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Bioethanol Production Potentials of Corn Cob, Waste Office Paper and Leaf of *Thaumatococcus daniellii*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GREEA, SAL and EIM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors EIM and SAL managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Lignocelluloses-based waste materials are prospective and renewable feedstocks for bioethanol production. Despite their widespread availability, little attention has been paid to their utilization as fermentation feedstocks for bioethanol production especially in developing countries like Nigeria. In this study, three lignocelluloses-based wastes: corn cobs, office paper waste, and *Thaumatococcus daniellii* leaves, commonly generated in Southwestern Nigeria were evaluated as fermentation feedstocks for bioethanol production.
Study Design: The study was a laboratory based experimental study which involved chemical hydrolysis and microbiological fermentation.

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Place and Duration of Study: Department of Environmental Health Sciences, Faculty of Public Health, University of Ibadan, Ibadan, Nigeria, between July 2014 and December 2014.
Methodology: Separate hydrolysis and fermentation method was adopted for the study. Hydrolysis of the feedstocks was carried out at H_2SO_4 concentration of 6 M, 9 M and 13 M at 100℃ for 60 min. Hydrolysates obtained were fermented at 30℃ for 72 hours using *Saccharomyces cerevisiae*.
Results: Reducing sugar yields in the hydrolysates ranged from 51.5 – 27.3%, with highest yields from all the feedstocks obtained at 9M treatment, suggesting that 9M is the optimal concentration of H_2SO_4 for hydrolysis of the tested feedstocks. Highest ethanol yields of 20.2, 16.8 and 15.9 g/L were obtained at 48-hour fermentation period from waste office paper, corn cob and leaves of *Thaumatococcus daniellii*, respectively.
Conclusion: The results thus indicate that 48 hours is the optimal fermentation period for the feedstocks using *Saccharomyces cerevisiae*. The study has demonstrated that ethanol could be produced from the paper, corncobs and leaves of *Thaumatococcus daniellii* leaves with maximum yield obtained from office paper waste.

Keywords: Bioethanol; office paper waste; agro-wastes; corn-cob; leaf of Thaumatococcus daniellii; concentrated acid hydrolysis.

1. INTRODUCTION

Globally, interest in renewable energy supplies has increased considerably in recent years for a number of reasons. Excessive usage of fossil fuels, which provide about 90% of world energy supply [1], has led to increase in atmospheric levels of greenhouse gases and other pollutants, with all their attendant problems which include global warming, acid rains and various health disorders. In addition, there are growing concerns over energy prices, increasing energy demand occasioned by expansion of industrial and transport systems, and dwindling fossil fuel reserves [2]. It is now generally accepted that replacement of fossil fuels with renewable energy supplies is an effective measure to mitigate negative impacts of overreliance on fossil fuels. Promoting the use of renewable energy can also stimulate economic growth through development of agricultural or manufacturing sector.

Biomass-based fuels (also known as Biofuels) are important forms of renewable energy that are receiving increasing attention worldwide. They include fuels like bioethanol, biomethanol, biodiesel, biogas, biosynthetic gas (bio-syngas), bio-char, Fischer–Tropsch liquids, and biohydrogen. Bioethanol (ethanol made biologically from biomass) is the most widely used biofuel for transportation worldwide [3]. It is a promising alternative to liquid fossil fuels as it is a renewable bio-based resource and has potential for low emissions when used in compression-ignition engines [4,5]. Bioethanol has been traditionally produced from food crops such as grains and sugarcane (first generation feedstocks). However, ethanol production from food crops may not be desirable, especially in developing countries, due to direct competition with food supply and its implications for food security. Hence, bioethanol production from non-food biomass sources (second generation feedstocks, mainly lignocellulosic biomass) is now considered essential in the move towards renewable energy supply [5].

