

Solvent-based Delignification of Rice straw for Efficient Enzymatic Hydrolysis of Cellulose and Bioethanol Production

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ABSTRACT: Lignocellulosic biomass consists of a variety of materials with distinctive physical and chemical characteristics. The conversion of Lignocellulosic biomass to ethanol involves pretreatment followed by polysaccharide hydrolysis to simple sugars followed by sugar fermentation to ethanol. Several solvents and ionic liquids, namely formic acid, concentrated phosphoric acid, were used to pretreat rice straw in order to increase cellulose digestibility for ethanol production. When being directly used to pretreat the raw rice straw under corresponding optimized conditions, the improvement of cellulose hydrolyzability followed the order of concentrated phosphoric acid more than formic acid. This greatly enhanced its susceptibility to enzymatic hydrolysis at 30 °C. The chemical pretreatments caused deep deacetylation and milder delignification of rice straw and did not cause an apparent loss of cellulose. In addition fungal treatment (*Trichoderma reesei*) of pretreated samples has been applied to improve the conversion of cellulosic material in sugar. The method of concentrated phosphoric acid pretreatment and subsequent fungal treatment (in different concentrations) results the highest conversion of lignocelluloses in rice straw to sugar and consequently, highest ethanol yields were 320 mg/gram dry biomass after 6 days fermentation with *Saccharomyces cerevisiae*.

KEYWORDS : Delignification, Bioethanol, Lignocellulosic biomass, phosphoric acid, formic acid, Fungal treatment, Fermentation.

I. INTRODUCTION

Lignocellulosic biomass has been considered as one of the most promising non-food feedstocks for producing second-generation bioethanol. However, release of fermentable sugars from lignocellulosic biomass has become a limiting step for efficient and economical production of ethanol from cellulose, mainly due to the biomass recalcitrance constructed by chemical components, cell wall structure as well as cellulose supramolecular structure [1]. Various chemical processes have been developed for removing lignin to increase cellulose accessibility, such as alkaline saponification[2] oxidative delignification [3] organic solvent extraction[4] and sulfite pulping [5] [6]. Among these pretreatments, the solvent (organosolv)-based process not only greatly improve cellulose accessibility but also achieve a biomass fractionation to lignin, hemicellulosic sugars, and a relatively pure cellulose fraction, all of which show promises for further production of biofuels and biochemicals in a biorefinery concept[7] [8]. Bioethanol can be produced from plentiful, domestic, cellulosic biomass resources such as herbaceous and woody plants, agricultural and forestry residues, and a large portion of municipal solid waste and industrial waste streams. To ensure that a low-cost energy feedstock is available, researchers are examining dedicated energy crops, wood and grass species that have been selected to produce high yields. Lignocellulose is an abundant material created from solar energy and renewable resources on earth, which

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makes them attractive for production of ethanol. Lignocellulose is composed of three main fractions like cellulose (~ 45% of dry weight), hemicellulose (~30% of dry weight) and lignin (~25% of dry weight). In these waste products, cellulose and hemicellulose are closely associated with lignin in the plant cell wall. Cellulose, the most abundant polymer on earth is composed of thousands of molecules of anhydroglucose linked by β (1, 4)-glycosidic bonds. Cellulose can be effectively hydrolyzed and depolymerized into fermentable sugars by the enzyme cellulase. The pretreated material is subjected to the enzymatic hydrolysis and the resultant hydrolysates are fermented to ethanol using fermenting microbes[9]. Cellulose can be effectively hydrolyzed and depolymerized into fermentable sugars by the enzyme cellulase[10] [11] [12] [13]. Currently most commercial cellulases are produced from *Trichoderma reesei* [14] [15] usually used to describe a mixture of cellulolytic enzymes action is required for effective breakdown of substrate to its monomeric units. The action of cellulases involves the concerted action of (i) endoglucanases (endo-1, 4- β -glucanases, EGs) can hydrolyze internal bonds preferably in cellulose amorphous regions releasing new terminal ends, which randomly attacks the internal, β 1,4-linkages (ii) cellobiohydrolase, (exo-1, 4 β --glucanases, CBHs) act on the existing or endoglucanase generated chain ends (iii) β 3-glucosidase, which hydrolyzes cellobiose to glucose.

II. MATERIAL AND METHODS

Raw materials

Rice straw from local mill powdered and sieved into a 1 mm seiver. Powder of the raw material was used as carbon source.

