



## Acid and enzyme hydrolysis to convert pretreated lignocellulosic materials into glucose for ethanol production

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### ABSTRACT

The current research investigates the use of acid and enzyme hydrolysis to produce glucose from pre-treated rice straw, banana plant waste and corn cob, as a lignocellulosic materials, to be a source for ethanol production. The agricultural biomasses were first tested, then a laboratory experimental set-up was designed in order to perform the necessary conversions. The biomass materials were characterized to contain 57.46–85.28% holocellulose and 14.55–26.12% lignin. Conversion of the cellulose to glucose was achieved by pre-treatment method for the agricultural residues first applying chemical pulping and steam explosion method as well as microwave treatment then followed by two processes, namely acid hydrolysis and enzyme hydrolysis. Sulfuric acid, 5%, was used in acid hydrolysis and *Trichoderma reesei* cellulases in enzyme hydrolysis. These experiments demonstrated that glucose concentration differs according to the type of pre-treatment and type of hydrolysis. Conversion of the glucose to ethanol during fermentation was accomplished by the action of yeasts from *Saccharomyces cerevisiae*. Ethanol production in the culture sample was monitored using gas chromatography. The results indicate that ethanol can be made from the above mentioned residues in a different yield according to the pre-treatment and the glucose produced from the hydrolysis method.

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### 1. Introduction

The production and utilization of bioethanol have attracted worldwide attention as a strategy for reducing global warming and improving global energy security. However, the feedstocks for bioethanol production should be derived from inedible parts of food crops, in order to avoid direct competition between bioethanol and food productions (Sakai et al., 2007). Since 2007, most bioethanol has been produced from sugar or starches obtained from fruits and grains. Ethanol can be produced from a number of renewable resources other than starches or sugars such as lignocellulosic materials. Lignocellulosic materials continue to be investigated as a source of fermentable sugars for biofuel (ethanol) production because of their high availability. In contrast, efficient conversion of lignocellulosic biomass to fermentable sugars is essential for the realization of economic bioethanol (Sheehan & Himmel, 1999).

Lignocellulose feedstocks, such as agricultural and forest residues, industrial and municipal wastes, and dedicated energy crops, by virtue of their high carbohydrate content, hold tremendous potential for large-scale bioethanol production (Farrell et al., 2006). Lignocellulosic waste materials contain cellulose in combination with lignin. Cellulose is the predominant polymer in lignocellulosic biomass, with hemicellulose and lignin found in smaller amounts. The cellulose component in these materials can be converted to ethanol in a two-step process where the cellulose is first converted to glucose sugars by hydrolysis; the resulting sugars can in turn be converted to ethanol by fermentation. However, due to the close association of cellulose and hemicellulose with lignin in the plant cell wall, pre-treatment is necessary to make these carbohydrates available for enzymatic hydrolysis and fermentation. For economic reasons, thermochemical pre-treatments, such as dilute acid hydrolysis and steam explosion, which solubilizes the hemicellulose components and increases cellulose accessibility, are commonly used to prepare lignocelluloses for enzymatic saccharification and fermentation (Endo, Nakamura, Ando, Tokuyasu, & Shima, 2008; Grethlein, Allen, & Converse, 1984; Meunier-Goddik & Penner,

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1999; Ucar & Fengel, 1988; Vlasenko, Ding, Labavitch, & Shoemaker, 1997).

A pre-requisite to the biological conversion of lignocellulosic biomass to ethanol is the release of the cellulose portion (and subsequently glucose) from the tightly woven lignocellulosic structure. For this, the biomass needs to be pre-treated, either physically, chemically, and/or biologically, to make the pre-treated biomass more amenable to subsequent cellulose hydrolysis. However, not every kind of lignocellulosic biomass can be pre-treated in the same way. While steam explosion has been tried and tested for agro residues like corn stover and rice straw, an additional acid hydrolysis step is needed for achieving high sugar yield from soft wood. Thus, significant amount of research has to be put in to determine the best pre-treatment strategy that can be the most economical, and at the same time can satisfy all the features of a good pre-treatment strategy (Cardona & Sanchez, 2007; Eggeman & Elander, 2005; Hendriks & Zeeman, 2009; Mosier et al., 2005; Wyman et al., 2005). Ethanol obtained from lignocellulosic biomass is favorable from a greenhouse gas (GHG) perspective, with around 85% net reduction in GHG emissions when replaced for gasoline.

The cellulose component resulted from the pre-treatment process can be converted to ethanol in a two-step process where the cellulose is first converted to glucose sugars by hydrolysis; the resulting sugars can in turn be converted to ethanol by fermentation. Acid catalyzed hydrolysis – concentrated and dilute – has been employed for yielding free sugars from the highly complex lignocellulosic biomass (Hamelinck, Hooijdonk, & Faaij, 2005; Xiang, Lee, Pettersson, & Torget, 2003). Dilute acid hydrolysis (0.7–3.0%) requires high operating temperatures (200–240 °C). Concentrated acid hydrolysis requires high amounts of acid and hence becomes uneconomical; acid recycling also entails considerable costs (Banerjee et al., 2010). On the other hand, enzymatic hydrolysis using cellulases does not generate inhibitors and the enzymes are very specific for cellulose. Cellulases, mainly derived from fungi like *Trichoderma reesei* and bacteria like *Cellulomonas fimi*, are a mixture of at least three different enzymes.