Lignocellulosic biomasess such as agricultural residues and waste materials are potentially inexpensive feedstocks for sustainable production of ethanol. However at present, they are often disposed of through open burning or dumping thereby causing various environmental problems. Their utilization for bioethanol production will generally open new economic opportunities and help improve energy security, especially in developing countries where waste management and energy insecurity are important environmental and socio-economic issues. The choice of suitable feedstock for bioethanol is determined by several factors which include less competition with food products, cost and availability. The interplay of these factors highlights importance of search for optimal feedstock in each country [6]. In Nigeria, maize is one of the most important cereal crops grown mainly for human consumption [7]. Being one of the leading corn producing countries in Africa, Nigeria produced 10.3million metric tons of maize in 2013 [8]. Maize production is accompanied by enormous amount of agro-wastes that are currently underutilized. Corn cobs, which form between 27 to 30% of maize agro-wastes [9,10], are a potential feedstock for

the production of bioethanol to fulfil the demand for biofuels.

Waste paper also represents another potentially cheap feedstock that is available on a renewable basis. Annual production of solid waste in Nigeria is about 25 million tonnnes [11] and paper waste accounts for between 7.5-23.1% of total solid waste in most cities [12]. *Thaumatococcus daniellii,* a non sacchariferous sweet plant that normally grows throughout the West African rain forest zone, is one of the many underutilized plants in Nigeria. It is grown predominantly in the cocoa-growing areas of South-Western Nigeria where it is called “Ewe-Eran” or “Adundunmitan” [13]. It grows in the wild and its cultivation by local farmers as part cropping system has been recommended to boost crop production, income generation and ensure sustainable fruit supply [14]. The leaves are mostly used for wrapping foods like bean pudding and are often discarded indiscriminately by the consumers of the food. The abundance of these waste materials in Nigeria coupled with their inexpensive nature provides an opportunity for their exploitation in the production of bio-ethanol. However, little attention has been paid to their assessment as fermentation feedstock in ethanol production.

The use of lignocellulosic biomass as a feedstock for ethanol production requires the hydrolysis of its cellulose to fermentable sugars. This could be done through acid hydrolysis and enzymatic hydrolysis, and the more common of the two is acid hydrolysis. Nearly all acid can be used; however, H_2SO_4 is most commonly used since it is usually the least expensive [15]. Several studies on bioethanol from corn cobs and waste paper using dilute acid and enzymatic hydrolysis have been carried out [16-18]. In this study the effect of concentrated H_2SO_4 on hydrolysis of corn cobs and office paper waste was evaluated. Advantages of concentrated acid process include high sugar recovery efficiency, which could be over 90% of both hemicellulose and cellulose sugars, and the use of relatively low cost materials [15]. To the best of our knowledge, no study has evaluated the ethanol production potential of *Thaumatococcus daniellii* leaves. The purpose of this study was to evaluate the ethanol production potentials of three lignocelluloses-based wastes: corn cob, office paper waste, and *Thaumatococcus daniellii* leaves, commonly generated in Southwestern Nigeria as a waste management strategy using concentrated H_2SO_4 hydrolysis. In addition, the optimal concentration of H_2SO_4 required for hydrolysis was determined.

2. MATERIALS AND METHODS

2.1 Material Collection and Preparation

Fresh leaves of *Thaumatococcus daniellii* and corn-cobs were collected from local market and waste dump site in Ibadan, Nigeria, respectively. After collection, they were taken to the laboratory in clean polythene bag where they were washed with tap water to remove dirt and soil. Subsequently, they were sun dried for about 3- 5 days to reduce the moisture content to about 10%. Office paper wastes were collected from waste bins located within the Ajose building, Faculty of Public Health, University of Ibadan, Nigeria. The materials were milled into powder using mortar and pistil. The materials were analysed for physiochemical parameters. The micro-organism used in this research work, *Saccharomyces cerevisiae*, was purchased from local market.

