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Full Length Research Paper

Dilute acid pretreatment of millet and guineacorn husks for bioethanol production

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Millet and guineacorn husks were pretreated with 3 %, 4 % and 5 % hydrochloric acid concentrations at 30 °C, 40 °C and 50 °C for 20, 25 and 30 minutes prior to bioethanol production over a seven day period using *Saccharomyces cerevisiae*, *Zymomonas mobilis* and the combination of the two organisms as the fermentative organisms. High reducing sugar yields (4.6 %) were obtained from millet husk hydrolysates at all the temperature regimes used (30 °C, 40 °C and 50 °C), most importantly at 4 % acid concentration. Also, the result revealed that the combination of *S. cerevisiae* and *Z. mobilis* produced a yield of 0.44 % after the seventh day period of fermentation from guineacorn husk. This indicated that dilute acid pretreatment could be a suitable option for freeing fermentable sugars from lignocellulosic wastes.

Keywords: Acid, bioethanol, lignocellulosic, pretreatment, reducing sugar

INTRODUCTION

Globally, efforts to substitute alternative fuels for petroleum are gaining attention in a world threatened by climate change, rural economic decline, and instability in major oil-producing countries. This has led to an increased interest in utilizing agricultural crops, residues and wastes to produce biofuels. Biofuel crops take in carbon dioxide from the atmosphere while they are growing, offsetting the greenhouse gases released when the fuel is subsequently burned. Replacing petroleum with biofuel can reduce air pollution, including emissions of fine particulates and carbon monoxide. Biofuel production also can improve rural economies by creating new jobs and raising farm incomes. As a locally produced renewable fuel, ethanol has the potential to diversify energy portfolios, lower dependence on foreign oil, and improve trade balances in oil-importing nations (Danielle, 2008).

However, a major obstacle in the utilization of these abundant raw materials is freeing the reducing sugar content of the plant biomass. Therefore, pretreatment is a necessary process for effective utilization of these lignocellulosic materials to obtain ultimately high degree of fermentable sugars (Seema *et al,* 2007). Therefore, the objective of this research is to consider the possibility of using dilute acid hydrolysis as a pretrreatment option in bioethanol production.

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MATERIALS AND METHODS

Collection and processing of samples

The agrowastes (millet husks and guineacorn husks) were collected from local milling centers in Sokoto metropolis of Sokoto State. One kilogram (1 kg) of each agrowaste sample was collected in a clean polythene bags and transported immediately to the Microbiology laboratory of Usmanu Danfodiyo University, Sokoto. The wastes were powdered using clean pestle and mortar, sieved using a sieve with a pore size of 2 mm.

Acid hydrolysis of raw materials

This was carried out according to the method described by Humphrey and Caritas (2007) and Gupta *et al.* (2009) as follows:

One hundred grams (100 g) of millet husks was weighed into 2 litre capacity conical flasks. Then 1 litre of varying dilute hydrochloric acid (HCl) concentrations of 3.0 %, 4.0 % and 5.0 % were added into the conical flasks. The flasks were covered with cotton wool, wrapped in aluminium foil, heated in a water bath for 20, 25 and 30 minutes at 30 ^{o}C, 40 ^{o}C and 50 ^{o}C and then autoclaved for 15 minutes at 121 ^{o}C. The flasks were allowed to cool, filtered through No1 Whatman filter paper and the pH was adjusted to 4.5 with 0.4M NaOH. The same procedure was repeated for guinea corn husks.

Determination of reducing sugar

The reducing sugar content following hydrolysis of the agrowastes was determined using the dinitrosalicylic acid colorimetric method of Miller (1959). The DNS reagent was prepared by adding 10 g of 3, 5- Dinitrosalicylic acid (DNS) , 2 g of phenol, 0.5 g of sodium sulfite and 10 g of sodium hydroxide to 1000 ml of distilled water. In a seperaate container 40 g of potassium sodium tartarate was dissolved into 100 ml of distilled water. The reducing sugar content of the hydrolysates was assayed by adding 3 ml of 3, 5- DNS reagents to 3 ml of the sample. The mixture was heated in boiling water for 10 minutes to develop the red-brown colour. Then 1 ml of 40 % potassium sodium tartarate solution was added to stabilize the colour and cooled to room temperature under running tap water. The absorbance of the samples was measured at 491 nm using UV-VIS spectrophotometer. The reducing sugar content was subsequently determined by making reference to a standard curve of known glucose concentrations.

