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Production of Biofuel from Agricultural Plant Wastes: Corn Stover and Sugarcane Bagasse

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Abstract Cellulosic ethanol is a biofuel, produced from different kinds of raw materials such as simple sugars, starch and lignocellulose because of their low cost and huge availability. In the present study lignocellulosic substances such as suger cane bagasse and corn stover are used as feedstocks for bioethanol production by using cellulolysis process. Though agricultural wastes are cost effective, renewable and abundant but there occur several challenges and limitations in the process of converting lignocellulosic materials to ethanol such as efficient pretreatment methods for lignin removal, hydrolysis of pretreated lignocellulosic materials, using enzymes to break complex cellulose into simple sugars such as glucose and followed by fermentation and distillation. The choice of pretreatment methods plays an important role to increase the efficiency of enzymatic scarification thereby making the whole process economically viable. The study was also carried out to determine the significant influences of pH and temperature on fermentation due to its effect on yeast growth and fermentation rate.

Keywords: *cellulosic ethanol, lignocellulosic materials, fermentation, distrillation and enzymatic scarification*

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1. Introduction

Global demand for energy continues to grow and still maintained from conventional fossil fuels such as oil, coal and natural gas. With Increase the world's energy demand and progressive depletion of oil reserves accelerate the search for alternative energy resources, especially for those derived from renewable materials such as biomass [1]. Excessive use of fossil fuels over the last century and following years has increased the level of greenhouse gasses in the earth's atmosphere drastically [2]. Global concern about climate change and the consequent need to diminish greenhouse gases emissions have encouraged the use of bioethanol as an energy source [3].

Bioethanol is a form of quasi-renewable energy that can be produced from agricultural feedstock. Lignocellulose is considered as an attractive feedstock for the production of fuel ethanol, because of its availability in large quantities at low cost [4,5]. In this context a series of researches have been carried out by different workers on ethanol production especially from lignocellulosic biomass. [6-11].

Cellulosic ethanol offers promise because cellulose fibers, a major and universal component in plant cells walls, can be used to produce ethanol. According to the International energy regency cellulosic ethanol could allow ethanol fuels to play a much bigger role in the future. Plant wastes from industrial processes (sawdust, paper pulp) and energy crops also grown specifically for fuel production, such as switch grass, miscanthus etc. Cellulosic biomass is composed of cellulose, hemicellulose and lignin, with smaller amounts of proteins, lipids (fats, waxes and oils) and ash. Roughly, two-thirds of the dry masses of cellulosic materials are present as cellulose and hemicellulose. Lignin makes up the bulk of the remaining dry mass. The residues from cellulosic ethanol plant can be reprocessed to produce biogas and final residues can be used as organic manure. The ideal organism for the production of ethanol would be the one which can utilize pentose and hexose sugars generated by lignocellulose hydrolysis [12,13,14]. In this paper we have reported cellulolysis processes which consist of hydrolysis of pretreated lignocellulosic materials, using enzymes to break complex cellulose into simple sugars such as glucose and followed by fermentation and distillation.

2. Materials and Methods

2.1. Chemicals

Hydrogen per oxide (H_2O_2), NaOH, cellulytic enzymes viz., alpha-amylase and glucoamylase (Cabiochem, USA), phosphate buffer and sodium acetate buffer .All the chemicals were used throughout the research work as analytical reagent grade.

2.2. Collection and Preparation of Raw Materials

Corn stover and sugarcane bagasse are the most favorable feedstocks for bioethanol production due to their

availability throughout the year. Though cellulose being the major component they also vary in chemical composition. The compositions of corn stover and sugarcane bagasse are shown in Table 1.