2.2 Material Pretreatment

Separate hydrolysis and fermentation method was adopted for this study. Each of the biomass was pretreated with acid hydrolysis. Twenty grams (20 g) of the ground biomass were hydrolyzed in 150 ml of various concentrations of H_2SO_4 (6 M, 9 M and 13 M) in a two-stage hydrolysis. In the first stage, the mixtures were heated at 100℃ for 60 mins. Thick gels obtained from the process were pressed into sieves to obtain the filtrates. The remaining solids after the first hydrolysis were mixed with 100 ml of various concentrations of H_2SO_4 (as used in the first stage), the mixtures were heated for 50 mins at 100℃ to hydrolyze further the biomasses. The resulting gels were pressed again as was done in the first stage to obtain the acid-sugar streams. The hydrolysates from the two steps were combined. The mixed hydrolysates were analyzed for total reducing sugar (TRS) and glucose.

The pH of mixed hydrolysates was adjusted to 5.5 by adding required volume of $Ca(OH)_2$. The precipitated $CaSO_4$ was removed from the solution by filtering it through Whatman Number1 filter paper. The filtrate which was a free sugar solution was tested for the presence of reducing sugar before it was used for fermentation.

2.3 Fermentation

The fermentation process was performed under an aseptic condition. Two grams (2 g) of

Saccharomyces cerevisiae was thoroughly mixed with each of the sugar solution obtained from the various substrates in 250 ml conical flask flamed around the mouth region with a lighter. After which it was sealed with a sterile cotton wool to make it air tight. The fermentation broths were placed in the incubator at a temperature of 30℃ for 72 hours to ensure maximum ethanol production. The effectiveness of the fermentation was checked by taking samples from the fermentation broth every 24 hours and testing for the presence of ethanol.

The fermented sample taken 24-hour interval was transferred into a round-bottomed flask and placed on a heating mantle which was fixed to a distillation column enclosed in running tap water. Another flask was fixed to the distillation column to collect distillate.

2.4 Analyses

Moisture content was determined by placing the samples in air-forced oven at 105℃ until a constant weight was achieved, after which the percentage of moisture content was calculated. The ash content was determined by burning the air-dried samples at 550℃ in a furnace until constant weight was attained. The samples were cooled in a desiccator and the percentage ash was calculated. To determine bulk density, an empty container (1000 ml) was weighed. It was filled with the sample, slightly compacted to ensure absence of large void spaces and the container with the sample was weighed again. The bulk density was calculated from the difference between the weight of filled container and that of empty container divided by the volume of the container.

Total organic carbon was determined by Walkey-black method. Total phosphorus was determined by Vanado-molybdate method [19]. Twenty milligram of each sample was digested with 5 ml of 2 M HCl solution and filtered through Whatman filter paper. The concentration of phosphorus in the filtrate was determined spectrophotometrically at a wavelength of 470 nm after the addition of Vanado-molybdate yellow solution. Total nitrogen was determined following Kjeldahl method. Total organic carbon was determined by Walkey-black method [19].

The AOAC method [20] was employed in the determination of glucose yield and total reducing sugar. The glucose yield in the hydrolystate was determined by using the ferric cyanide method while the total reducing sugar content was determined quantitatively by using the Phenol-sulphuric acid method. The amount of reducing sugar released was colorimetrically determined using UV spectrophotometer at a wavelength of 420 nm. A calibration curve was obtained using D- glucose as standard. Presence of reducing sugar in the filtrate of neutralization process was determined using Fehling test.

The concentration of ethanol in the distillate was determined following the AOAC methods [19]. The distillate was collected in an acid solution of potassium dichromate where it was oxidized by acetic acid at 60℃. The residual dichromate was determined by back titration with ferrous sulphate in a strong acid solution using ferroin indicator (1,10-phenanthroline ferrous sulphate complex). The quantity of ethanol produced in g/L was obtained by multiplying the volume of ethanol obtained by the density of ethanol (0.8033 g/mL). The g/L is equivalent to the yield of 100 g of dried substrate [21].

2.5 Statistical Analysis

All data was summarized using descriptive statistics such as mean and standard deviation. A simple linear regression model was used to indicate the relationship between intermediate products and the ethanol yield of the substrates.

