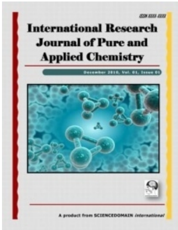

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Bio-ethanol Production from Thermally Pre-treated Corn Chaff and Cassava Waste Water

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Authors' contributions

The first and corresponding author CNI designed the study, wrote the protocol and first draft of the manuscript. Authors CNI, COBO and AUO managed the analyses of the study. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The production of bio-ethanol from corn chaff and cassava waste water was carried out after pretreatment with liquid hot water. Proximate analysis was carried out on the wastes using standard methods and the results obtained for corn chaff were carbohydrate (1.64%), fibre (22%), moisture (60.31%), ash (6.40%), fat (1.56%) and protein (9.09%) while the cassava waste water had carbohydrate (0.78%), fibre (21%), moisture (63.31%), ash (12.32%), fat (0.35%) and protein (2.24%). They were separately pretreated with liquid hot water, hydrolysed enzymatically, fermented with *Saccharomyces cerevisiae* and distilled to produce bio-ethanol. The specific gravity and refractive index were monitored during fermentation, both decreasing as fermentation progressed. The yield of bio-ethanol was 5% (v/v) for corn chaff and 4.2% (v/v) for cassava waste water which is low for sustainable ethanol production. Analysis of the fermentation broth and bio-ethanol produced were carried out using standard methods. Density (0.9218g/cm^3), refractive index (1.3521), viscosity (0.014 Pas) and boiling point (79.5°C) were obtained for bio-ethanol from corn chaff while density (0.9622g/cm^3), refractive index (1.3501), viscosity (0.013 Pas) and boiling point (80.2°C) were obtained for the bio-ethanol from cassava waste water. These were not close to the values expected for pure ethanol. The bio-ethanol produced from these two feedstocks were not of satisfactory quality even after the liquid hot water pretreatment, although that of the

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corn chaff was of better quality. Hence the need to exploit other optimization techniques for the production of bio-ethanol from corn chaff and cassava waste water.

Keywords: Bio-ethanol; corn chaff; cassava waste water; liquid hot water; pretreatment.

1. INTRODUCTION

Bio-ethanol is an important renewable fuel contributing to the reduction of negative environmental impact generated by the daily utilization of fossil fuels. As a result, production and use of biofuels have increased. Although, at the moment bio-ethanol is mainly used in blends with gasoline as E10 and E20, the demand has soared. For instance, consumption of bio-ethanol in most countries of the European Union is far greater than the quantity produced in those countries. According to a study by Hart's Global Biofuels Center (a division of Hart Energy Publishing LP, one of the world's largest energy industry publishers), the Global biofuel use may double from 2009 to 2015 [1].

Bio-ethanol can be obtained from a variety of feedstocks using cellulosic, starchy and sugar sources. These feedstocks include sugar beet, corn, sugar cane, bagasse, sorghum, switch grass, barley, hemp, potatoes, wheat, wood, paper, straw, cotton and other biomass. When ethanol fuel is produced from lignocellulosic materials such as wood, herbaceous plants, wild tubers, agricultural and forestry wastes, its use provides new markets for depressed farm economies. Although each source of biomass represents a technological challenge, the diversity of raw materials will allow the decentralization of fuel production with geopolitical, economical and social benefits [2]. A lot of agricultural raw materials rich in fermentable carbohydrates are being used worldwide for bio-ethanol production, but the costs of these raw materials have become a limiting factor for large scale production. Since the price of feedstock contributes more than 55% to the production cost, inexpensive feedstock such as lignocelluloses biomass and agric-food waste, are being considered to make bio ethanol competitive in the open market [3]. In addition, the use of food materials will put pressure on the cost with attendant food scarcity. Therefore there is the need for sourcing of ethanol from non-food materials. These lignocelluloses biomass and agric-food wastes can be used as potential feed stock for bio-ethanol production and could also be an attractive alternative for disposal of the polluting residues [4]. However most of the wastes are not as viable as expected and therefore needs to be optimized for large production.

Processing of lignocellulosics to ethanol involves pretreatment, hydrolysis, fermentation and separation/purification. Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its sub–microscopic chemical composition and structure so that hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved and more rapidly with a greater yield [5]. It is also necessary for degrading the lignin, decrease cellulose crystallinity, and increase the surface area for enzymatic activity [6]. There are several pre-treatment methods, using acid, steam and liquid hot water (LHW). However, LHW has the major advantage that the solubilised hemicellulose and lignin products are present in lower concentrations, when compared to steam pretreatment, due to higher water input. Due to these lower concentrations, the risk on degradation products like furfural and the condensation and precipitation of lignin compounds is reduced. Weil et al. [7] had a 2- to 5-fold increase in enzymatic hydrolysis of their substrate after LHW pretreatment.

