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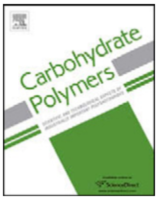

Acid and enzyme hydrolysis to convert pretreated lignocellulosic materials into glucose for ethanol production

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ABSTRACT

The current research investigates the use of acid and enzyme hydrolysis to produce glucose from pretreated 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.

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 lignocelulosic 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 α-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:

$$\begin{aligned}&\text{Percentage of the crystallinity (DC\%)}\\&\quad = 100 - \text{percentage of the amorphous fraction}\end{aligned}$$

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) was used for the hydrolysis. The pre-treated fibers (10 g odf) in 100 ml sodium acetate buffer at pH 4.7 were sterilized first at 121 °C for 20 min. The enzyme was added after cooling to the above samples. The mixture was incubated in a shaker bath at 50 °C and 75 rpm for four days. After a reaction time of 1, 2 and 3 days, the reaction was stopped by placing the sample in a boiling beaker of water for 5 min. The mixture was vacuum-filtered and the glucose concentration was analyzed using the enzymatic colorimetric method.

2.6. *Glucose assay*

The total reducing sugars produced from the enzymatic hydrolysis of cellulose can be measured by the dinitrosalicylic acid (DNS) method. Samples were retrieved at 0, 5, 10 and 24 h. Upon sampling, the hydrolysis reaction was quenched by immersing samples in boiling water for 5 min and then centrifuged at 11,000 × *g* for 10 min. The supernatants was collected and analyzed for sugar contents using UV measurement. Perkin-Elmer Lambda 6 UV/vis spectrophotometer with PECS 5 software was used in scanning colorimetric absorbances between 400 nm and 650 nm. Readings were taken at 550 nm in accordance with manufacturer specifications. Glucose standard samples were prepared for determination.

2.7. *Enzyme hydrolysis calculations*

Data from the enzyme hydrolysis time study were analyzed to provide information on enzyme hydrolysis rates. Enzyme hydrolysis rates were computed as concentration of glucose released per hydrolysis time:

$$\nu = \frac{dS}{dt} = \frac{\mathrm{Glu}_t - \mathrm{Glu}_0}{t - t_0}$$

where, ν = enzyme hydrolysis rate (mg/ml glucose per hour), Glu_t = concentration of glucose at time t (mg/ml), Glu_0 = initial glucose concentration at time = 0 h (mg/ml), t = hydrolysis time (h), and t_0 = time = 0 hour (h).

2.8. *Fermentation*

The experiment for the fermentation was carried out to convert the resulting sugars into ethanol. *S. cerevisiae*, which is an organism for ethanol production, was used for fermentation. A growth medium was prepared and fresh colonies from agar plate were used to inoculate 50 ml of the growth medium in 250 ml Erlenmeyer flasks. The cultures were grown in a shaker bath. The cells were harvested, centrifuged at 11,000 × *g* under sterile conditions, and re-suspended in 2 ml of deionized water.

The 1 l flask containing the enzyme-hydrolyzed pre-treated materials were supplemented with 5 g yeast extract/50 ml and inoculated with *S. cerevisiae*. The initial pH of the fermentation broth was 6.0 and the fermentation was carried out at 35 °C and 75 rpm for 72 h under an aerobic condition. The supernatants were collected after the end of the three days and analyzed by chromatography for ethanol and sugar contents.

2.9. *Ethanol analysis*

Quantitative monitoring of ethanol production in the fermentation systems was performed on the Shimadzu GC-14A Gas Chromatograph with a Restek RTX-5 capillary column and Fisher 1-butanol A383-1 as the internal standard.

3. Results and discussion

3.1. *Acid and enzyme hydrolysis*

Ethanol or ethyl alcohol, CH_3CH_2OH, has been described as one of the most exotic synthetic oxygen-containing organic chemicals because of its unique combination of properties as a solvent, a germicide, a beverage, an antifreeze, a fuel, a depressant, and especially because of its versatility as a chemical intermediate for other organic chemicals.