3. RESULTS AND DISCUSSION

3.1 Compositions of Feedstocks

Compositions of feedstock are useful in understanding the decomposition process, predicting the energy yield and other technical aspect related to conversion of particular feedstock to useful energy products. Prior to acid pretreatment of the materials and their usage as fermentation feedstocks, their moisture contents, ash contents, bulk densities, total organic carbons, nitrogen contents and phosphorus contents were determined. The results are presented in Table 1. As it can be seen from the table, the level of moisture content in corn cobs was comparable to that found in *Thaumatococcus daniellii* leaves while office paper waste recorded the least level among the feedstocks. The ash contents, residues of lignocellulosic biomass from high temperature treatment containing inorganic mineral elements (major elements: Si, Na, K, Mg and Ca. and minor elements: Al, Fe, Mn, P and S) [22], were less than 10%, with lowest value observed in corn cobs. The values of bulk density and total organic carbon varied considerably among the

feedstocks, with highest values of bulk density and total organic carbon observed in corn cob and office paper waste, respectively. The nitrogen content was generally low in all the feedstocks (<1.0%) while phosphorus was considerably higher in *Thaumatococcus daniellii* leaves and paper waste than in corn cob.

3.2 Production of Reducing Sugars from the Feedstocks

Acid hydrolysis of the feedstocks, using three different concentrations of H_2SO_4, resulted in production of various amounts of reducing sugar, as presented in Fig. 1. Reducing sugar yields showed a similar trend for all the concentrations of acid used (Fig. 1a). The yield was highest in office paper waste for all the concentrations of acid used; suggesting that waste office paper is more susceptible to acid hydrolysis than other feedstocks tested. Comparison of yields across different concentrations of acid used showed that the highest value was obtained in 9M treatment for all the feedstocks and was followed by appreciable reduction at higher concentration; indicating that 9M of H_2SO_4 is the optimal acid treatment for the all the feedstocks tested. Glucose constituted more than half of total reducing sugar obtained in all the feedstocks (Fig. 1b). Interestingly, glucose yield was highest in 9 M of H_2SO_4, which supports earlier proposition that 9M is the optimal acid treatment for all the feedstock tested.

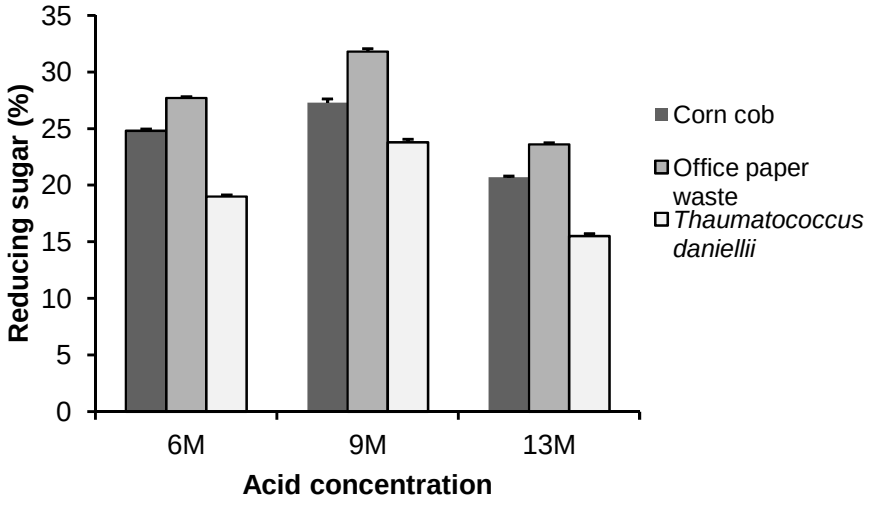
The use of lignocellulosic biomass as a feedstock for ethanol production requires the hydrolysis of cellulose to fermentable sugars. Therefore, the significance of particular lignocellulosic biomass for ethanol production is determined from its ability to produce sufficient amount of reducing sugar and high amount of glucose [23]. Generally, the production of considerable amount of reducing sugars from all the feedstocks tested demonstrates their potentials for bioethanol production. It also confirms the importance of preparative procedure for hydrolysis of feedstock. All the feedstocks were crushed to powdery forms before being used for hydrolysis, which explains recovery of considerable amount of reducing sugar from all the feedstocks. This observation is in line with the findings of previous study [6], where different preparatory steps employed for hydrolysis of different parts of cassava were observed to influence amount of reducing sugar obtained.