Cassava and corn are starchy food crops and are expected to have high sugar content. However, it is not the case with their wastes such as the cassava waste water and corn chaff. Hence, the present study was carried out to evaluate the chemical composition of corn chaff and cassava waste water in order to explore their potential in bio-ethanol production via LHW pretreatment.

2. MATERIALS AND METHODS

This study was carried out at the National Centre for Energy Research and Development, University of Nigeria Nsukka. Nsukka is 419.4m above sea level. Corn chaff was collected in a container from "*pap*" sellers in the local market while cassava waste water was from a local "*garri*" processing mill. "*Pap*" is locally processed corn and "*garri*" is locally processed cassava. Analytical grade reagents were utilized for this study. The enzymes α-amylase, β-amylase and glucoamylase were produced in the Microbiology laboratory of the University of Nigeria Nsukka. Other materials used were Equitron autoclave, model 7406 ST, water bath, Abbe Refract meter, model WYA - 2S, Jenway pH meter model 3510, hydrometer, beakers, weighing balance and magnetic stirrer.

2.1 Characterization of Corn Chaff and Cassava Waste Water

Proximate analyses of the corn chaff and cassava waste water were carried out. They were characterized for moisture content by the oven dry method, ash content by heating at $550^{\circ}C$ for 24 hours in furnace, crude protein content by the standard Kjeldahl method, fat content using the Soxhlet extraction method and insoluble dietary fiber (IDF) fractions were separated by filtration and the filtrate was subjected to alcohol precipitation to obtain soluble dietary fiber (SDF). The fractions were dried, weighed and expressed as %DM (% Dry matter). The % carbohydrate was calculated by difference of total proximate parameters carried out from 100% [8].

2.2 Thermal Pretreatment

The corn chaff was slurried with water in a 1:2 ratio. The cassava waste water and the slurried corn chaff were separately autoclaved at 135ºC, 103.422×10^{3} Pa for 15 mins. The thermally pre-treated samples were cooled to room temperature and used for further processing.

2.3 Enzymatic Saccharification

The hydrolysates obtained from the pretreatments were subjected to enzymatic saccharification. The crude enzyme filtrate of α-amylase, β-amylase and glucoamylase was weighed and added into a 1000ml beaker flask containing the hydrolysates at 0.5g/ml. The mixture was heated to $65^{\circ}C$ for 1 hour. The temperature of the reaction mixture was cooled to $60^{\circ}C$ and maintained for 1 hour in order to facilitate the enzyme catalysis. The hydrolysis was performed in a flask within a thermostated water bath with stirring. The insoluble residue was removed by filtration and the clear hydrolysates were used for further fermentation studies [9].

2.4 Test for Reducing Sugar

Benedict qualitative reagent (2 ml) was added to five drops of the hydrolysates and boiled for two minutes. A dirty brown precipitate was formed indicating the presence of reducing sugar.

2.5 Fermentation and Ethanol Production

The fermentation was carried out using baker's yeast (*Saccharomyces cerevisiae*). The hydrolysates were autoclaved at 121°C for 15minutes and then cooled to room temperature. The pH of the media was adjusted to 5 using dilute H_2S0_4 and 1% yeast added [10]. The flask was properly connected to lime water through a rubber tubing to ensure that carbon (IV) oxide leaves the flask while trapping water vapour or any volatile alcohol. The end of fermentation was determined by carbon (IV) oxide evolution using lime water. Fermentation was stopped when no more carbon dioxide was coming out of the fermentation medium. The parameters measured daily were pH using a pH meter, density using a hydrometer, refractive index and Brix using an Abbe's refractometer. The fermentation lasted for 72 hours (3 days) under room temperature.

2.6 Distillation and Rectification of Ethanol from Fermented Worth

The fermentation broths were distilled to obtain the bio-ethanol produced. The bio-ethanol was redistilled three more times in order to obtain more concentrated alcohol. It was characterized for boiling point, refractive index using a refractometer, specific gravity using a specific gravity bottle, kinematic viscosity using a viscometer and determination of ethanol content was carried out using Specific gravity method [11].