Table 1. Chemical Composition of corn stover and sugarcane bagasse

Raw materials	Cellulose (%)	Hemicellulose (%)	Protein (%)	Lignin (%)	Ash (%)	Reference
Corn stover	42.6	21.3	4.0	15.1	4.3	[7,15]
Sugarcane bagasse	50	25	3.0	18.4	2.8	[16,17]

Corn stover was collected from farmer's field after cob harvesting. The stalks were defoliated and cut in to small pieces. The pieces were then oven dried at 60 °C for 72 h (6 – 8 % moisture). It was treated at 60 °C because if the temperature was higher, it will affect the enzymes in the corn stover. Once dried, the materials were grinded using the grinding machine. The grounded sample was sealed in the seal bag or poly bag and stored in room conditions. The sugarcane bagasses were collected from the North Bengal Sugar Mill. The bagasse were also oven dried at 60°C for 72 h like corn stover, milled, packed in concealed poly bags and stored in room condition.

Lignocellulose is a complex carbohydrate polymer of cellulose, hemicellulose and lignin. Cellulose is linear and crystalline. It is a homopolymer of repeating sugar units of glucose linked by b-1,4 glycosidic bonds. Hemicellulose is a short and highly branched polymer. It is a heteropolymer of D-xylose, D-arabinose, D-glucose, D-galactose, and D-mannose. Lignin is hydrophobic in nature and is tightly bound to these two carbohydrate polymers. It thus protects these polymers from microbial attack. It is a three-dimensional aromatic polymer of p, hydroxyphenylpropanoid units connected by C-C and C-O-C links. Sugar compositions of various agro wastes (rice straw, wheat straw, corn straw, bagasse) are given in Table 2 [16]. Lignocellulosics are processed for bioethanol production through three major operations: pretreatment for delignification is necessary to liberate cellulose and hemicellulose before hydrolysis; hydrolysis of cellulose and hemicellulose to produce fermentable sugars including glucose, xylose, arabinose, galactose, mannose and fermentation of reducing sugars.

Table 2. Carbohydrate content of corn stover and sugarcane bagasse (%)

Raw materials	Glucose	Xylose	Arabinose	Galactose	Mannose	Reference
Corn stover	39.0	14.8	3.2	0.8	0.3	[16]
Sugarcane bagasse	38.1	23.3	2.5	1.1	-	[16]

2.3. Hydrogen Peroxide Pretreatment for Lignin Removal

The removal of lignin is necessary for cellulose to become readily available for the enzymes for asccharification, which permit the yeast to convert the glucose into ethanol [18]. In the present study the delignification was done with the pretreatment of feedstock with H_2O_2 in alkaline condition according to method developed by Dawson and Boopathy [19]. Milled corn stover and sugarcane baggase were slurried in water (12.5%,w/v unless otherwise stated) containing H_2O_2 (0-5%, v/v) were placed in 400ml Boro-silicate bottles (Boro 3.3, Isolab,Gemany) and adjusted to pH of the solution was 11.5 using NaOH and shaken in an incubator at 150 rpm at 25°C and 35°C for 6-24 h. Each H_2O_2 treatment combination was repeated four times. After required time of soaking, the residue was removed from the solution by filtering through cheesecloth. The residues were washed three times with deionized water. The residues were then dried in an oven at 60°C for 12 h. Finally, the residues were re-weighed. The weight difference was equated to the amount of lignin removed. The both types of delignified feed stock materials were adjusted to P^H 4.5 using concentrated HCL before enzymatic saccharification. Although lignocellulose is the most abundant plant material resource, its susceptibility has been curtailed by its rigid structure. As the result, an effective pretreatment is needed to liberate the cellulose from the lignin seal and its crystalline structure so as to render it accessible for a subsequent hydrolysis step [20]. By far, most pretreatments are done through physical or chemical means. In order to achieve higher efficiency, both physical and chemical pretreatment are required. Physical pretreatment is often called size reduction to reduce biomass physical size. Chemical pretreatment is to remove chemical barriers so that the enzymes can access to cellulose for microbial destruction.

