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Improvement of acetone, butanol and ethanol production from rice straw by acid and alkaline pretreatments

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HIGHLIGHTS

- ABE production from rice straw was significantly improved by alkali and acid pretreatments.
- After pretreatments, over 67% of glucan and 17% of xylan were recovered.
- After pretreatment, more than 163 g glucose was produced from each kg of rice straw.
- More than 44 g butanol and 17 g acetone were produced from each kg of rice straw.

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ABSTRACT

Rice straw was hydrolyzed and fermented to acetone, butanol, and ethanol by *Clostridium acetobutylicum* bacterium. Concentrated phosphoric acid and alkaline treatment with NaOH were used for pretreatment of the straw prior to enzymatic hydrolysis using commercial cellulase and β-glucosidase. The enzymatic hydrolysates were then anaerobically fermented by *C. acetobutylicum*. Hydrolysis of the alkaline pretreated straw resulted in production of 163.5 g glucose from each kg of untreated rice straw which was then fermented to 45.2 g butanol, 17.7 g acetone, and 1.2 g ethanol. Additionally, concentrated phosphoric acid pretreatment and subsequent hydrolysis resulted in production of 192.3 g glucose from each kg straw from which 44.2 g butanol, 18.2 g acetone, and 0.6 g ethanol were produced after 72 h fermentation. Increasing the produced ABE from less than 10 g to higher than 62 g from each kg straw by the treatments suggested the alkaline and phosphoric acid pretreatments as promising processes for efficient production of ABE from rice straw.

1. Introduction

Butanol, main product of acetone butanol ethanol (ABE) fermentation, is a solvent, a chemical intermediate, an extractant, and more importantly a potential biofuel [1]. By 1950, ABE fermentation by solventogenic *Clostridium* species using corn starch and molasses as substrates was one of the main fermentative products in industrial scale [2]. However, many ABE plants were closed during the 1960s as a result of increased price of corn and molasses and availability of cheaper petrochemical derived butanol [2]. Growing concerns regarding volatility of oil supply and global warming result in recent expansion in research relating to ABE fermentation for production of butanol which has unique characteristics as a potential biofuel [1].

Even though biological production of butanol through ABE fermentation is challenging due to its commercial obstacles, interesting attributes of biobutanol as a biofuel represents its potential new market [3]. Not only biobutanol is less volatile, explosive, corrosive, and hygroscopic than ethanol, but also it has more energy content and could easily mix with gasoline in any portion [3].

Considering the important effects of substrate cost on economical feasibility of biobutanol production, efficient utilization of lignocellulosic wastes instead of costly food-based substrates, e.g., corn and molasses, was suggested to make the butanol production economically viable [4,5].

A number of lignocellulosic residues such as wheat straw [6], barely straw [7], corn stover [8], and switchgrass [8] have previously been used for biobutanol production. Among agricultural wastes, rice straw is one of the low cost and mainly useless lignocellulosic materials that may properly be used for biological

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butanol production. Additionally, rice straw is potentially one of the most favorable feedstocks in terms of quantity as a substrate for biological products [9].

Solventogenic *Clostridia* used in ABE fermentation are able to ferment a wide variety of carbohydrates including lactose, sucrose, glucose, fructose, mannose, dextrin, starch, xylose, arabinose, and inulin [4]. Therefore, the prerequisite in the utilization of lignocelluloses for butanol production is to prepare a hydrolysate rich in fermentable sugars. Application of enzymes for the hydrolysis of lignocellulose offers several advantages of higher yields, minimal byproduct formation, low energy requirements, mild operating conditions, and environmentally friendly processing over the other chemical conversion routes. However, the recalcitrant structure of the native straw, similar to other lignocelluloses, makes its enzymatic hydrolysis inefficient [10]. Dilute sulfuric acid pretreatment, which is among the best pretreatment methods for improvement of cellulosic ethanol production, has recently been used for improvement of ABE fermentation from lignocellulosic resources [6,7]. However, generation of different byproducts in this process has inhibited the ABE producing microorganism growth and fermentation [8]. Overliming of the dilute acid pretreated hydrolysates was also applied to reduce the negative effects of the inhibitors; however, lime treatment of the hydrolysates resulted in reduction of the produced sugars levels and also inefficient fermentation [8]. Furthermore, steam explosion without addition of any acid was also used for pretreatment of ABE production from lignocelluloses. However, the explosion process should be conducted at high pressures and temperatures [11].

Different pretreatment processes have been developed for improvement of ethanol production from lignocelluloses [10]. Alkaline pretreatment is one of the most promising technologies for improvement of agricultural residues hydrolysis. The process resulted in increasing the swelling capacity and internal surface area, decreasing cellulose crystallinity and degree of polymerization, and also disrupting the compact lignin-carbohydrate structure [12]. On the other hand, the concentrated phosphoric acid pretreatment at modest reaction conditions has been recently demonstrated to be an effective method for improvement of hydrolysis of lignocelluloses [13]. These pretreatment technologies may also be applied prior to ABE fermentation. To our knowledge, there is no publication on utilization of these pretreatments prior to ABE fermentation.