Fermentation

The fermentation of the hydrolysed samples was carried out in accordance with the methods described by Brooks (2008) as follows: Fifty milliliters (50 ml) of the millet husk hydrolysates was dispensed into seven 100 ml capacity conical flasks. Each conical flask was replicated seven times. The flasks were then covered with cotton wool, wrapped in aluminium foil, and autoclaved at 120 ^{o}C for 15 minutes. The tubes were allowed to cool at room temperature and aseptically inoculated with the fermentative organisms (6.00×10^{2}cfu/ml for each organism) as follows:

A: inoculated with *Saccharomyces cerevisiae*

B: inoculated with *Zymomonas mobilis*

C: inoculated with *Saccharomyces cerevisiae* and *Zymomonas mobilis*

All the flasks were incubated anaerobically at 30 ^{o}C. One flask was removed every twenty-four hour for a period of seven days. The same procedure was repeated for the guinea corn husk hydrolysates. The fermented broth was distillated at 78 ^{o}C and the distillate collected for further analysis

Determination of concentration of bioethanol produced

This was carried out using UV-VIS quantitative analysis of alcohols using chromium VI reagent according to the methods described by Oyeleke and Jibrin (2009) as follows: 1 ml of standard ethanol was diluted with 100 ml of distilled water to give a concentration of 1 %. Then each of 0, 2, 4, 6 and 8 mls of the 1% ethanol was diluted to 10 mls with distilled water to produce 0, 0.2, 0.4, 0.6 and 0.8 % of the ethanol. To each of the varying ethanol concentrations 2 mls of chromium reagent was added and allowed to stand for an hour for colour development. The absorbance of each concentration was measured at 588 nm using UV-VIS spectrophotometer and the readings used to developed standard ethanol curve. Then 4 mls of each bioethanol samples were put in test tubes and treated with 2 mls of the chromium reagent. The mixture was allowed to stand for an hour and the absorbance measured at 588 nm using the UV-VIS spectrophotometer.

Statistical analysis

Data obtained were statistically analyzed by one-way analysis of variance. Comparison of means were made by the New Duncan's multiple range test (P = 0.05).

RESULTS

The result of the reducing sugar yield obtained from the hydrolysates after hydrolysis with 3 %, 4 % and 5% HCl at 30 ^{o}C, 40 ^{o}C and 50 ^{o}C for 20, 25 and 30 minutes are presented in Figures 1-3. The highest yield of 4. 60% was obtained from millet husk hydrolysates at 40 ^{o}C for 20, 25 and 30 minutes. The lowest yield of 0.118% was obtained from guineacorn husk hydrolysates at 30 ^{o}C for 20 minutes. When 4 % HCl was used the highest yield of 4.60% was obtained from millet husk at 40 ^{o}C for 20, 25 and 30 minutes. This was followed by a yield of 3.70 % obtained from guineacorn husk hydrolysates at 30 ^{o}C for 20 minutes. The lowest yield of 0.04 % was obtained from guineacorn husk at 50^{o}C for 25 minutes. When 5 % HCl was used the highest yield of 4.60 % was obtained from millet husk and guineacorn husk hydrolysates at 30 ^{o}C and 50 ^{o}C for 25 and 30 minutes respectively. This was followed by a yield of 2.88 % from millet husk hydrolysates at 30 ^{o}C for 20 minutes, 40 ^{o}C for all the time regime and 50 ^{o}C for 20 minutes. The lowest yield of 0.19 % was obtained from guineacorn husk at 30^{o}C for 30 minutes. The result of the concentration of bioethanol