The many and varied raw materials used in the manufacture of ethanol via fermentation are conveniently classified under three types of agricultural raw materials: sugar, starches, and cellulose materials. Cellulose from wood, agricultural residues, waste sulfite liquor from pulp and paper mills must likewise be converted to sugars, generally by the action of mineral acids. In our study, agricultural residues from rice straw, banana plant waste and corn cob are our cellulosic source for ethanol production. There are two major ways of converting cellulose to glucose: chemical versus enzymatic.

The first step in producing ethanol from lignocellulosic materials is to open the bundles of lignocelluloses in order to access the polymer chains of cellulose and hemicellulose by a process so-called pretreatment. Thus, the pretreatment process is possibly the most important factor in the ethanol production process because it influences waste treatment, cellulose conversion rates and hydrolysis of hemicellulose sugars and their subsequent fermentation.

For this, the selected agricultural biomass were pretreated, as described in a previous work (El-Zawawy et al., 2009), with alkaline pulping, steam explosion and alkali microwave treatment. The major components of pre-treated rice straw, banana plant waste and corn cob were lignin (22.71%, 27.11% and 20.00% for steam explosion treatment, respectively, 1.56%, 10.74% and 1.77% for alkaline pulping pre-treatment, respectively, and 6.04%, 15.22% and 6.71% for alkali microwave treatment, respectively), holocellulose (55.31%, 42.01% and 61.31% for steam explosion treatment, respectively, 86.83%, 69.78% and 83.99% for alkaline pulping pre-treatment, respectively, and 82.16%, 55.62% and 89.33% for alkali microwave treatment, respectively) and α-cellulose content (44.95%, 30.13% and 50.34% for steam explosion pre-treatment, respectively, 58.37%, 51.52% and 47.53% for alkaline pulping pre-treatment, respectively, and 53.90%, 48.16% and 59.28% for alkali microwave treatment, respectively). The results for water hydrolysis pre-treatment are given in Table 1.

The results indicated that the holocellulose for the alkaline pulping and microwave treatment for rice straw and corn cob are higher than those of the steam exploded and water hydrolyzed biomasses. For banana plant waste, the holocellulose was higher in case of alkaline pulping followed by water hydrolysis pre-treatment. The term "holocellulose" is often used to describe the total carbohydrate contained in a plant or microbial cell. Holocellulose is therefore comprised of cellulose and hemicellulose in lignocellulosic materials.

Steam explosion applies high-pressure steam to the biomass for a few minutes, followed by explosive decompression. The rapid thermal expansion opens up the biomass structure and hydrolyzes the acetyl groups of hemicelluloses, causing hemicel-

Table 1
Chemical analysis for different agricultural residues after water hydrolysis pre-treatment.

Lignocellulosic biomass	Holocellulose (%)	Acid insoluble (%)	DC (%)
Rice straw	75.79	28.2	78.64
Banana plant waste	68.79	32.66	76.02
Corn cob	81.22	41.46	73.84

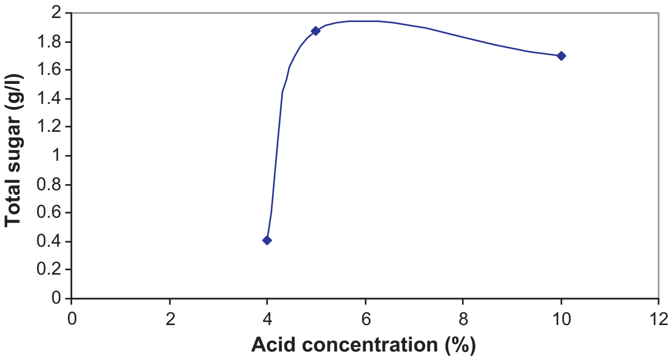

Fig. 1. Effect of different acid concentrations on the total sugar.

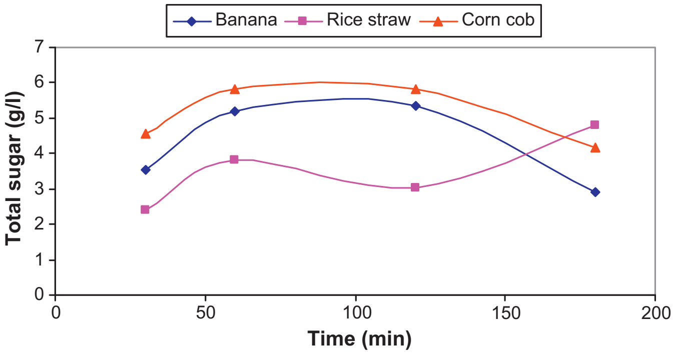

Fig. 2. Effect of hydrolysis time on total sugar.