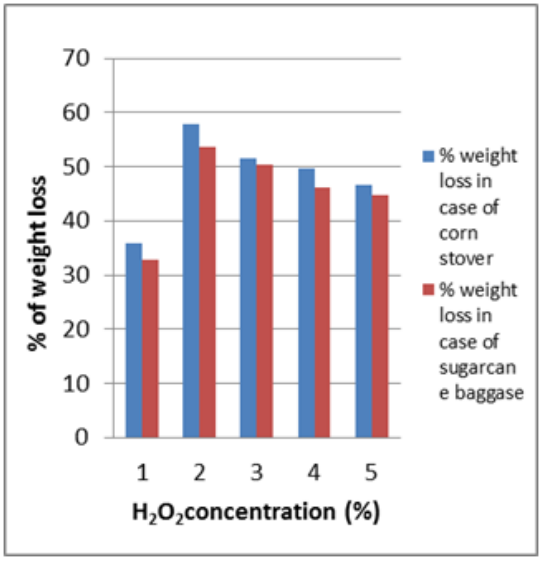

Figure 1. Effect of H_2O_2 concentration (0-5%, v/v) on lignin removal of corn stover and sugarcane baggase

The effect of H_2O_2 pretreatment of corn stover and sugarcane bagasse in alkaline solution on lignin removal is shown in Figure 1. It shows that the pretreatment of both types' raw materials with 2% H_2O_2 at pH 11.5 and soaking for 48 h remove the most lignin content effectively. This treatment was chosen for the rest of the fermentation experiment. The amount of weight loss after H_2O_2 pretreatment was due to lignin removal [18,19]. The % of weight loss indicate that the removal of lignin from total amount of lignin (15.1% for corn stover and 18.4% for sugarcane baggase).

2.4. Enzymatic Saccharification of Delignified Sugarcane Bagasse and Corn Stover

The enzymatic saccharification of the alkaline peroxide pretreated corn stover and sugarcane baggase were performed by shaking gently (150 rpm) at 37°C in an incubator after adjusting the pH to 4.5 with HCl. An enzyme mix consist of two types of cellulytic enzymes viz., alpha-amylase and glucoamylase (Cabiochem, USA) were used for enzymatic saccharification of both types delignified feed stock materials. Saccharification was done in two steps. In first step 1µl of enzyme alpha-amylase diluted with phosphate buffer was added to the slury and incubated at 37°C in an incubated shaker at 150 rpm for 24 h. Then, 1 µL of secondary enzyme, glucoamylase diluted with acetic acid with sodium acetate buffer was added to the mixture. The mixture was maintained at 37°C for 24-120 h as the glucoamylase hydrolyzed the dextrin to fermentable glucose. Samples (1 mL) were withdrawn and kept at -20°C before analysis.

2.5. Preparation of Buffer

Buffer was used to dilute the enzyme alpha-amylase and glucoamylase. Enzymatic reaction will be more effective if dilute with buffer compared to distilled water. There were two types of buffers prepared which were phosphate buffer for α-amylase and acetic acid with sodium acetate buffer for glucoamylase. The prepared buffer was covered with aluminum foil and kept at room temperature for further use.

2.6. Microbial Source

Baker's yeast (*Saccharomyces cerevisiea)* produced by S.I. Lesaffre, France, was prepared on agar plate prior to cultivation. *S. cerevisiea* culture was maintained in tryptic soy broth (TSB) and a fresh one week old culture grown on TSB was used in the fermentation experiments.

2.7. Fermentation Experiments

The hydrolysates from corn stover and sugarcane bagasse were filtered and 800 ml of filtered hydrolysate were transferred to one liter anaerobic fermentator. The pH of the hydrolysate was to 3.0, 3.5, 4.0, 4.5 and 5.0 (Table 4). The the volume of the hydrolysate was adjusted to one liter and autoclaved at 121°C (1.5 psi) for 20 min. After autoclaving the mixture was cooled down to 32°C and 10 ml of activated *Saccharomyces cerevisiea* (baker yeast) was added to the hydrolysate. The openings of each of the fermenator bottle were concealed with air lock system for ensuring anaerobic condition in side fermentator bottle. Then the fermentators were incubated at range of temperature from 25 to 50°C (Table 5) for 120 h.

2.8. Distillation of Ethanol

After 120 hours, the sample was filtered using Whatman Filter Paper to separate the ethanol from the residue. The bioethanol was distilled using rotary evaporator. The sample was heated at 80°C to get the bioethanol.