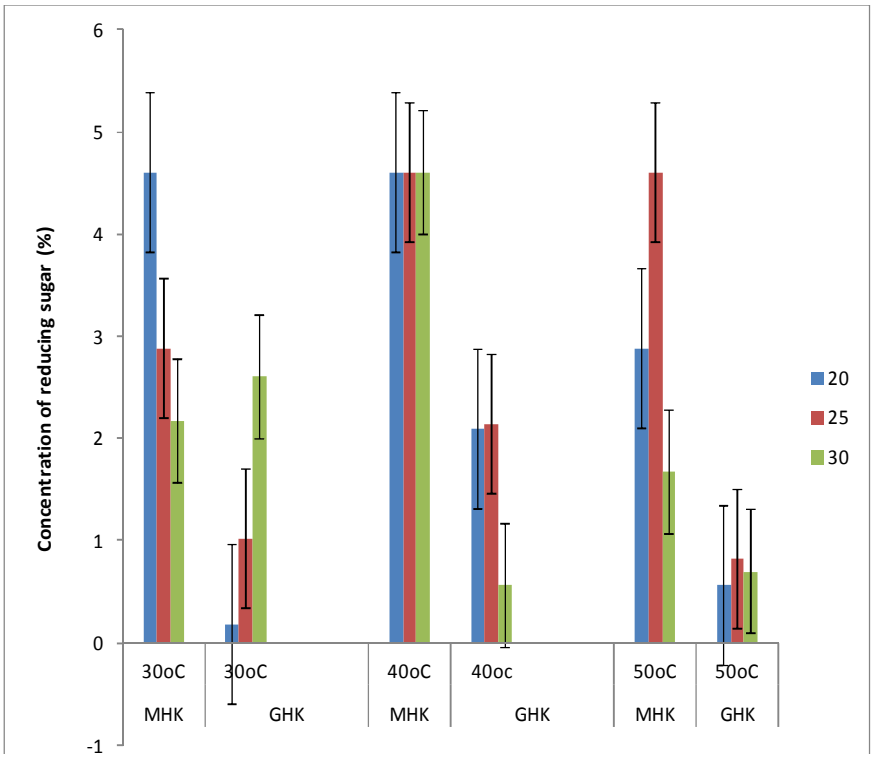

Figure 1. Reducing sugar yield of hydrolysates after hydrolysis with 3% HCl at 30, 40 and 50°C for 20, 25 and 30 minutes

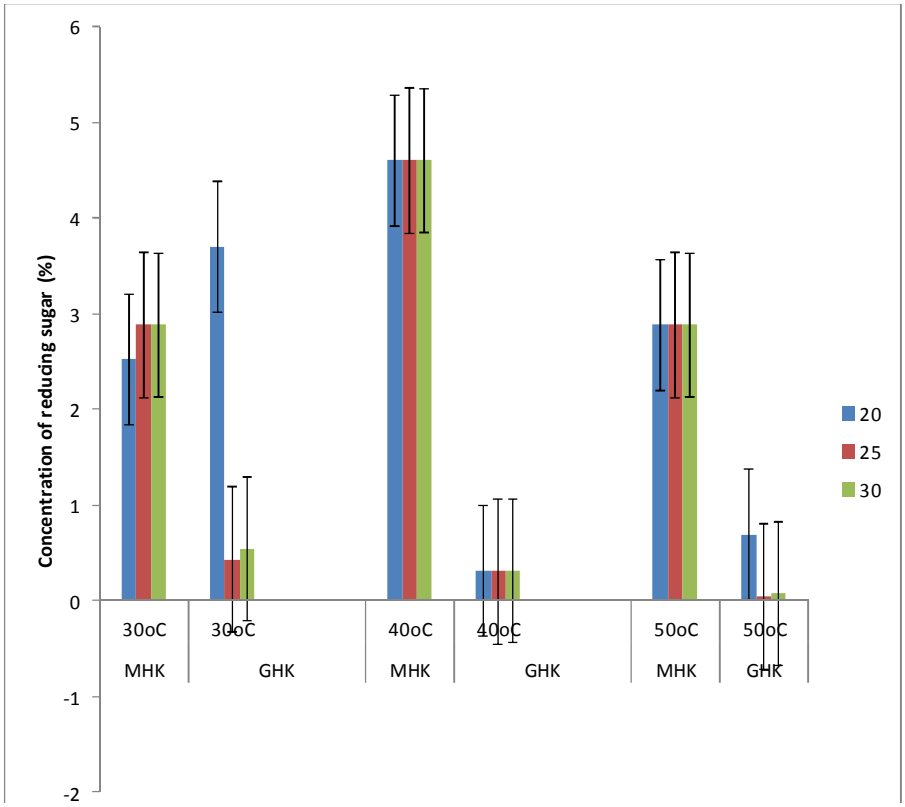

Figure 2. Reducing sugar yield of hydrolysates after hydrolysis with 4% HCl at 30, 40 and 50°C for 20, 25 and 30 minutes