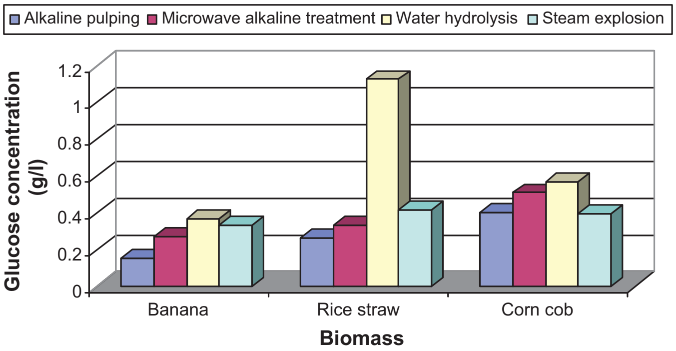

Fig. 3. Effect of pre-treatment on glucose concentration.

lulose solubilization. Thus, during steam pre-treatment, parts of the hemicellulose hydrolyze.

During the alkali microwave treatment, the acid insoluble residues are higher for all the biomasses, except for rice straw, as can be seen in Table 2. On the other hand, the lignin content was lower in alkaline pulping pre-treatment, where usually the lignin dissolved and separated in the resulted black liquor, and thus can facilitate the hydrolysis of the resulted cellulose.

Moreover, it can be seen from Table 2 that the degree of crystallinity (DC) for the steam exploded rice straw, corn cob and banana are lower compared to the pulped, microwave treated, or water hydrolyzed (Table 1) fibers. This can be ascribed to the degradation of the cellulose and hemicellulose parts during the steam explosion process which one can expecting an enhancement for the hydrolysis of the resulted cellulose by cellulase.

On the other hand, the resulted cellulosic polymers, after the pre-treatment for the lignocellulosic materials, were converted to simple sugars, before fermentation, by a hydrolysis process. Acid hydrolysis – concentrated and diluted – has been applied for yielding free sugars from the highly complex pre-treated lignocellulosic biomass. Different concentrations from sulfuric acid were applied to convert the lignocellulosic biomass into sugars. The results represented in Fig. 1 indicated that a suitable acid concentration was 5%.

Furthermore, different time of hydrolysis was carried out using 5% sulfuric acid from 30 min to 180 min. The results represented in Fig. 2 clarify that the best condition to get higher sugar was applying 2 h for dilute acid hydrolysis.

The data of the glucose concentration resulted from the acid hydrolysis of the pretreated rice straw, corn cob and banana plant waste were found to depend on the pretreatment method. Glucose data is graphically represented in Fig. 3, where the graph clearly shows that the glucose concentration is higher for the acid hydrolyzed water pulped biomass, i.e. the water hydrolysis pulping pre-treatment facilitate the acid hydrolysis step followed by the steam explosion then the microwave alkali treatment and the alkaline pulping. On the other hand, further study was carried out by running the acid hydrolysis under microwave condition for different times. It was noticed that one can reach a maximum sugar conversion within 10 min hydrolysis under microwave process. A comparison was carried between the acid hydrolysis for 2 h and that carried out in microwave for 10 min. The results were illustrated in Fig. 4. It was surprise that acid hydrolysis in microwave for 10 min results in a higher glucose concentration compared to that carried out for 2 h in the traditional method. Also, it was noticed that higher glucose content was resulted from corn cob followed by rice straw then banana plant waste. This may be due to the fact that corn cob contains higher holocellulose content (85.28%) followed by rice straw (68.09%) then banana plant waste (57.46%) and thus can produces higher sugar content under the same conditions.