Microorganisms

Trichoderma reesei (MTCC-4876) were obtained from, MTCC Chandigarh. The fungi produces cellulolytic enzymes that converted carbohydrate polymers into fermentable sugars Later *Saccharomyces cerevisiae* was inoculated to utilize reducing sugars to ethanol.

Inoculum-preparation

Fungal cultures were inoculated onto PDA medium in the Petri plate. Spores scraped out from 7 days old slants were dispersed in desired quantity of sterile distilled water containing 0.1% Tween 80 and vortexed. Spore count was measured with haemocytometer and adjusted to 2×10^6 spores/ml by adjustment of optical density.

Culture-medium

30 g screened rice straw was packed into the flask followed by addition of 300 mL 80 wt% formic acid and phosphoric acid solutions respectively. Electrical stirring with a Teflon paddle was used at 300 rpm for keeping the system as homogeneous as possible. The delignification was controlled at the boiling point of the formic acid and phosphoric acid solutions under atmospheric pressure (~107 °C) for 1 h. After delignification, the mixture was filtered under vacuum and it was taken up in conical flask (500 ml). The conical flasks were plugged with cotton and sterilized at 15lbs per sq.inch for 30 minutes. The flasks were inoculated with the fungal strains in their different concentrations (ml). These flasks were incubated at room temperature for 6 days on an orbital shaker. After six days mycelium was separated by filtration through Whatman filter paper. The filtrate was used for further studies [16].

Fermentation of Hydrolysates to Ethanol

After saccharification, *Saccharomyces cerevisiae* was inoculated for utilization of reducing sugars to ethanol. The microorganisms strain was grown in SDDL broth (glucose 20.0, yeast extract, 5.0 g L^{-1}) at 30°C for 48 hrs. The number of viable cells 10^9 (cfu/ml) was determined by the agar plate method using Schreder agar ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5, $(\text{NH}_4)_2\text{SO}_4$ 1.0, KH_2PO_4 1.0, yeast extract 1.0, sucrose 20, agar 15 g L^{-1}) incubated for 24 hrs at 30°C.

Analytical Methods

The carbohydrate content of pretreated raw materials in the culture broth was measured by Anthrone method [17]. The amount of reducing sugars in pretreated raw materials in the culture broth were determined by dinitrosalicylic acid (DNS) method [18] with glucose as standard. The amount of Non-reducing sugar present in the sample was calculated by subtracting Reducing sugars from Total soluble sugars. Soluble protein was analyzed by the method of

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Lowry *et al.* [19]. The estimation of pentose sugars was carried out using the fuming Ferric chloride (Iron solution) and orcinol reagent, as described by Standing *et al.* [20]. Determination of ethanol content was done by spectrophotometric method [21]. Cellulase enzyme production was studied by FPU assay [22].

III. RESULTS AND DISCUSSION

Cellulose, the dominant structural polysaccharide of plant cell walls, was ranging from 35 to 50%, followed by hemicelluloses (20-35%) and lignin (10-25%). Among various temperature range tested, the maximum microbial saccharification of delignified cellulosic biomass was achieved at 30°C. The results revealed that pH 5.5 was the optimum pH for the saccharification of delignified cellulosic substrates. Among different concentrations used, saccharification was observed to release the optimum amount of sugars (225.36 mg/g). *S. cerevisiae* has already been accepted as the most promising yeast for pentose fermentation, as it produced considerably more ethanol from pentose and hexose sugar mixture, showed higher volumetric rate and yield of ethanol production [23]. The fermentation process would be economically viable only if both hexose and pentose sugars present in the hydrolysates are converted to ethanol. Ethanol production increased with increase in the incubation time till 6 days of incubation and the maximum ethanol obtained was 3.20 ± 0.36 g/l.

IV. CONCLUSION

In the present study, bioethanol production process from lignocellulosic biomass i.e, Rice straw was developed. In order to fractionate the lignin and hemicellulose from the lignocellulosic substrate, formic acid, concentrated phosphoric acid pretreatment was adopted, which offers the possibility of producing cellulosic material that eventually will be highly amenable for microbial enzymatic hydrolysis. Thereafter the saccharification was taken forward with *Trichoderma reesei* and ethanol production done by *Saccharomyces cerevisiae*.

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