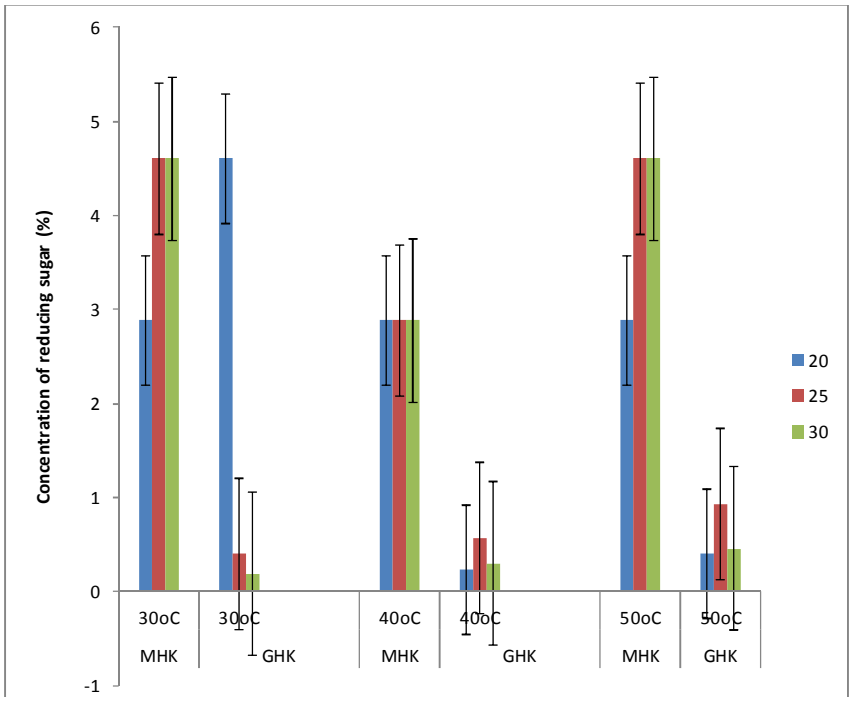

Figure 3. Reducing sugar yield of hydrolysates after hydrolysis with 5% HCl at 30, 40 and 50°C for 20, 25 and 30 minutes

Table 1. Concentration of bioethanol produced from millet husk using *Saccharomyces cerevisiae, Zymomonas mobilis* and the combination of *Saccharomyces cerevisiae* and *Zymomonas mobilis* (%)

Days	*S. cerevisiae*	*Z. mobilis*	*S. cerevisiae + Z. mobilis*
1	$0.03^{c} \pm 0.02$	0.06 ± 0.02	$0.06^{ab} \pm 0.01$
2	$0.13^{a} \pm 0.05$	0.10 ± 0.04	$0.09^{a} \pm 0.04$
3	$0.05^{bc} \pm 0.02$	0.05 ± 0.02	$0.03^{b} \pm 0.01$
4	$0.08^{abc} \pm 0.02$	0.07 ± 0.01	$0.07^{ab} \pm 0.01$
5	$0.12^{a} \pm 0.03$	0.07 ± 0.02	$0.09^{a} \pm 0.01$
6	$0.08^{abc} \pm 0.02$	0.06 ± 0.03	$0.05^{ab} \pm 0.03$
7	$0.11^{ab} \pm 0.04$	0.08 ± 0.02	$0.09^{a} \pm 0.06$
$LSD_{0.05}$	0.05	0.04	0.05

a,b,c means within a column with different superscripts are significantly different (P<0.05)
Values are mean ± standard deviations of three replications

produced from millet husk using *Saccharomyces cerevisiae, Zymomonas mobilis* and the combination of *Saccharomyces cerevisiae* and *Zymomonas mobilis* in a 7 days fermentation period is presented in Table 1. The result revealed that *Saccharomyces cerevisiae* produced the highest bioethanol concentration of 0.13 % after 2 days of fermentation. Similarly, *Zymomonas mobilis* produced the highest bioethanol concentration of 0.10 % after second day of fermentation while the combination of the two organisms produced the highest yield of 0.09 % after seventh day of fermentation. The result of the concentration of bioethanol produced from guineacorn husk using *Saccharomyces cerevisiae, Zymomonas mobilis* and the combination of *Saccharomyces cerevisiae* and *Zymomonas mobilis* in a 7 days fermentation period is presented in Table 2. The result revealed that *Saccharomyces cerevisiae* produced the highest bioethanol concentration of 0.23 % after 6 days of fermentation. Similarly, *Zymomonas mobilis* produced the highest bioethanol concentration of 0.28 % after third day of fermentation while the combination of the two organisms produced their highest yield of 0.44 % after