3. RESULTS AND DISCUSSION

The Corn chaff and Cassava waste water were analysed for various parameters to estimate their potential as good feedstocks for bio-ethanol production. The nutritive content of the feedstock utilized for bio-ethanol production should be sufficient to sustain the enzymes needed for the process. Infact, the nutrients significantly increases the ethanol concentration and fermentation rate [12].

The results of the proximate analysis are given in Table 1.

Table 1. Proximate analysis of corn chaff and cassava waste water

Parameters Sample	Fibre (%)	Moisture content (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)
Corn chaff	22.00	60.31	6.40	1.56	9.09	1.64
Cassava waste water	21.00	63.31	12.32	0.35	2.24	0.78

The protein content of cassava waste water was within the range of 1.7-3.8% which was also obtained by Breuninger et al., [13] for cassava roots but was low when compared to the value obtained by Essien et al. [14], who reported 7.8% protein content in banana peels. The carbohydrate contents of both wastes were quite low which is an indication of the level of sugar available for conversion to bio-ethanol. Comparing the two wastes, the corn chaff had more than twice the amount of carbohydrate contained in the cassava waste water. Again, the lipid content of the feedstocks were lower than 2.2% for citrus [15] but was slightly above 0.2 to 1.4% obtained by Breuninger et al., [13] for cassava roots. In view of the results of the proximate analysis, the pre-treatment was employed to improve the total yield of sugars in the hydrolysis step and also the rate of bio-ethanol production, as the fermentation broth was estimated not to be nutritive enough. Table 2 and 3 show the readings obtained during fermentation at 12 hours interval for three days.

Table 2. Temperature, Brix, Density, pH and refractive indices for the corn chaff

Parameters Days	Temperature (°C)	Brix	Density (g/cm^3)	pH reading	Refractive Index
1	28.1	2.6	1.10	5.79	1.3361
	30.3	2.5	1.07	5.80	1.3361
2	30.9	2.2	1.06	5.96	1.3361
	31.0	2.1	1.05	5.99	1.3362
3	31.1	2.0	1.02	6.10	1.3364
	31.1	2.0	1.02	6.10	1.3367

Table 3. Temperature, Brix, Density, pH and refractive indices for cassava waste water

Days Parameters	Temperature (°C)	Brix	Density (g/cm^3)	pH reading	Refractive index
1.	28.2	7.1	1.12	5.98	1.3364
	30.3	6.8	1.10	5.79	1.3382
2.	31.1	6.6	1.07	6.10	1.3421
	31.3	6.5	1.05	6.10	1.3425
3.	31.3	6.4	1.03	6.29	1.3427
	32.2	5.2	1.03	6.38	1.3450

Table 4. Properties of bio-ethanol produced from corn chaff and cassava waste water

Parameters Bio ethanol	Boiling Point (°C)	Viscosity (centistokes)	Density (g/cm^3)	Conductivity (μs/cm)	Refractive index
Corn chaff	79.5	0.014	0.9218	40	1.3521
Cassava waste water	80.2	0.013	0.9622	120	1.3501

It was observed that there was variation of temperature as a result of activities of yeast cell during fermentation. Temperature varied for the different days with an average of 30°C which was found to be favourable for fermentation. The pH was between 6.1 to 6.4, a range that may not have been favourable for bio-ethanol production as the maximum ethanol yield of bio-ethanol for some studies were obtained at pH 6 [16].

For both wastes, the brix value which is the total sugar content decreased from the first to the third day as expected showing increasing production of ethanol. As fermentation progressed, the depletion of sugar and the accumulation of alcohol pushed the refractive index in the opposite direction. Refractive index also increased to 1.3367 for corn chaff and 1.3450 for cassava waste water by the end of fermentation.

The density decreased from the first to the second day but remained constant at 1.02 and 1.03 for both wastes on the third day for the next 12 hours interval. This indicates progressive production of bio-ethanol. The yield of bio-ethanol from the corn chaff and cassava waste water were 5 % (v/v) and 4.2 % (v/v) which is low for sustainability of bio-ethanol production, as the recommendation of suitable bio-ethanol yield is between the range of 9 – 16% [17]. The specific gravity of the bio-ethanol produced was 0.9218 for corn chaff and 0.9622 for cassava waste water. These specific gravity values were related to the Standard United State Bureau of International Tables, which relates specific gravities to alcohol content. The specific gravity gave alcohol content of about 28% for cassava waste water and 45% for corn chaff.

