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A Comparative Study on the Effects of Chemical and Hydrothermal Pretreatment Methods on the Valorization of Lignocellulose Agricultural Waste (Sugarcane Bagasse)

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ABSTRACT

To effectively valorize agricultural waste (sugarcane bagasse) through biorefinery process, pretreatment which can be carried out in several ways is inevitable. This study explored the effects of chemical (acid and alkaline) and hydrothermal (steaming and autoclave) pretreatment methods on reducible sugar and ethanol yield from sugarcane bagasse. Acid pretreatment chemicals which include hydrogen peroxide and acetic acid were mixed together with sugarcane residue and heated at 85 ^{O}C for one hour. In alkaline pretreatment sodium hydroxide was mixed with the sugarcane waste and allow to incubate at 50 ^{O}C for one hour. The steaming approach involved using a steam distillation equipment. The sugarcane bagasse was first placed in a steam bag and then into a steam distillation unit which was steamed over a heating mantle for an hour. Autoclave pretreatment method used a stove type autoclave to disintegrate the processed sugarcane residue at 121^{O}C. Each of these pretreated samples underwent independent hydrolysis and simultaneous saccharification and fermentation operations. Sugar yield was determined by the 3,5-dinitrosalicylic acid method, whereas the bioethanol yield was determined from refractometer readings. Sugarcane bagasse pretreated with acids gave a sugar yield of 995.567 mg/l and ethanol concentrations of 1.7303% in both enzymatic and acid hydrolysis, and 2.0758% ethanol concentration in simultaneous saccharification and fermentation process. Alkaline-pretreated sample gave a fermentable sugar yield of 441.2591 mg/l with 0.8655%, 0.6938%, and 1.73033% ethanol concentrations from enzymatic hydrolysis, acid hydrolysis and simultaneous saccharification and fermentation processes respectively. Steaming yielded 913.4849 mg/l of fermentable sugar, whereas the ethanol concentrations in the enzymatic hydrolysis, acid hydrolysis and simultaneous saccharification and fermentation methods were 1.2121%, 0.6938%, and 0.8666% respectively. Autoclave pretreatment resulted in a fermentable sugar yield of 800.9 mg/l with ethanol concentration of 1.3848% in both the enzymatic and acid hydrolysis, while that of simultaneous saccharification and fermentation was 1.0393%.

Keywords: Sugarcane bagasse, pretreatment, acid hydrolysis, enzymatic hydrolysis, reducing sugar yield, ethanol yield, simultaneous saccharification, and fermentation

INTRODUCTION

Lignocellulose agricultural waste valorization, which promotes the reuse or recycling of residues from farm activities into useful products, has attracted great attention in different sectors. However, waste-to-energy features have increasingly become relevant because of the speedy depletion of natural resources, the

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increase in waste creation, and the increase in demand for food, which was initially used for first-generation biofuel. Agricultural residues are important lignocellulose biomass that find application as biofuel feedstock [1]. Efforts to generate and exploit agricultural leftovers are critical to addressing the existing and future energy supply-demand gap [2].

Sugarcane bagasse (SCB) is one of the major lignocellulose agricultural wastes produced in Nigeria. It is one of the most available agricultural residues worldwide, with over 180 million tons [3]. According to [4], [5], [6], SCB contains over 40% cellulose, 30% hemicellulose carbohydrate, and 25.4% lignin. Due to its high cellulose content, it has become a potential material in biorefinery, which is also very cost-effective [7] raw material for bioproducts. Converting this residue into energy will not only promote clean and sustainable energy production but will simultaneously keep the environment away from trash and pollution. According to [8], creating bioenergy from plant feedstocks can help minimize greenhouse gas emissions and ensure a reliable biofuel supply. Although SCB has attracted attention in biorefinery, the benefits can be harnessed by making the fermentable sugar available for hydrolysis. According to [9], only a small percentage (20%) of natural biomass is hydrolyzed without deconstructing the recalcitrant structure of the lignocellulose biomass (LB). Therefore, reducible sugar can be made accessible by breaking the tight bond holding these biomass components together.