2.9. Analytical Procedure

Sugars, furfural, HMF, acetic acid, ethanol, and succinic acid were analyzed by high-pressure liquid chromatography (HPLC) [21]. The separation system consisted of a solvent delivery system (P2000 pump, Spectra-Physics, San Jose, CA) equipped with an autosampler (717, Waters Chromatography Division, Millipore Corp., Milford, MA), a refractive index detector (410 differential refractometer, Waters), a dual absorbance detector (2487, Waters), and a computer software based integration system (Chromquest 4.0, Spectra- Physics). Two ion moderated partition chromatography columns (Aminex HPX-87P with De-ashing and Carbo-P micro-guard cartridges, Aminex HPX 87H with Cation H micro-guard cartridge) were used. The Aminex HPX-87P column was maintained at 85 °C, and the sugars were eluted with Milli-Q filtered water at a flow rate of 0.6 mL/min. The Aminex HPX-87H column was maintained at 65 °C, and the sugars, organic acids, furfural, HMF, and ethanol were eluted with 10 mM HNO3 prepared using Milli-Q filtered water at a flow rate of 0.6 mL/min. Peaks were detected by refractive index or UV absorption (277 nm) and were identified and quantified by comparison to retention times of authentic standards (glucose, xylose, galactose, arabinose, furfural, HMF, acetic acid, succinic acid, and ethanol).

3. Results and Discussion

3.1. Effect of Alkaline Peroxide Concentration on Pretreatment and Enzymatic Saccharification

Initially, the effects of alkaline H_2O_2 level (0-5%, v/v) on the pretreatment of wheat straw 12.5%, w/v) at pH 11.5 and 35°C for 24 h were evaluated. The resultant glucose, xylose, arabinose, and total sugars yield in terms of mg per g of corn stover and sugarcane baggase after enzymatic saccharification using *Saccharomyces cerevisiea* enzyme at 37°C, pH 4.5, for 120 h is shown in Figure 1 and Figure 2. It was found that the sugar yield increased with increasing H_2O_2 up to 2.0% (v/v). There is not much difference between 2.0 and 5.0% (v/v) H_2O_2 pretreatment on each individual sugar as well as total sugar yields. Thus, it was decided to use 2.0% (v/v) H_2O_2 concentration for subsequent pretreatment studies.

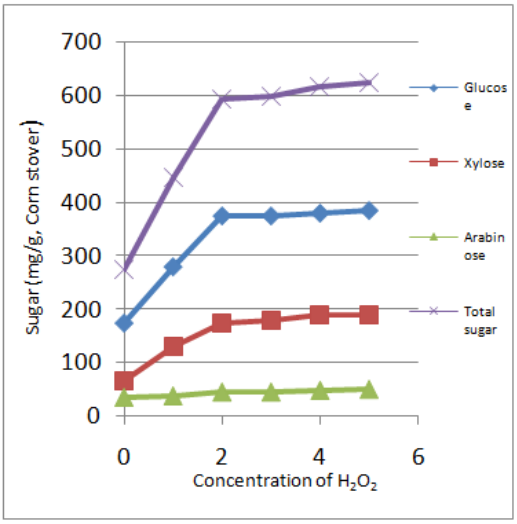

Figure 2. Effect of H_2O_2 level (0-5%, v/v) for the pretreatment (pH 11.5, 35°C, 24 h) of corn stover (12.5%, w/v) on its enzymatic saccharification (37°C, pH 4.5 , 120 h). The data presented are averages of two separate experiments

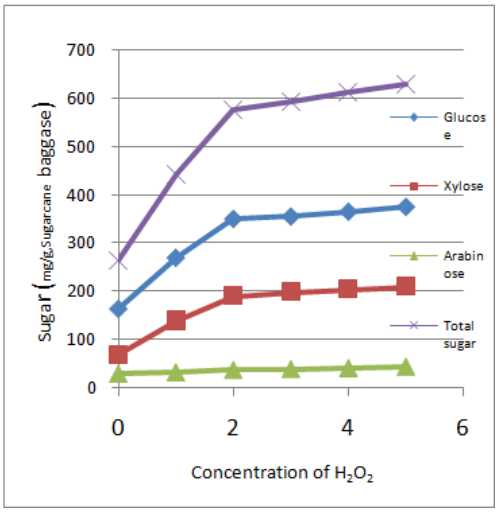

Figure 3. Effect of H_2O_2 level (0-5%, v/v) for the pretreatment (pH 11.5, 35 °C, 24 h) of sugarcane baggase (12.5%, w/v) on its enzymatic saccharification (37 °C, pH 4.5, 120 h). The data presented are averages of two separate experiments

