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Evaluation of pretreatment methods for enzymatic saccharification of wheat straw for bioethanol production

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ABSTRACT

Pretreatment is an essential step in the enzymatic hydrolysis of biomass and subsequent production of bioethanol. The current study is focused on two different pretreatment methods of wheat straw using mild temperatures (100 °C for 2 h and RT for overnight). In one method, native substrate was treated with 1.5% (w/v) NaOH at two different above mentioned conditions followed by acid hydrolysis (0.75% (v/v) sulfuric acid at 100 °C for 2 h). In another method, the native substrate was initially treated with acid (0.75% (v/v) sulfuric acid at 100 °C for 2 h) followed by treatment with 1.5% (w/v) NaOH at two different above conditions. After the pretreatments, the residues were treated with Accellerase 1500 (26 U/g) and maximum yield of glucose (65.2 g L^{-1}) were found with 0.75% sulfuric acid (100 °C for 2 h) followed by alkali (1.5% NaOH at 100 °C for 2 h). Fermentation of this hydrolyzate using *Saccharomyces cerevisiae* strain produced 24.4 g L^{-1} of ethanol with corresponding yield of 0.44 g/g.

1. Introduction

Ethanol from renewable resources has been of interest in recent decades as an alternative fuel to the current fossil fuels. Its production has gained importance in the last few years due to the increased dependency on oil and conventional fuels (Ramanathan, 2000). At present ethanol is produced from molasses. The cost of production increases as the demand for molasses has increased. Hence, it is necessary to search for alternate source for ethanol production. Lignocellulosic biomasses like wood and agricultural crops are abundantly available having rich source of sugars e.g., straw and sugar beet pulp which are potential raw materials for producing several high-value products like fuel ethanol and biodiesel. Up to 80% of the lignocelluloses are polysaccharide. The cost of ethanol production from lignocellulosic materials is relatively high based on current technologies, and the main challenges are the low yield and high cost of the hydrolysis process. Considerable research efforts have been made to improve the hydrolysis of lignocellulosic materials (Sun & Cheng, 2002). The current research investigates the use of acid and enzymes to saccharify lignocellulosic materials and to produce glucose from pretreated lignocellulosic materials, to be a source for ethanol production.

Lignocellulosic material consists of mainly three different types of polymers, namely cellulose, hemicellulose and lignin, which are associated with each other. Wheat straw is the most attractive low cost feedstock for production of fuel alcohol because of abundance, renewability and low lignin content. It has a huge potential for bioethanol production. Wheat straw is an abundant by-product from wheat production, which is annually generated worldwide (529 million tons/year). Asia is the first largest producing region with 43% of global wheat production. The average yield of wheat straw is 1.3–1.4 kg per kg of wheat grain. Wheat straw has to be pretreated before enzymatic hydrolysis since it contains lignin and hemicellulose that protect the cellulose. Research has been done on the separation of cellulose, hemicellulose and lignin components from wheat straw and structural characterization of the hemicellulose fraction (Sun & Cheng, 2002) and also the production of ethanol from wheat straw hydrolyzates (Klinke, Olsson, Thomsen, & Ahrin, 2003; Nigam, 2001).

Pre-treatment disrupts the naturally resistant carbohydrate–lignin shield that limits the accessibility of enzymes or chemicals to the cellulose and hemicellulose. The main goal of pretreatment is to increase the enzyme accessibility and improving digestibility of cellulose (Mosier et al., 2005). Each pretreatment has a specific effect on the cellulose, hemicellulose and lignin fraction thus, different pretreatment methods and conditions should be chosen according to the process configuration selected for the subsequent hydrolysis (Alvira, Tomás-Pejó, Ballesteros, & Negro, 2010).

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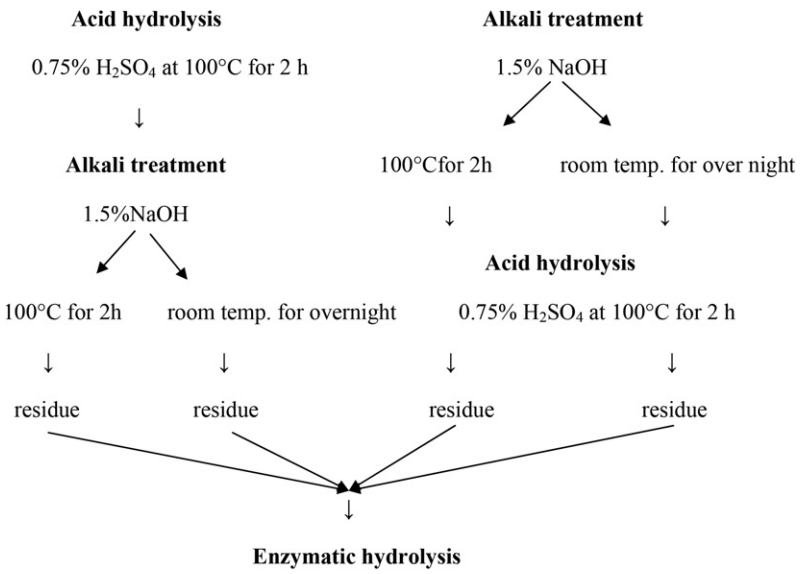

Fig. 1. Flow chart for pretreatment by acid followed by alkali and alkali followed by acid at different conditions using wheat straw.