Fig. 5 shows the maximum ethanol yield resulted after hydrolysis by acid for 2 h and for 10 min in microwave. Although, the sugar content for the hydrolysis in microwave results in higher value compared to the hydrolysis for 2 h, but the actual ethanol yield was lower after fermentation. This can be explained by the production of large number of inhibitors byproducts like furans, organic acids, and phenolics through the microwave acid hydrolysis in the short time compared to the longer acid hydrolysis time and thus this byproduct inhibited the fermentation process which lead to a lower ethanol yield resulted from microwave hydrolysis compared to the two hours acid hydrolysis.

On the other hand, enzymatic hydrolysis using cellulases does not generate inhibitors and the enzymes are very specific for cellulose. Cellulases are a mixture of at least three different enzymes: (1) endoglucanase (EG, endo-1,4-D-glucanohydrolase, or EC 3.2.1.4.) which attacks regions of low crystallinity in the cellulose fiber, creating free chain-ends; (2) exoglucanase or cellobiohydrolase (CBH,

Table 2
Acid insoluble and degree of crystallinity for different agricultural residues after different pre-treatments.

Lignocellulosic biomass	Steam explosion		Alkaline pulping		Alkali microwave	
	Acid insoluble (%)	DC (%)	Acid insoluble (%)	DC (%)	Acid insoluble (%)	DC (%)
Rice straw	8.20	60.10	9.50	76.55	17.50	65.85
Banana plant waste	23.93	47.49	17.83	63.29	36.83	62.79
Corn cob	41.30	39.85	37.26	75.02	35.63	58.71