Pretreatment is a known and important method to disintegrate this tight bond and increase the availability of simple sugar for hydrolysis. According to several reports, different pretreatment methods can be used to achieve this. These include; physical, chemical, physiochemical, and biological pretreatment [10]. Chemical pretreatment hydrolyzes lignin, hemicellulose, and cellulose. Physiochemical/hydrothermal pretreatments are known to break down lignin-holocellulose linkages while biological pretreatment works by degrading lignin from holocellulose components. Chemical pretreatment entails exposing LB to a concentrated or dilute chemical environment to make the available sugar accessible for saccharification.

Acid pretreatment, a type of chemical pretreatment, entails mixing with an acid solution, heating, stirring at a specific temperature at a given stirring speed for a set amount of time, and performing solid-liquid separation to produce a solid substance that is a fiber sample containing residual lignin. The alkali pretreatment method entails using chemicals like the hydroxides of ammonium, potassium, sodium, and calcium to distort the multiplex structure of lignocellulose biomass (LB) [11]. This pretreatment solubilizes or disrupts the lignin as a result of its susceptibility to alkaline conditions in comparison with cellulose and hemicellulose [12]. It also reduces the crystallinity and degree of polymerization of an LB, which happens by cellulose swelling [13], [14], [15], [16]. Physiochemical pretreatment uses high temperatures to disintegrate LB. Steam explosion is one of the pretreatment techniques that is carried out under high pressure. This process depolymerizes lignin and explodes the cellulose fibrils [17]. In the biological pretreatment method, microorganisms attack the structure of LB thereby gradually disintegrating the natural structure of LB. Bacterial and fungal pretreatments are some of the ways to achieve this. In bacteria pretreatment, bacteria species are used to disintegrate the lignin. Bacteria, which have mostly been isolated from the environment, have strong viability [18]. Bacillus strains include enough genes for glycoside hydrolases and glycosyl transferases, which are needed for cellulose breakdown [19].

Each of the pretreatment methods discussed above has its merits and demerits. The ability of the processes to produce a higher percentage of reducing sugar and ethanol from SCB was the only consideration in this work. This work only concentrated on the ability of the processes to valorize and improve the fermentable sugar yield from SCB without considering other factors such as the production of inhibitors, cost, equipment requirement, time, and the possibility of pollution. The main objective of this paper was to study the efficiency of different pretreatment methods in SCB valorization. Another objective was to determine which saccharification method produced more ethanol yield for a particular pretreatment method. Our previous report investigated the effect of autoclave pretreatment on SCB, and the method was found to be effective

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with a sugar yield of 800.9 mg/l. In this study, acid, alkali, steam and autoclave pretreatment processes were developed for sugarcane bagasse (SCB) to support the conversion of this agricultural waste into a valuable product while also improving the sugar yield. The processes were applied for subsequent ethanol conversion by separate saccharification and fermentation (acid hydrolysis and enzymatic hydrolysis) and simultaneous saccharification and fermentation (SSF) processes. The work compared sugar yield and ethanol yield from the different processes, and provided the best method for ethanol production from SCB. This work also serves as a basis for further study on SCB and other agricultural waste valorization in biorefineries.

MATERIAL AND METHODS

Feed Preparation

The sugarcane bagasse (SCB) obtained from Douglas Market Owerri, Imo State, Nigeria was processed to remove dust, and moisture, and increase the surface area for an effective pretreatment by washing the SCB with distilled water, and sun drying to remove the moisture content. The drying process carried out facilitated the grinding operation and, also prevented the SCB from developing molds. The dried SCB was ground in an attrition mill and sieved through a 1mm screen according to the method reported by [20]. The SCB was evaluated for its stability, thermal tolerance, chemical composition, and surface morphology before pretreatment. The results were reported by [4].

Material setup

Twelve (12) 500ml Erlenmeyer flasks were thoroughly washed and autoclaved at 121 ^{o}C for 15 minutes. 20 g of the processed sugarcane bagasse was introduced into each of the flasks. Each pretreatment process was carried out in three portions which were used for three different hydrolysis methods. All the experiments were carried out at Luco Scientific Laboratory Benin City, Edo State, Nigeria.

