Beckman Coulter SA3100). Crystallinity index (CrI) was determined by measuring X-ray diffraction (XRD) using a powder X-ray diffractometer (Rigaku, D/Max-2500, Japan). A sample was scanned at a rate of 2°/min in the 20 range of 3–60° and CrI was estimated according to Eq. (1)

CrI (%) =
$$(I00_2 - I_{am}/I00_2) \times 100$$
 (1)

where $I00_2$ and I_{am} are the intensity for the peak at $2\theta = 22.12$ and I_{am} is the intensity of $2\theta = 15.79$.

Microstructures and the surface property of both the untreated and treated samples were observed using scanning electron microscopy (SEM) (Hitachi S-4800) at an accelerated voltage of 1.0–5.0 kV. The samples were prepared by air drying and covering them with a thin layer of platinum before making the final analysis. Digital images were obtained at magnification of 50 μ m and 100 μ m.

2.7. Experimental design

Response surface methodology (RSM) was used to attain the optimal pretreatment conditions aiming at the highest enzymatic digestibility (Y). Three process variables reaction temperature (X₁), catalyst concentration (X₂) and no. of cycles (X₃) were optimized. Ranges of tested variables were as follows: $50-90 \degree C$ for temperature, 2–6% for NaOH concentrations and 30–60 for no. of cycles. All the experiments were performed with 25% solids loading and at a fixed 82 rpm of the twin gear reactor. Box-Behnken design (BBD) was used to design the experiments and all the experiments were performed in triplicate. Data analysis was done with the help of Design Expert software (Version 8.0, State-Ease, Inc., USA) [19].

3. Results and discussion

3.1. Effect of twin gear pretreatment

Pretreatment of rice straw using a twin gear extruder was executed at various conditions, and the results, particularly in terms of solids composition and lignin removal are summarized in Table 1. Glucan recovery ranged from 40.86% to 63.16% during

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