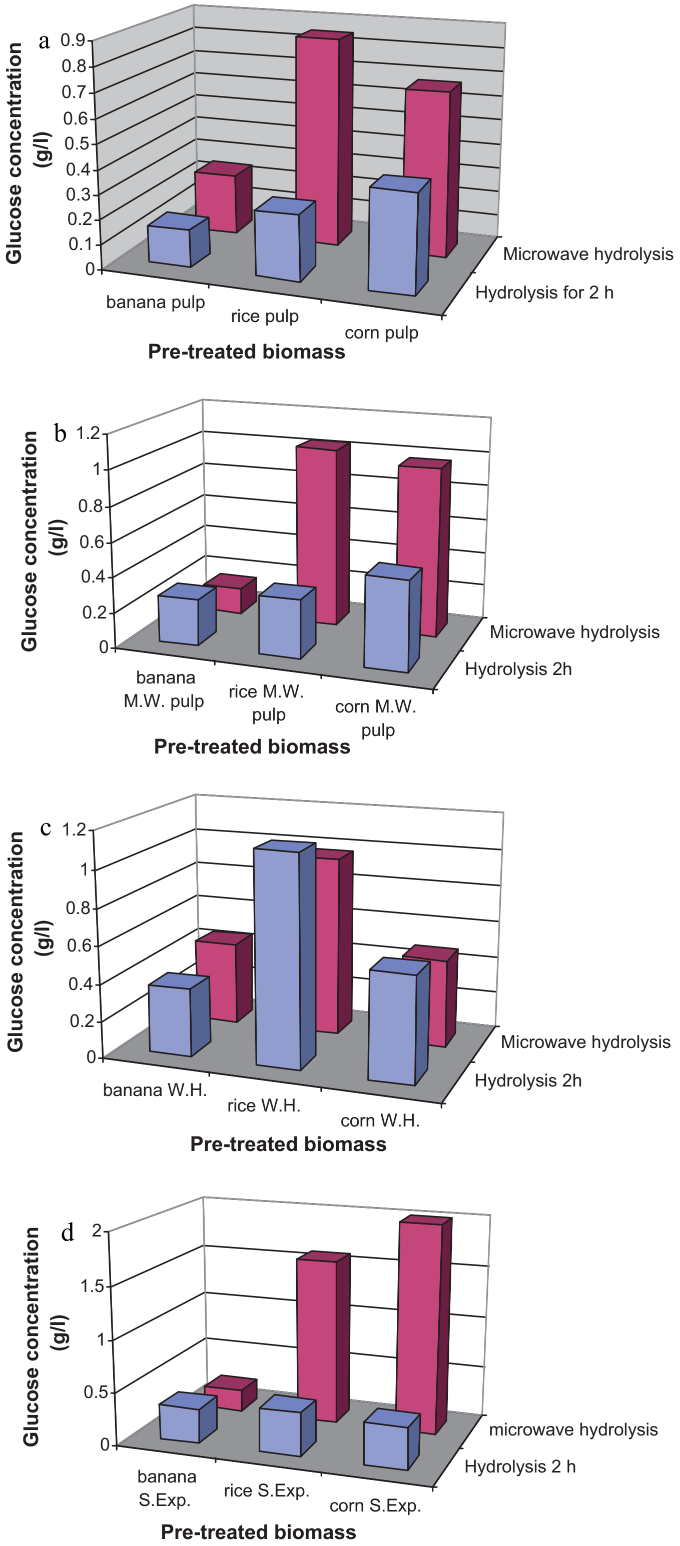

Fig. 4. Glucose concentration resulted from acid hydrolysis carried out for 2 h and 10 min in microwave after different pre-treatments, i.e. (a) alkaline pulping pre-treatment, (b) alkali microwave treatment, (c) water hydrolysis pre-treatment and (d) steam explosion pre-treatment.