Acid hydrolysis removes the hemicellulosic portion and some fraction of lignin the remainder of the lignin remains intact to the cellulosic substrate (Kaya, Heitmann, & Thomas, 2000). During enzymatic hydrolysis of lignocellulosic biomass, cellulase components, β-glucosidase and endoglucanase have more binding affinity towards lignin than to the carbohydrates, resulting in a lower efficiency of saccharification. Hence, to achieve maximum hydrolysis of cellulosics, which is a prerequisite for ethanol fermentation, an appropriate delignification treatment of biomass is required (Rishi, Krishna Kant, & Kuhad, 2009).

Alkali treatment disrupts the cell wall through dissolving hemicellulose, lignin, silica, and hydrolyzing uronic and acetic acid esters. Alkali swells cellulose, decreasing the crystallinity of cellulose (Sun, Lawther, & Banks, 1995). All of the ester-linked substituents of the hemicellulose and other cell wall components can be cleaved by alkali (Ternrud, 1987).

Enzymatic hydrolysis of cellulose to glucose is carried out by cellulase enzymes which are highly specific catalysts. The conversion of native cellulose to sugar can be achieved by applying different pretreatment methods namely chemical, acid and enzyme (Waleed et al., 2011). It is necessary to increase the rate of hydrolysis of cellulose to fermentable sugars. Enzyme hydrolysis is performed under mild conditions (e.g., pH 4.5–5.0 and temperature 40–50 °C) and it is possible to obtain cellulose hydrolysis close to 100% (Ogier, Ballerini, Leygue, Rigal, & Pourquie, 1999). Therefore one may expect low corrosion problems, low utility consumption, and low toxicity of the hydrolyzates which is the main advantage of this process (Lee, Iyer, & Torget, 1999; Taherzadeh, 1999). Addition of surfactants during hydrolysis can modify the cellulose surface properties. Among the different surfactants, fatty acid esters of sorbitan polyethoxylates (Tween® 20 and 80) and polyethylene glycol are reported as most effective for enzymatic hydrolysis (Kim, Kim, & Kim, 2006; Börjesson, Peterson, & Tjerneld, 2007).

Several species of bacteria and fungi are able to produce cellulases and hemicellulases (Sun & Cheng, 2002). Fungi like *Trichoderma reesei* or *Trichoderma viride* have been the most broadly studied and best characterized and the best vehicles for cellulase production (Tengborg, Galbe, & Zacchi, 2001; Xia & Shen, 2004). A full complement production of cellulase, stability under the enzymatic hydrolysis conditions, and resistance of the enzyme to chemical inhibitors are the advantages of the cellulase produced by *Trichoderma* (Hari Krishna, Janardhan Reddy, & Chowdary, 2001; Itoh, Wada, Honda, Kuwahara, & Watanabe, 2003). Fuel ethanol production from plant biomass hydrolyzates by *Saccharomyces cerevisiae* is of great economic and environmental significance. The demands on the microorganisms that perform this reaction are more complicated than those for conventional ethanol production from hexoses or their disaccharides, which uses exclusively *Saccharomyces* yeasts (Klinke et al., 2003).

In the present study, effect of pretreatment conditions with alkali, acid and enzymatic saccharification for high sugar yield from wheat straw was examined (Fig. 1). The work reported here is on the investigation of the order of pretreatment method for higher saccharification and also subsequent fermentation of sugars to ethanol.

2. Materials and methods

2.1. Raw material

Wheat straw was collected from Aligarh, Uttar Pradesh, India. The straw was dried and cut into 1–3 cm fiber in a laboratory pulverizer followed by sieving to make the straw dust free and then dried at 60 ± 0.5 °C for overnight. All the chemicals used were of analytical or reagent grade and all the experiments were performed in triplicates and the results are presented as mean ± standard deviation.

2.2. Microorganism

The organism *S. cerevisiae*, identified as VS3 strain in our lab (Kiransree, Sridhar, Suresh, Banat, & Venkateswar, 2000) was used for fermentation studies. Stock cultures of *S. cerevisiae* were maintained and grown on YEPD agar. (yeast extract, 10 g/L; peptone, 20 g/L; glucose, 20 g/L; and agar, 25 g/L, pH: 5.0 ± 0.2. Stock cultures were stored at 4 °C.)

2.2.1. Inoculum media for organism

Medium used for the inoculum preparation for ethanol fermentation contained (g/L): yeast extract, 10; peptone, 20; dextrose, 20, pH: 5.00 ± 0.5 for 24 h at 28 ± 0.5 °C and 150 rpm (Pasha, Valli, & Rao, 2007).

2.3. Fermentation media

The hydrolyzate was taken along with the supplementation of (g/L): yeast extract, 1.5; $(NH_4)2SO_4$, 1; K_2HPO_4, 0.5; peptone, 1;