3.2. Effect of Duration of Alkaline Peroxide Pretreatment at Two Temperatures

The effects of duration of alkaline peroxide pretreatment on the enzymatic saccharification of wheat straw at 25 and 35°C were investigated. The results are presented in Figure 4 and Figure 5. It is evident that the effect of pretreatment time is more pronounced at 25°C than at 35°C. In case of corn stover the increase of formation of total sugars 80± 3 mg per g of corn stover by increasing the pretreatment time from 6 to 24 h at 25°C, whereas the increase of total sugars was only 30± 4 mg per g of corn stover within the same time period at 35°C. On the other hand in case of sugarcane baggase the increase of formation of total sugars 75± 3 mg per g of sugarcane baggase by increasing the pretreatment time from 6 to 24 h at 25°C, whereas the increase of total sugars was only 25± 4 mg per g of sugarcane baggase within the same time period at 35°C. However, the longer is the pretreatment time; the better is the yield of sugars by enzymatic saccharification. Galactose was not detected in any of these hydrolyzates. The reason for this is not clear.

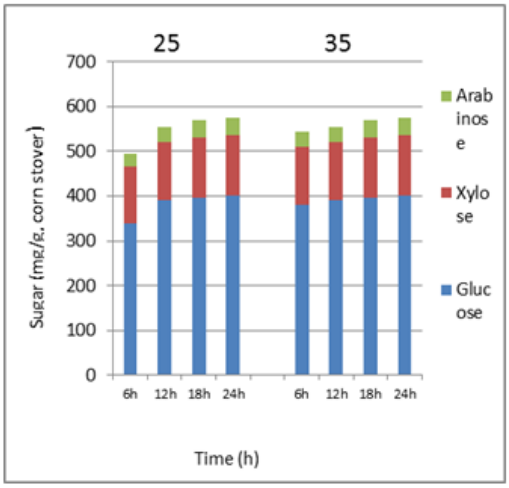

Figure 4. Effect of duration of alkaline H_2O_2 pretreatment (2.0%, v/v; pH 11.5) of corn stover (12.5%, w/v) at two temperatures (25 and 35 °C) on its enzymatic saccharification (37 °C, pH 4.5, 6-24 h). The data presented are averages of two separate experiments

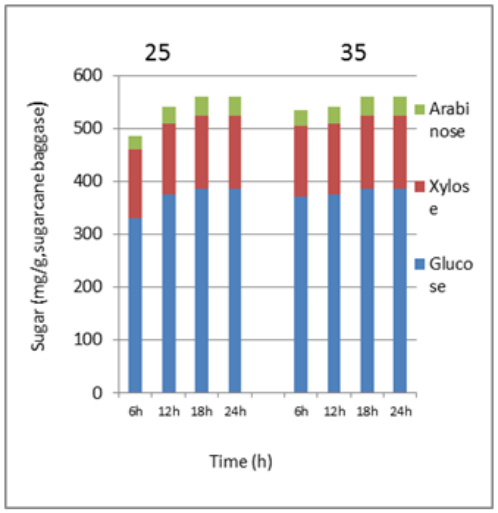

Figure 5. Effect of duration of alkaline H_2O_2 pretreatment (2.0%, v/v; pH 11.5) of sugarcane baggase (12.5%, w/v) at two temperatures (25 and 35 °C) on its enzymatic saccharification (37 °C, pH 4.5, 6-24 h). The data presented are averages of two separate experiments