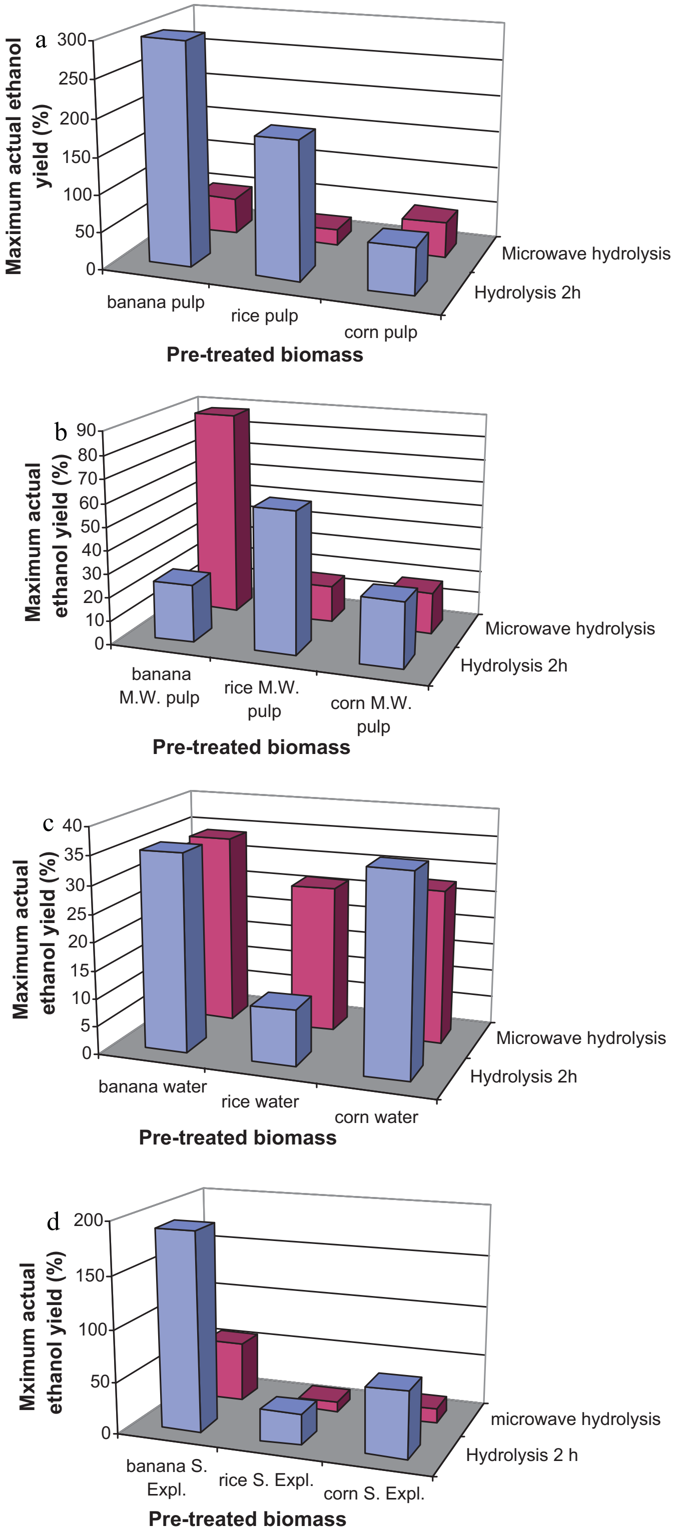

Fig. 5. Maximum actual ethanol yield resulted from acid hydrolysis carried out for 2 h and 10 min in microwave after different pre-treatments, i.e. (a) alkaline pulping pre-treatment, (b) alkali microwave treatment, (c) water hydrolysis pre-treatment and (d) steam explosion pre-treatment.

1,4-β-D-glucan cellobiohydrolase, or EC 3.2.1.91.) which degrade the molecule further by removing cellobiose units from the free chain-ends; and (3) β-glucosidase (EC 3.2.1.21.) which hydrolyzes cellobiose to produce glucose, in much smaller amounts (Sun & Cheng, 2003).

The sugar content production depends on the enzyme used for hydrolysis. The data of the glucose concentration resulted from the enzyme hydrolysis of the pre-treated rice straw, corn cob and banana plant waste using cellulase from *T. reesei* indicate a depends on the pre-treatment method. Glucose data is graphically

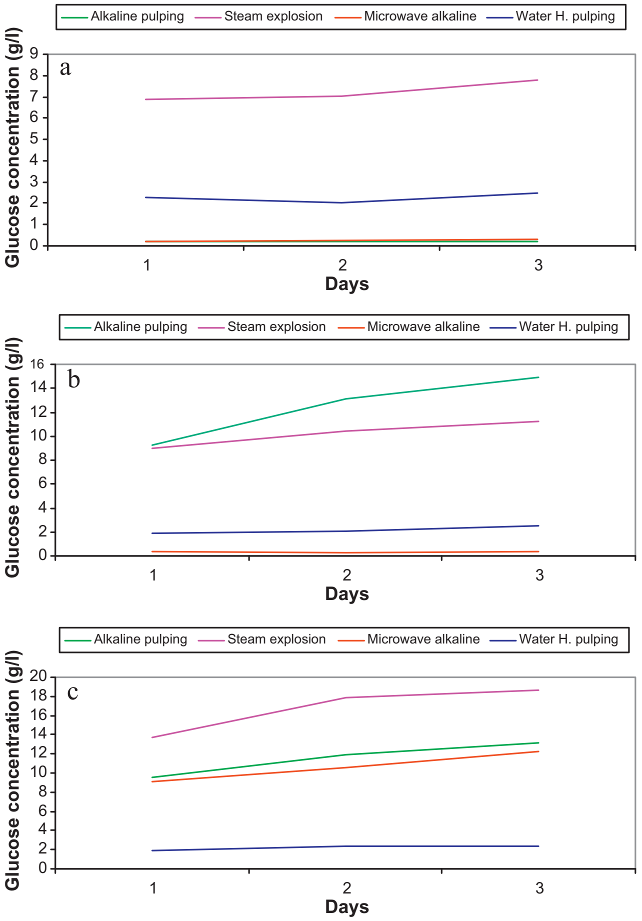

Fig. 6. Effect of different pre-treatment on glucose production by enzymatic hydrolysis for (a) banana plant waste, (b) rice straw and (c) corn cob.

represented in Fig. 6. The graph clearly shows that the glucose concentration is higher for the enzymatically hydrolyzed steam exploded banana plant waste (Fig. 6a) and corn cob biomasses (Fig. 6c) followed by alkaline pulping for rice straw biomass (Fig. 6b). This can be explained by the effect of the steam explosion treatment on the degree of crystallinity. Where, as indicated above in Tables 1 and 2, the steam exploded biomass resulted with a lower degree of crystallinity which enhances the enzymatic hydrolysis and hence facilitate the conversion of the cellulose into glucose.