The results of the refractive index, boiling point and viscosity of the bio-ethanol produced were 1.3521, 79.5°C and 0.014 Pas for corn chaff and 1.3501, 80.2°C and 0.013 Pas for cassava waste water. Comparing these with 1.36, 78°C and 0.0012 Pas for 100% ethanol respectively, it is clear that the bio-ethanol produced still has some level of impurities such as water and may be some higher aldehydes and oil thereby accounting for the deviation from expected values.

4. CONCLUSION

Bio-ethanol produced from cassava waste water and corn chaff were not of very good quality even after the thermal pretreatment. Therefore, other optimization techniques should be exploited for the production of bio-ethanol from processed cassava waste water and corn chaff. The raw material is cheap to acquire and also readily available in relatively large quantities in Nigeria. Hence the need to embark on large scale production of bio-ethanol form these wastes deserves attention. This is because of its economic value which will ensure that the bioethanol product is obtained at far-reduced prices compared with other edible feedstocks.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Johnson CA. Global biofuel use may double by 2015. Ethanol Producer Magazine. Accessed from www.globalbiofuelscenter.com on 1st of October 2009.
2. Bon EPS, Ferrara MA. Bio-ethanol production via enzymatic hydrolysis of cellulosic biomass document prepared for The Role of Agricultural Biotechnologies for Production of Bio energy in Developing Countries, an FAO seminar held in Rome on 12 October 2007, Accessed 20th March, 2011.
   Available: http://www.fao.org/biotech/seminaroct2007.htm.
3. Petersson A, Thomsen MH, Nielsen BA. Potential Bio ethanol production using biomass from banana fruit. Biomass and Bio energy. 2009;31:812-819.
4. Pramanik K, Rao DE. Kinetic study on ethanol fermentation of banana waste using Saccharomyces cerevisiae yeast isolated from toddy. IFCI Journal. 2005;85: 53-58.
5. Mosier N, Wyman C, Dale B, Elander R, Lee YY, Mark H, Ladisch M. Features of promising technologies for pretreatment of lignocelluloses biomass. Bioresource technol. 2005;96: 673–686.
6. Krishna SH, Chowdary GV. Optimization of simultaneous saccharification and fermentation for the production of ethanol from lignocellulosic biomass. J Agricul Food Chem. 2000;48:1971–1976.
7. Weil JR, Sarikaya A, Rau SL, Goebz J, Lasisch CM, Brwer M, Hendrickson R, Tadisch, MR, Pretreatment of corn fiber by pressure cooking in water. Appl Biochem Biotechnol. 1998;73:1–17.

8. AOAC (Association of Official Analytical chemist). Official methods of analysis (15^{th} -ed) Washington, VA: AOAC; 1990.
9. Aden A, Ruth M, Issen K, Neeres K. Ligno cellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis for corn stover. Technical Report NREL/TP 51023438. Golden, co (USA), National Renewable Energy Lab. 2002;143.
10. Hossain ABMS, Ahmed SA, Ahmed M. Alshammari, Faris MA Adnan, Annuar MSM., Hadeel Mustafa, Norah Hammad Bio-ethanol fuel production from rotten banana as an environmental waste management and sustainable energy Afri J Microbiol Res. 2011;5(6):586-598. ISSN: 1996-0808
11. Mojovic L, Nikolic S, Marica R, Vukasinovic M. Production of bio-ethanol from corn meal hydrolyzates. Fuel. 2006; 85:1750-1755.
12. Reddy LV, Reddy OVS, Wee Y. Production of ethanol from mango (*Mangifera indica* L.) peel by Saccharomyces cerevisiae CFTRI101. Afr J Biotechnol. 2011;10(20):4183-4189.
13. Breuninger WF, Piyachomkwan K, Sriroth K. Tapioca/cassava Starch: Production and use. In: Starch chemistry and technology, Be Miller, J. & Whistler, R. 3rd ed. Academic press, New York. 2009;544.
14. Essien JP, Akpan EJ, Essien EP. Studies on mould growth and biomass production using waste banana peel. Technol., 2005; 96:1451:1456.
15. Chau CF, Huang YL. Comparison of the chemical composition and physicochemical properties of different fibers prepared from the peel of Citrus sinensis L., Liucheng, J Agri. Food Chem. 2003; 51: 215: 218.
16. Neelakandan T, Usharani G. Optimization and Production of Bio-ethanol from Cashew Apple Juice Using Immobilized Yeast Cells by Saccharomyces cerevisiae. American-Eurasian J Scientific Res. 2009;4(2):85-88. ISSN 1818-6785.
17. Singh A, Das K, Sharma DK. Production of fermentable sugars and ethanol from agricultural residues. Afri J Biotechnol. 1984;34 A:51-61.

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