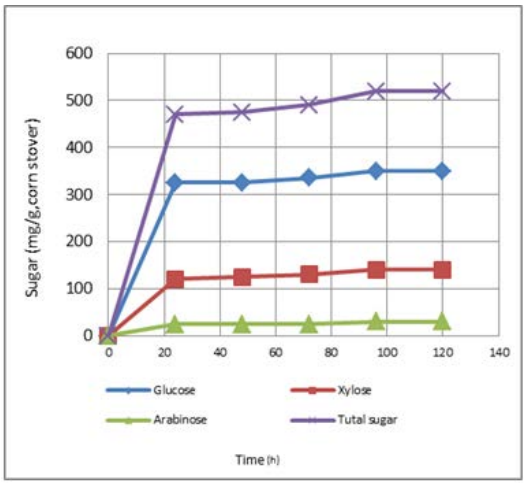

Figure 6. Time course of enzymatic hydrolysis (37°C, pH 4.5) of alkaline H_2O_2 pretreated (pH 11.5, 35°C, 120 h) corn stover (12.5%, w/v) using *Saccharomyces cerevisiea* enzyme. The data presented are averages of two separate experiments

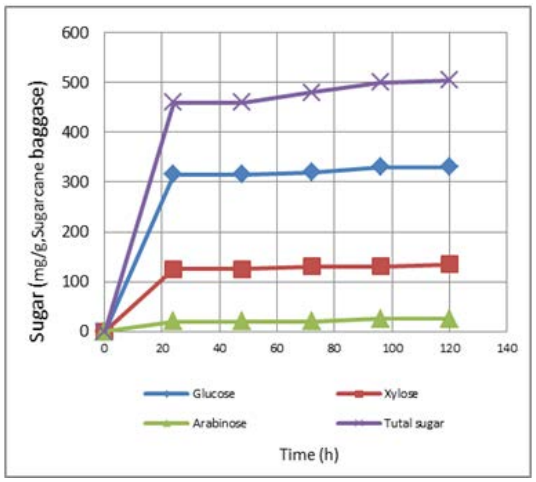

Figure 7. Time course of enzymatic hydrolysis (37°C, pH 4.5) of alkaline H_2O_2 pretreated (pH 11.5, 35°C, 120 h) sugarcane baggase (12.5%, w/v) using *Saccharomyces cerevisiea* enzyme. The data presented are averages of two separate experiments

The time course of enzymatic hydrolysis of the alkaline H_2O_2 pretreated (2.0%, v/v; pH 11.5; 35°C, 120 h) of corn

stover and sugarcane baggase is presented in Figure 6 and Figure 7. Most of the sugars were released within 24 h. However, the sugar yield increased very slowly up to 120 h, after which there was no increase in sugar concentration. This indicates that there is a long incubation time needed for getting the maximum sugar yield under the conditions used.

3.3. Fermentation of Corn Stover and Sugarcane Baggase Hydrolyzates and Production of Ethanol

The results of fermentation of alkaline peroxide pretreated and enzyme saccharified corn stover and sugarcane baggase by the recombinant *Saccaharomyces cerevisiea* enzyme are summarized in Table 3. The yeast produced small amounts of succinic and acetic acids as byproducts in addition to ethanol. It is clear that the separate hydrolysis and fermentation (SHF) approach worked better than the simultaneous saccharification and fermentation (SSF) method with respect to ethanol yield. The maximum concentration of ethanol (per L) from corn stover (66.0 g) hydrolyzate by recombinant *Saccaharomyces cerevisiea* enzyme was 13.75 (0.38 g per g of available sugars in corn stover) by SHF. For SSF, the maximum concentration of ethanol was 11.2 (0.32 g per g of available sugars based on separate hydrolysis data). On the other hand the maximum concentration of ethanol (per L) from sugarcane baggase (66.0 g) hydrolyzate by recombinant *Saccaharomyces cerevisiea* enzyme was 14.20 (0.40 g per g of available sugars in sugarcane baggase) by SHF. For SSF, the maximum concentration of ethanol was 12.9 (0.35 g per g of available sugars based on separate hydrolysis data). Both SHF and SSF were completed within 48 h of fermentation even though the yield of ethanol was higher in the case of SHF than SSF. However, ethanol production by the above process is lower than the ethanol production from AFEX-pretreated corn stover by recombinant *E.coli* stains K011 and SL40 and by *K. oxytoca* strain P2, under P^H-controlled conditions [22].