The results for the glucose concentration also indicated that each agricultural biomass require different pre-treatment in order to produce higher glucose content. From Fig. 6, one can noticed that for rice straw the best pre-treatment is applying alkaline pulping process, while for corn cob the higher cellulose conversion results from applying steam explosion pre-treatment. In conclusion, not every kind of lignocellulosic biomass can be pre-treated in the same way.

Furthermore, the calculated rate of enzyme hydrolysis was gathered in Table 3 from which one can noticed that the higher rate of enzyme hydrolysis was obtained for the steam exploded corn cob. As mentioned above, the degree of crystallinity can play a role thus the lower degree of crystallinity resulted from the steam explosion pretreatment affects the rate of enzyme hydrolysis.

3.2. Ethanol production

S. cerevisiae is by far the most commonly used microbial species for industrial ethanol production from sugar- and starch-based raw materials. It produces ethanol with stoichiometric yields and tolerates a wide spectrum of inhibitors and elevated osmotic pressure (Hahn-Hagerdal, Karhumaa, Fonseca, Spencer-Martins, & Gorwa-Grauslund, 2007). For acid hydrolysis, Fig. 7 clearly shows that alkaline pulping and steam explosion pre-treatments had an effect on conversion of sugars in agricultural biomass to ethanol. Fermentation of alkaline pulped banana plant waste yielded higher ethanol from the converted cellulose into glucose. Similar to the banana plant waste cellulose conversion, alkaline pulped rice straw had the second ethanol yields from the converted corn cob cellulose into glucose. The samples treated with alkaline pulping showed improved ethanol concentrations compared to the other treated methods, while that treated with alkali-microwave and water hydrolysis resulted with the lower ethanol concentrations. Earlier, it was noted that glucose yields from acid hydrolysis is steam explosion dependent, where higher glucose yields were obtained after biomass treated with steam explosion (Fig. 3). Subsequently, it was also noted that sugar to ethanol conversion is alkaline pulping dependent, where the treatment favored higher conversion. From the data given here, the steam explosion results in a better degradation of the cellulosic materials and thus can be easily converted into sugars due to the lower degree of crystallinity of the produced biomass (Tables 1 and 2). At the same time, the steam explosion treatment results in a higher lignin content compared to the alkaline pulping pre-treatment, as previously mentioned (El-Zawawy et al., 2009). This higher lignin content may affect the production of ethanol during the acid hydrolysis process.

Table 3
Rate of enzyme hydrolysis for pre-treated biomass materials using *Trichoderma reesei*.

Pretreated biomass	Rate of enzyme hydrolysis		
	1st day	2nd day	3rd day
Steam exploded corn	0.57	0.37	0.26
Steam exploded rice	0.37	0.22	0.15
Steam exploded banana	0.28	0.14	0.11
Alkaline pulped corn	0.40	0.25	0.18
Alkaline pulped rice	0.39	0.27	0.21
Alkaline pulped banana	0.00625	0.00312	0.00194
Microwave corn	0.38	0.22	0.17
Microwave rice	0.014	0.00583	0.00431
Microwave banana	0.00758	0.00504	0.00392

For the enzyme hydrolysis, the effect of alkaline pulping, steam explosion, water hydrolysis and alkali-microwave treatment on ethanol yields from fermentation of rice straw, corn cob and banana plant waste was analyzed. Fig. 8 clearly shows that both steam explosion and alkaline pulping pretreatments had an effect on conversion of sugars in agricultural biomass to ethanol. Fermentation of the alkaline pulped rice straw yield 6.07 g/l of ethanol from the

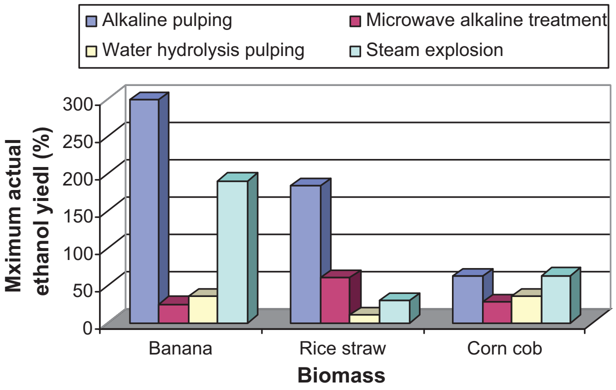

Fig. 7. Maximum actual ethanol yield resulted from different pre-treatments after acid hydrolysis.