In the current study, alkaline and concentrated phosphoric acid pretreatments were evaluated for production of acetone, butanol, and ethanol from rice straw. The pretreated straw was hydrolyzed by two commercial hydrolytic enzymes and fermented by *C. acetobutylicum*.

2. Materials and methods

2.1. Rice straw and enzymes

Rice straw used in all the experiments was obtained from Lenjan field (Isfahan province, Iran). It was dried at 60 ± 5 °C for 1 day. The straw with original length between 20 and 50 mm was partially ball-milled and screened to achieve a size of less than 833 μm (20 mesh) and larger than 299 μm (48 mesh) prior to the enzymatic hydrolysis.

Two commercial enzymes, cellulase (Celluclast 1.5L, Novozyme, Denmark) and β-glucosidase (Novozyme 188, Novozyme, Denmark), were used for hydrolysis. The cellulase activity was 80 FPU/ml, measured by the method presented by Adney and Baker [14] and it contained 42 mg/ml protein, as measured by Bradford assay [15]. The β-glucosidase activity was 240 IU/ml according to method presented by Ximenes et al. [16].

2.2. Pretreatment methods

Prior to enzymatic hydrolysis, alkaline and concentrated phosphoric acid pretreatments were used. Alkaline pretreatment was performed using 12% w/v NaOH with 5% w/w solids loading at 0 °C for 3 h. After the pretreatment, the mixture was washed with distilled water until pH 7 was reached. The solid was then filtered and dried at 50 °C [17].

Concentrated phosphoric acid pretreatment was performed by mixing one gram straw with 8 ml of H_3PO_4 (85%) at 50 °C for 30 min. Pre-cold acetone was consequently added for quenching the mixture. After centrifugation for 10 min, the supernatant was collected. The solid pellet was suspended in 40 ml acetone and centrifuged three times. The precipitated solids were consequently washed three times with excess distilled water and centrifuged [13]. The treated solid was then dried at 50 °C.

2.3. Enzymatic hydrolysis

Pretreated and untreated rice straws were enzymatically hydrolyzed in 50 mM sodium citrate buffer (pH 4.8) using cellulase and β-glucosidase. The straw was soaked in the buffer for 4 h prior to enzymatic hydrolysis. Hydrolysis was performed at 140 rpm and 45 °C with 2% solid loading for 72 h. The enzyme loadings were 25 FPU cellulase and 50 IU β-glucosidase per gram of biomass.

2.4. Microorganism and inoculum preparation

2.4.1. Clostridium acetobutylicum

NRRL B-591 was obtained from Persian type culture collection (PTCC) (Iranian Research Organization for Science and Technology, Tehran, Iran). The culture was stored in sterile distilled water at 2–4 °C. In order to prepare the inoculum, 2.5 g cooked meat medium (Sigma–Aldrich) was soaked in 15–18 ml distilled water. After addition of 0.2 g glucose, the prepared medium was autoclaved at 121 °C for 20 min and cooled to 75 °C. One half ml of spore suspension was added to the medium and heat shocked at 75 °C for 2 min, and the mixture was then cooled in ice-cold water for 1 min. The heat shocked spores were then incubated in an anaerobic jar at 35 °C for 24 h. For cultivation of the strain, a 100 ml medium containing 30 g/L glucose, 5 g/L yeast extract, 2 g/L ammonium acetate, 1 g/L sodium chloride, 0.75 g/L KH_2PO_4, 0.75 g/L K_2HPO_4, 0.2 g/L $MgSO_4$, 0.01 g/L $MnSO_4{\cdot}7H_2O$, and 0.01 g/L $FeSO_4{\cdot}7H_2O$ was prepared and autoclaved at 121 °C for 20 min. After cooling to 35 °C, 0.5 g/L cysteine $HCl{\cdot}H_2O$ was filtered (0.45 μm Millipore filter, GEMA Medical SL) and added to the medium. About 5 ml of the prepared bacterial culture was then added to the medium and the growth was conducted at 37 °C for 24 h [18].

2.5. ABE fermentation

Fermentation of 50 ml of the enzymatic hydrolysate was performed in 118 ml serum bottles (717561, Pajuhesh Setayesh Sepahan, Isfahan, Iran) sealed with butyl rubber and aluminum crimp cap. After addition of 0.05 g yeast extract to each bottle, pH of the solutions was adjusted to 6.5 using 5 M NaOH. These solutions were sterilized at 121 °C for 20 min followed by cooling to room temperature. Prior to inoculation, 0.5 ml of P2 stock solution was filter sterilized (Millipore filter; 0.22 μm) and added to each bottle. P2 stock solutions contained a buffer (50 g/L KH_2PO_4, 50 g/L K_2HPO_4, and 220 g/L CH_3COONH_4), vitamin (0.1 g/L para-aminobenzoic acid, 0.1 g/L thiamin, and 0.001 g/L biotin), and mineral (20 g/L $MgSO_4{\cdot}7H_2O$, 1 g/L $MnSO_4{\cdot}H2O$, 1 g/L $FeSO_4{\cdot}7H_2O$, 1 g/L NaCl) solutions [19]. The bottles were then inoculated with 6 ml of actively growing culture (optical density 1.2–1.6 at 610 nm). These bottles were sparged with pure nitrogen in order to provide