Table 3. Ethanol production from corn stover and sugarcane baggase hydrolyzate by recombinant Saccharomyces cerevisiea enzyme at 37°C

Raw materials	Hydrolyzate	Fermentation time	Sugar (g/L)	Ethanol (g/L)	Ethanol (g/g of sugar)
Corn stover	Separate hydrolysis and fermentation	48	34.5±0.8	13.75±0.5	0.38
	Simultaneous saccharification and fermentation	48	--------	11.2±0.3	0.32
Sugarcane baggase	Separate hydrolysis and fermentation	48	36.4±0.9	14.26±0.6	0.40
	Simultaneous saccharification and fermentation	48	--------	12.9±0.2	0.35

The medium contained hydrolyzates from 50 g of corn stover and sugarcane baggase per L. For pretreatment, corn stover and sugarcane baggase (12.56%, w/w) was treated with 2.0% H_2O_2 (v/v) at 35 °C for 24 h. Separate enzymatic saccharification was performed using *Saccaharomyces cerevisiea* at 37 °C and pH 4.5 for 120 h. Enzyme used 2 µL/50 gm of each raw materials. Fermentation experiments were performed at pH 6.5 for SHF and pH 6.0 for SSF. The data presented are averages of two separate experiments.

3.4. Effects of pH of Fermentation Process on Ethanol Concentration in Water

The study was carried out to determine the significant influences of pH on fermentation due to its effect on yeast growth and fermentation rate. The sample was fermented at different pH values from 3, 3.5, 4, 4.5 and 5 while the temperature was kept constant at 37°C to obtain maximum yield of bioethanol. The total bioethanol content in each sample was determined and recorded in the Table 4.

Table 4. The Effects of pH on Ethanol Concentration (%) in water

pH	Ethanol concentration in water (%)	
	Corn stover	Sugarcane bagasse
3.0	9.57	8.55
3.5	9.86	10.42
4.0	11.32	11.94
4.5	13.75	14.26
5.0	6.58	5.87

Based on the results obtained, pH 4.5 showed the highest ethanol content in water which is 14.26 %, followed by pH 4.0 which is 11.94 %, then pH 3.5 at 10.4 % and pH 3.0 at 8.55 % in case of sugarcane bagasse. The lowest ethanol concentration in water was achieved at pH 5.0. The result reveals that ethanol concentration in water gradually increases along with the increases in pH and reaches a maximum percentage of ethanol production when pH is equals to 4.5 and later it start to declining. In case corn stover material the highest ethanol content in water was 13.75 at pH 4.5 followed by 11.32 at pH 4.0.

The maximum ethanol concentration in water at pH 4.5 reflects enzyme function in an environment [23] while the lower ethanol concentration in water at pH reflects lesser yeast activity. The maximum ethanol productivity was observed at pH of 4.2 to 4.5 [24]. Furthermore, the increase in ethanol concentration in water is more efficient with the increase in pH from 4.0-4.5 and also found that the optimum pH range for *S.cerevisiae* to be pH 4.5 [25].

In general, yeast is an acidophilic organism and as such, grows better under acidic condition. The optimum pH range for yeast growth can vary from pH 4.0 to 6.0, depending on the temperature, the presence of oxygen and strain of yeast. Optimum pH values are required for the activity of plasma membrane bound proteins, including enzymes and transport proteins [26]. During growth, it is important for the yeast to maintain a constant intercellular pH.

There are many enzymes functioning during within yeast cell during growth and its metabolism. Each enzyme works best at its optimal pH, which is acidic because of the acidophilic nature of the yeast itself. When the extracellular enzymes pH changes from the optimal level, the yeast cell required using energy to either pump in or pump out the hydrogen ions in order to maintain the optimum intercellular pH [27].