Table 2. Concentration of bioethanol produced from guineacorn husk using *Saccharomyces cerevisiae, Zymomonas mobilis* and the combination of *Saccharomyces cerevisiae* and *Zymomonas mobilis* (%)

Days	*S. cerevisiae*	*Z. mobilis*	*S. cerevisiae + Z. Mobilis*
1	0.20 ± 0.08	0.18^{ab} ± 0.01	0.23 ± 0.08
2	0.18 ± 0.09	0.24^{a} ± 0.04	0.26 ± 0.04
3	0.19 ± 0.02	0.25^{a} ± 0.04	0.22 ± 0.06
4	0.17 ± 0.05	0.16^{b} ± 0.02	0.25 ± 0.01
5	0.20 ± 0.05	0.20^{ab} ± 0.03	0.28 ± 0.13
6	0.23 ± 0.18	0.14^{b} ± 0.02	0.16 ± 0.03
7	0.20 ± 0.076	0.16^{b} ± 0.07	0.44 ± 0.39
$LSD_{(0.05)}$	0.16	0.07	0.28

a,b,c means within a column with different superscripts are significantly different (P<0.05)
Values are mean ± standard deviations of three replications

the seventh day of fermentation.

DISCUSSION

The results on the hydrolysis of the agrowastes using different acid concentration and temperature treatments revealed high yield of reducing sugars. High yields were obtained from millet husk hydrolysates at all the temperature regimes used (30 ^{o}C, 40 ^{o}C and 50 ^{o}C), most importantly at 4 % acid concentration. However, at 5 % acid concentration, high yields were obtained from millet husk hydrolysates at 30 ^{o}C for 25 and 30 minutes and at 5 % acid concentration at 50 ^{o}C for 25 and 30 minutes. The results revealed a high presence of carbohydrate in millet husks than that obtained from guineacorn husk. Almost all the treatment temperatures produced high yield of reducing sugar. The implication of these findings is that the various temperature regimes used in this study proved to be suitable for hydrolysis of these agrowastes. The result adequately correspond with the report of Mazmanci (2011) who reported that temperature values of 20 ^{o}C, 30 ^{o}C and 40 ^{o}C were less effective for solubility of reducing sugar. When treatment time were considered in all the reducing sugar yield, on the average the highest yields were obtained at 30 minutes treatment period. This is in conformity with the work of Pessoa *et al.* (1997) who reported that reducing sugar yield tend to increase upward at 10 minutes intervals. *S. cerevisiae* produced the highest bioethanol concentration from millet husk after 48 hours of fermentation and it decreased significantly ($p<0.05$) throughout the period of fermentation. However, *Zymomonas mobilis* produced the highest concentration of bioethanol from guineacorn husk after 72 hours of fermentation. The combination of *S. cerevisiae* and *Z. mobilis* produced the highest concentration of bioethanol from guineacorn husk after the seventh day period of fermentation. Similarly, there was no significant difference ($p<0.05$) between the concentrations of bioethanol produced by the two organisms on the first, second and third day of fermentation. The organisms used in the study produced highest concentration of bioethanol at the third day of fermentation. This is attributable to the fact that the organisms reached their growth density within these periods. Obire (2005) reported that *Z. mobilis* reaches its growth density between 12-15 hours of fermentation. Therefore, this could be a reason for its low ethanol yield as the fermentation takes longer period of time.

CONCLUSION

Conclusively, dilute acid pretreatment could serve as a pretreatment option in freeing reducing sugar content of lignocellulosic biomass for bioethanol production. Similarly, the combination of *Saccharomyces cerevisiae* and *Zymomonas mobilis* may serve as a good mixed culture for the production of bioethanol. Also, the study indicated the potentiality of millet and guineacorn husks as cheap raw materials for the bioethanol production process.

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