Chemical pretreatment

1) Acid Pretreatment: At temperatures below 100^{o}C acid concentration of 30–70% is normally used for LB pretreatment but with dilute acid of 0.1-10% pretreatment is exposed to high temperatures of 100-250 ^{o} C [21]. SCB was pretreated using a combination of hydrogen peroxide (H_2O_2) (30% w/w) and acetic acid (CH_3COOH) (99% w/w) (HPAC)[22]. HPAC was prepared in the ratio of 1:1 (v/v) H_2O_2 and CH_3COOH [23] of which 200 ml was poured into the flask containing the processed SCB. After stirring the sample, the flask was placed in a thermostatic water bath and heated at 85 °C for one hour. The pretreated sample was neutralized by washing in distilled water. The neutralized sample was then filtered using filter paper. The pretreated hydrolysate obtained was analyzed for total reducing sugar by DNS method according to [24] as described earlier in [4].

2) Alkali pretreatment: To 20 g of processed SCB, 250 mL of 1% (w/v) NaOH was added in a 500 mL Erlenmeyer flask and incubated at 50 °C at 80 rpm. The process was carried out for 1 hour. The pretreated SCB pulp was washed with distilled water until a neutral pH of 7.0 was achieved [5]. The neutralized sample was then filtered using filter paper. The pretreated hydrolysate obtained was analyzed for the estimation of total reducing sugar (TRS) by the DNS method.

Hydrothermal Pretreatment

1) Autoclave pretreatment: SCB was exposed to high temperatures to disintegrate the rigid structure of lignocellulose biomass. A stove-type autoclave was used to treat the processed SCB at a temperature of 121 ^{o}C for a period of 20 mins at a pressure of 15psi as reported by [4].

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2) Steam pretreatment: In the steam explosion, biomass is heated in the presence of steam at high temperatures in the range of 160–280 ?C for 10–30 min under high pressure (i.e., 0.7–4.8 MPa). The water present in the substrate expands through evaporation process leading to the hydrolysis of the sample to a great extent. Pressure reduction to atmospheric level improved the disintegration of biomass [17], [23], which also leads to the decrease in cellulose crystallinity [23], [24]. 20 g of SCB was poured into a sample bag and placed inside a steam distillation apparatus. The sample was then steamed by placing the apparatus on a heating mantle for 1 hour. After an hour of steaming, the sample was removed from the steam apparatus, and allowed to cool at room temperature before hydrolysis.

Saccharification Processes

Separate hydrolysis (acid and enzymatic hydrolysis) and simultaneous saccharification and fermentation were employed in the hydrolysis of the pretreated SCB.

1) Acid hydrolysis: The acid hydrolysis process was performed using the procedure reported by [20]. To make 1 M of H_2SO_4, 2.717 ml of pure H_2SO_4 was diluted in 1000 ml of purified water. To begin acid hydrolysis, 200 ml of diluted acid was placed into a SCB beaker and capped. The sample was then placed in a thermostatic water bath and heated to 85 degrees Celsius for one hour. This was left to cool at room temperature. The sample was then poured into a 1000ml beaker and analyzed for acidity using pH paper. A basic sodium hydroxide (NaOH) (1 M) solution was prepared and used to dilute the acidic solution until it was neutral. The 1 g of activated carbon was combined with 10 ml of distilled water before being placed into the detoxifying solution. The 3,5-dinitrosalicylic acid (DNS) technique was used to determine fermentable sugar levels.

2) Enzymatic hydrolysis: After autoclaving the samples and allowing them to cool at room temperature for an hour, the sample was placed in a thermostatic water bath at 40°C and heated for 20 minutes. 1 g of cellulase (10 FPU/g mass) was measured using filter paper, placed into a 50 ml beaker containing 10 ml of water, and mixed with a stirring rod to make a homogenous mixture. After 20 minutes of heating, the cellulase solution was poured into the sample, which was sealed with a rubber stopper and allowed to heat for another 20 minutes, thus activating the cellulase enzyme. The sample was then incubated using an incubator for 66 hours at 40 ^{O}C this method was reported by [20]. A 3,5-dinitrosalicylic acid reagent and a reference glucose curve were used to detect reducing sugar. The fermentable sugar yield was determined according to the method reported by [24] as reported in [4].

3)Simultaneous saccharification and fermentation: During this procedure, 1 g of cellulase enzyme (10 FPU/g mass) was measured into a 50 ml beaker containing 10 ml distilled water, and swirled with a stirring rod to create a homogeneous mixture. Similarly, 1 g of dried yeast (Saccharomyces cerevisiae KCTC 7906) was also measured into a separate 50 ml beaker and combined with 10 ml of distilled water. Both cellulase enzyme (10 FPU/g mass) and Saccharomyces cerevisiae (KCTC 7906) solutions were added to the sample at the same time, which was sealed and heated for 20 minutes at 40 degrees Celsius to activate the enzymes. The sample was then cultured in an incubator for 66 hours [20]. After this procedure, the ethanol concentration was determined from a refractometer.