Enzymatic hydrolysis is the most promising alternative to the use of dilute acid, but it is certainly not a replacement process. We aimed to compare between using the acid and enzyme for the pre-treated agricultural residues to convert the resulted cellulose into glucose. Thus, the present work summarizes recent research focusing on ethanol production from different agricultural residues, namely rice straw, corn cob and banana plant waste. Their major components are lignin (14.55%, 15.83% and 20.31%, respectively), holocellulose (68.09%, 85.28% and 57.46%, respectively), and ash (16.34%, 1.32% and 13.63%, respectively). Different pre-treatment processes, namely alkaline pulping, steam explosion and alkali microwave treatment were applied to the different agricultural biomass and were carried out in a previous work (El-Zawawy, Ibrahim, Abdel-Fattah, Soliman, & Mahmoud, 2009). The pretreated raw materials were subjected to both acid and enzyme hydrolysis followed fermentation using yeast, *Saccharomyces cerevisiae*. The sugar contents were determined using enzymatic colorimetric method. The results indicated that ethanol can be made from the three different biomasses, but the ethanol yield was varied according to the pretreatment and hydrolysis applied.

## 2. Experimental

### 2.1. Materials and chemicals

Lignocellulosic residues from rice straw, banana plant waste and corn cob were used as the lignocellulosic feedstock source for ethanol production. Sulfuric acid and cellulase from *T. reesei* ATCC 26921 (SIGMA) were used for the hydrolysis.

### 2.2. Pre-treatment

The pre-treatment of any lignocellulosic biomass is crucial before hydrolysis. The objective of pre-treatment is to decrease the crystallinity of cellulose which enhances the hydrolysis of cellulose. Steam explosion, alkaline pulping with 10% sodium hydroxide (wt/wt) and alkali microwave treatment of the rice straw, corn cob and banana plant waste were carried out as a pre-treatment process as described previously (El-Zawawy et al., 2009).

Water hydrolysis was done as another pre-treatment process on a closed autoclave for corn cob, banana plant waste and rice straw. The fibers were cooked at 170 °C using water in a liquor ratio of 1:10 for 2 h. After cooking, the pressure was released to atmosphere and the pulped fiber was washed with water then air dried.

### 2.3. Fiber characterization

The chemical composition of pre-treated agricultural residues was determined by methods shown in the following sequence: holocellulose and  $\alpha$ -cellulose (TAPPI T257 om-85) and Klason lignin (TAPPI T222 om-88).

The acid insoluble residue and ash contents of the samples were determined by ASTM method E1721-95. Samples of biomass (0.3 g) were hydrolyzed with 72% sulfuric acid for 2 h at 30 °C. The hydrolyzed samples were diluted with distilled water to 3% sulfuric acid and autoclaved at 121 °C for 1 h. The hydrolyzates were filtered through filtering crucibles. The residues were dried overnight at 105 °C in a laboratory oven and weighed. The residues were then ashed in a muffle furnace at 575 °C for 3 h, cooled in a desiccator and weighed. The ash and acid insoluble residue content were calculated on oven dry basis.

The degree of crystallinity (DC) was measured using the iodine absorption method (Kortschagin, Sokolowa, & Schikanowa, 1991) in order to determine the accessible portion of the fibers, i.e. the amorphous regions. The percentage of the crystallinity was calculated as:

$$\text{Percentage of the crystallinity (DC\%)} \\ = 100 - \text{percentage of the amorphous fraction}$$

### 2.4. Acid hydrolysis (sulfuric acid)

Pre-treated rice straw, banana plant waste and corn cob were used as cellulosic sources for the ethanol production. Different concentrations from sulfuric acid, namely 4%, 5% and 10%, were applied first to determine the best concentration and the reaction was carried at different times from 30 min to 180 min until we come to a conclusion to use 5% sulfuric acid for 2 h hydrolysis. At the end of the hydrolysis time, the glucose concentration was measured.

A microwave processing set up was applied to hydrolyze the pre-treated agricultural wastes using microwave Home Model of frequency 2.45 GHz multimode cavity and 900 W applying the optimum hydrolysis condition of using 5% sulfuric acid but for different times from 10 to 30 min. At the end of the hydrolysis time, the glucose concentration was measured and it was noticed that one can reach a maximum sugar conversion within 10 min hydrolysis under microwave process.

### 2.5. Enzyme hydrolysis

Samples of rice straw, banana plant waste and corn cob, at different pre-treatments, i.e. steam explosion, alkaline pulping, water hydrolysis and alkali treatment under microwave were selected for enzyme hydrolysis. Cellulase from *T. reesei* ATCC 26921 (SIGMA)

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