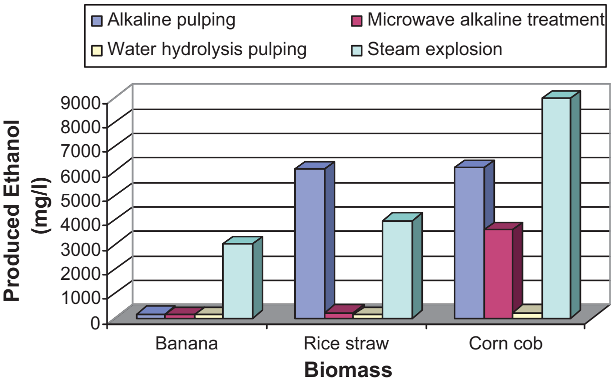

Fig. 8. Effect of pre-treatment process on ethanol production.

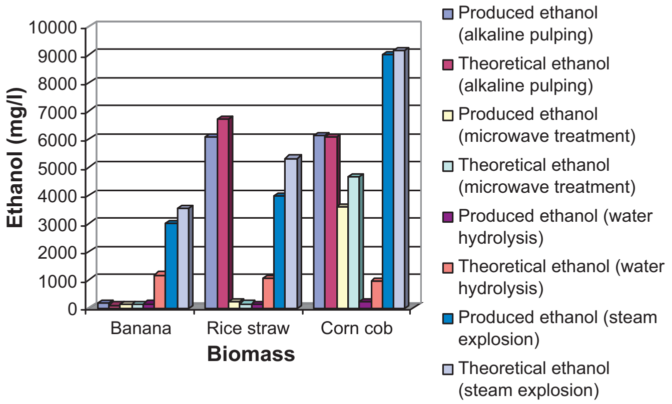

Fig. 9. Effect of pre-treatment process on actual and theoretical ethanol yield.

converted cellulose into glucose. Similar to the rice straw cellulose conversion, alkaline pulped corn cob had 6.13 g/l ethanol yields from the converted corn cob cellulose into glucose. The corn cob samples treated with steam explosion showed improved ethanol concentrations compared to the other treated methods, where it had 8.99 g/l ethanol yields from the converted corn cob into glucose, while that treated with alkali-microwave resulted with the lower ethanol concentrations for all samples.

From the above results, it was noted that glucose yield from enzyme hydrolysis differs according to the pre-treatment process. It was also noted that sugar to ethanol conversion is a pre-treatment dependent besides lignocellulosic source (Fig. 8). One can mention that pre-treatment can improve the potential for agricultural biomass to ethanol according to the biomass used.

For the theoretical calculation, it is shown that the alkaline pulped rice straw produces a maximum of 7.61 g/l ethanol compared to 5.72 g/l ethanol for steam exploded rice straw (Fig. 9). Fig. 9 shows the theoretical ethanol and the actual ethanol concentration, where one can again notice that the production of ethanol depends on the pretreatment method. From the data given here on ethanol concentration, it is evident that steam explosion treatment is favored for corn cob biomass, while alkaline pulping pre-treatment can improve the potential for rice straw to ethanol conversion.

4. Conclusions

Enzymatic hydrolysis of cellulose to glucose is carried out by cellulase enzyme that is highly specific catalysts. The hydrolysis is performed under mild conditions (e.g. pH 4.5–5.0 and temperature 40–50 °C). Therefore, one may expect low corrosion problems, low utility consumption, and low toxicity of the hydrolyzates as the main advantages of this process compared to the acid hydrolysis carried out by dilute acid at higher temperature.

The results indicated that the higher ethanol concentration can be obtained from the enzyme hydrolyzed steam exploded corn cob followed by the rice straw, while for alkaline pulping pre-treatment it is higher for rice straw followed by the corn cob. This can be explained to the fact that each pre-treatment can affect the cellulosic region differently and hence facilitates the enzyme treatment and thus the fermentation to produce the ethanol.

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