Fermentation

After the enzymatic and acid hydrolysis methods respectively, fermentation was carried out on the samples. The samples were once again placed in a thermostatic water bath and heated at 40 ^{O}C for 20 minutes. 10 ml solution of 1 g dry yeast (*S. cerevisiae* KCTC 7906) was poured into the sample in a conical flask and was sealed. The sample was heated for an additional 20 minutes before placing it in an incubator and allowed to ferment for 66 hours. Alcohol was tested by adding iodine and sodium hydroxide to 3 ml of the mixture at a

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temperature of 40 ^{O}C. The formation of a yellow precipitate indicates the presence of alcohol [20]. The brix value was also recorded and was used to determine the ethanol concentration.

RESULTS AND DISCUSSION

Effect of Pretreatment on Reducing Sugar Yield

The fermentable sugar yield obtained through the DNS method using a reference glucose curve (figure 1) and ethanol concentration from different saccharification methods obtained using a reference concentration curve (figure 2) were reported in table I. The sugar yield obtained from base, acid, autoclave, and steaming pretreated SCB were 356.14 mg/l, 829.48 mg/l, 800.90 mg/l, and 799.95 m/l respectively.

Table I: Fermentable Sugar Yield and Ethanol Concentration from SCB

PT	AB (540nm)	FS (mg/l)	HS	BV (%)	ETC(%)
Base	0.880	356.14	SSF	6	1.7303
			Acid	3	0.6938
			Enzyme	3.5	0.86655
Acid	1.874	829.48	SSF	7	2.0758
			Acid	6	1.7303
			Enzyme	6	1.7303
Autoclave	1.814	800.9	SSF	4	1.0393
			Acid	5	1.3848
			Enzyme	5	1.3848
Steaming	1.812	799.95	SSF	3.5	0.86655
			Acid	3	0.6938
			Enzyme	4.5	1.21205

PT = pretreatment, AB = absorbance, RS = reducing sugar, HS = hydrolysis, BV = Brix value in Fermentation Broth, ETC = Ethanol Conc. in Fermented broth

Among the different pretreatment methods used in this study, acid pretreatment gave the highest value (829.48 mg/l) of reducing sugar. This implies that with acid pretreatment, more reducing sugar can be harnessed from SCB, which is in line with [27]. This is followed by autoclave pretreatment with sugar yield of 800.90 mg/l, with base pretreatment having the least sugar yield of 356.14 mg/l.

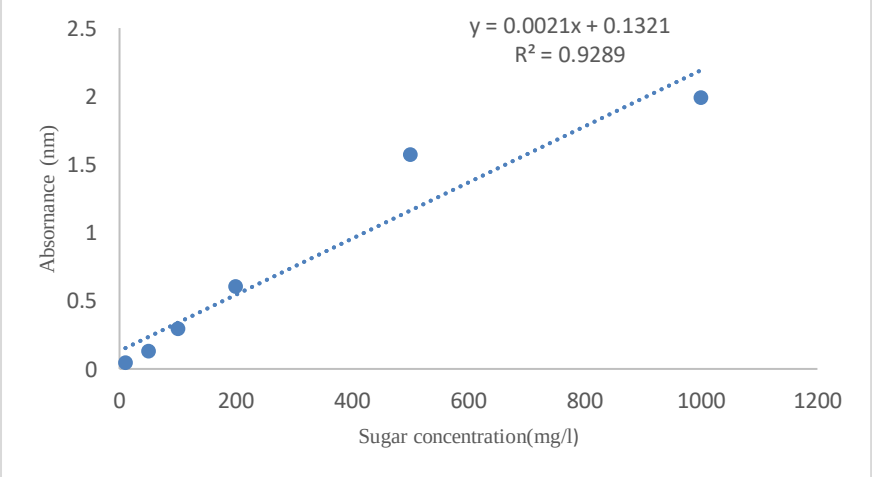

Figure 1:Glucose curve for determining the sugar yield. Extracted from [4]

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Effect of pretreatment on ethanol yield

It was observed that the rate of the reducing sugar conversion from base pretreatment using SSF was the highest with a sugar yield of 356.14 mg/l and 1.7303 % v/v ethanol concentration. In comparison with [28], where two different yeasts were used for fermentation of fruits, S. cerevisiae CAT-1 and S. cerevisiae Angel, the former produced 28.02 g/L of ethanol, resulting in 98% consumption of 359.38 mg/L of reducing sugar produced, while the later resulted in 97% consumption of the reducing sugar produced and 31.87 g/L ethanol production. This suggests that the saccharification and fermentation processes utilized in this work were not duly optimized.