If the extracellular pH changes too much from the optimum pH range, it may too difficult for the cell to maintain constant intracellular pH and the enzyme may not function normally. Furthermore, if the enzymes are deactivated, the yeast cell will not be able to grow and make ethanol efficiently [26].This is the most likely explanation for the observed reduction in ethanol production when the initial medium pH was at 3.0. There

were also low carbon dioxide productions at pH 3.0 because the low pH encourages the production of acid instead of alcohol [28].

However this study shows lowest ethanol concentration in water at pH 5. This may be due to the disability of the yeast strain to tolerate at pH 5. Different yeast strain has different pH range to activate and produce ethanol. There are other possibilities; the yeast that was used to conduct the experiment may be old. Old yeast will not carry out fermentation process efficiently compared to new yeast according to [29]. There is increased rate of ethanol production at pH 5. This statement is not applicable for this study since at pH 5 there is no ethanol production.

3.5. Effects of Temperature of Fermentation Process on Ethanol Concentration in Water

Temperature is one of the major factors that determine the ethanol production. Table 5 showed the ethanol concentration in water (%) that obtained at different temperature. Based on the result obtain, no ethanol concentration in water was observed at 25 and 30°C.

Table 5. Effects of temperature on ethanol concentration in water (%)

Temperature (°C)	Ethanol concentration in water with water (%)	
	Corn stover	Sugarcane bagasse
25	5.76	5.22
30	7.45	6.86
35	13.75	14.26
40	12.78	13.62
45	11.45	12.34

However, as the temperature increases beyond 30°C it showed increase in production of ethanol. At 35°C ethanol concentration in water were maximum and turned out to be 13.7% followed by 40°C where 12.3% ethanol was obtained. Fermentation process required a suitable temperature for the yeast to react. Temperature that is too high kills yeast, and low temperature slows down yeast activity. Thus, to keep a specific range of temperature were required.

However the ethanol concentration in water was decreased at 45°C. This indicates that 35°C were the optimum temperature for ethanol production. This finding is in agreeable with last studies about temperature on ethanol concentration in water [30]. This studies result also denied the study of [31]. Who found optimum temperature of ethanol production to be 25°C.

From the result we can conclude that higher the temperature, lower the ethanol concentration. The rate of enzyme catalyzed reaction increases with temperature up to a certain temperature and then the enzymes begins to denature. Higher temperature inhibits the growth of the cells and fermentation significantly decreases. In this study, ethanol concentration in water declined considerably at 40°C, which showed the inhibition effects on the cell growth at higher temperature.

This statement supported by study from [26]. Based on the high temperature might denature the ribosome and enzymes. Furthermore, higher temperature would alter the structure of the membrane and decreases its functionality [32]. Above the optimum temperature, the enzyme reaction drops precipitously as the enzyme denatures [33].

Enzymes are sensitive to temperature changes. At temperature above 40°C the rate of respiration slows down and drops. This was because all the enzymes are made up of the protein chains of amino acid. It exists in the form of a helix structure with hydrogen bonds holding them together. When heat was applied to the enzyme, energy was given off. The active enzyme cell deforms and the hydrogen bonds break, denature the yeast enzyme. This process called as denaturizing. The optimum temperature in which yeast enzyme work best is around 35°C, below this temperature the rate of reaction was slow and above 45°C the yeast enzyme would denature.

At low temperature the cells showed no ethanol concentration. This may be due to enzymes low tolerance to produce ethanol at lower temperature. Furthermore, at low temperature the enzyme deactivated and reaction slow down or stop altogether. At lower temperature, the molecules move slower than at higher temperature. These explain that the enzyme may not have enough energy to cause chemical reaction. Overall we can conclude that temperature 35°C was the optimum temperature for ethanol production.

4. Conclusion

This preliminary study showed that ethanol production from postharvest corn stover and sugarcane bagasse residue is possible through the delignification of the ligonocellulosic materials with hydrogen peroxide followed by enzymatic hydrolysis and yeast fermentation. Though the ethanol production lowers than the theoretical yield as per National Renewable Energy Laboratory (NREL, USA) calculation but the production cost is low. Therefore, further study is necessary for the optimization of ethanol production from corn stover and sugarcane bagasse like materials which in abundance in Bangladesh.

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