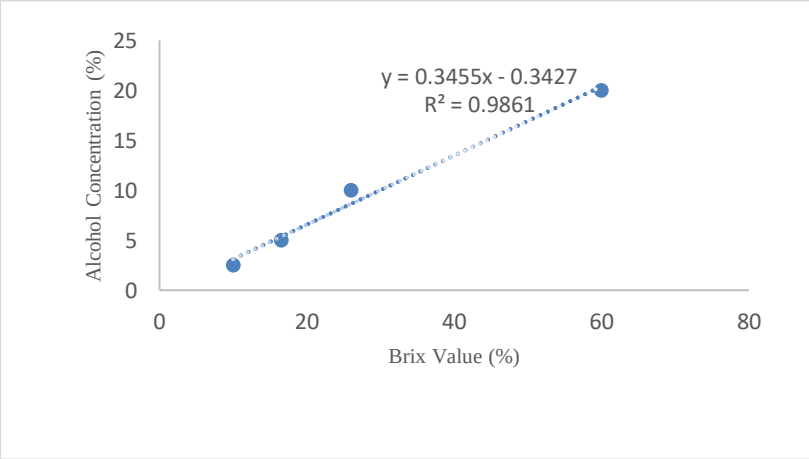

Figure 2: Ethanol concentration curve. Extracted from [4]

Figures 1 and 2 show the reference curves with which the fermentable sugar and ethanol concentrations were determined. The regression equations (1) and (2) were used to calculate the sugar and ethanol yields respectively. This has an R^2 of close to unity (0.9289), which indicates the fitness of the regression model, an indication that the line was properly fitted and therefore the model is valid.

$y=0.0021x+0.1321$ (1)

$y=0.3455x-0.3427$ (2)

Effect of the saccharification method on the concentration of ethanol

Different ethanol samples were produced through separate saccharification and simultaneous saccharification and fermentation methods. The result of the saccharification process shows that SSF gave the highest ethanol concentration of 2.08% v/v in acid-pretreated SCB. Enzymatic saccharification provided the highest ethanol yield (1.21205% v/v) from steaming pretreated SCB. According to the previous report, after 12 hrs. of simultaneous saccharification and fermentation of pawpaw seed, the highest ethanol concentration (4.33% v/v) was achieved [29] which is higher than the results (2.0758% v/v) obtained in this work after 66 hrs. The reduction in ethanol production after 12 hrs. could be a result of catabolite repression [30], of which this work did not monitor the ethanol production before 66 hrs.

CONCLUSION

This work has provided a great insight into the valorization of agricultural waste into bioethanol. It has shown the potential of sugarcane waste in biorefinery. The comparative study showed that SSF has more potential in ethanol conversion when compared to other saccharification methods. However, careful attention should be of great importance during this process to prevent possible negative effects from the interaction between cellulase and yeast. The locally available SCB offers promise for bioethanol production.

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However, the procedures must be enhanced and optimized before SCB-based bioethanol may be commercially viable.

RECOMMENDATION

1. Although acid pretreatment produced the highest sugar yield, chemical pretreatment is known to have its drawbacks, such as pollution, possible corrosion on the reactor, high cost of material for reactor construction to prevent corrosion, formation of degradation components, and vice versa. It is recommended that a proper optimization study be carried out to ascertain which conditions will support the production of sugar from chemical pretreatment while at the same time reducing the disadvantages.
2. Using the perfect enzymes or a combination of enzymes for enzymatic hydrolysis can improve the ethanol concentration, thereby supporting enzymatic hydrolysis against acid hydrolysis, which has some limitations.
3. Simultaneous saccharification and fermentation save a lot of time and resources. Therefore, optimization of the process to ensure high sugar yield and conversion will be of great benefit.
4. A study on the conversion of SCB into other value-added bioproducts is also recommended.

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