Lignocellulosic biomass consists mainly of cellulose, hemicellulose and lignin interlinked in a hetero-matrix structure; approximately 90% of dry matter consists of cellulose, hemicellulose and lignin, while the remaining 10% comprises of ash and extractives [22,24]. The composition of cellulose, hemicellulose and lignin may vary widely among lignocellulosic biomass. The differences observed in reducing sugar yields from the feedstocks may be explained by compositional differences since different compositional characteristics of feedstocks influence their hydrolysis and reducing sugars yields [25]. Highest yields of reducing sugar obtained from office paper waste as compared to other feedstocks may be attributed to high cellulose content of office paper waste. Cellulose molecules consist of long chains of glucose molecules [15], thus high cellulose content will imply high glucose recovery. Though not determined in the present study, higher cellulose content (85-95% of the total weight) has been reported for paper waste [26,27] compared to 26-69.2% of the total weight that have been reported for corn cob [26,28,29]. The results of total organic carbon where the highest organic carbon content was in the order of office paper waste, corn cobs and *Thaumatococcus daniellii* leaves further confirm the statement. In addition, least amount of reducing sugar obtained from *Thaumatococcus daniellii* leaves may also be attributed to its high level of proteinaceous and fat matter [30]. This observation corroborates the finding of previous study [6], where leaves of cassava with high proteinaceous and lipid matter produced low amount of reducing sugar.

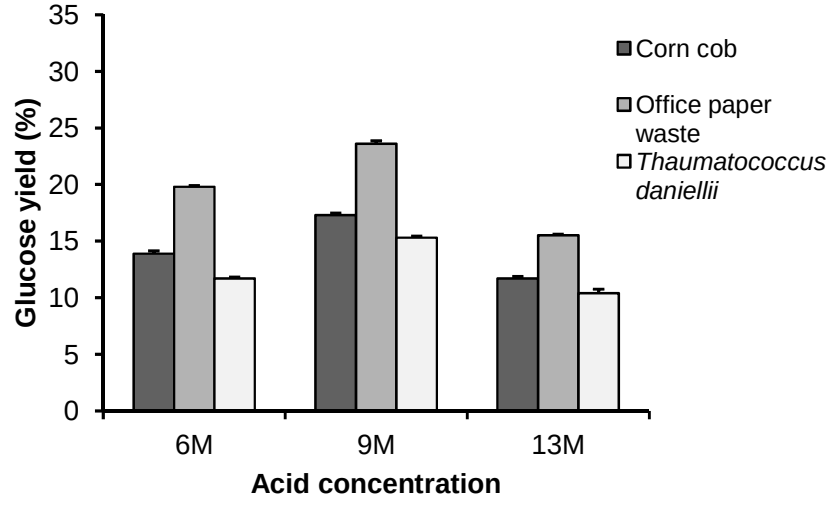
Table 1. Compositions of feedstocks

Feedstock	Moisture content (%)	Ash content (%)	Bulk density (g/cm^3)	TOC (%)	N (%)	P (%)
Corn cob	10.4±1.7[a]	1.4±1.3	265.6±1.5	26.6±0.7	0.4±1.2	0.3±1.5
Office paper waste	5.5±1.3	6.9±1.6	118.9±1.2	38.5±1.3	0.4±1.3	11.2±0.6
Leaves *of Thaumatococcus daniellii*	10.9±1.4	9.7±1.2	169.2±1.0	12.7±1.3	0.3±0.5	6.8±1.0

[a]*Data are mean ± standard deviations (n=3)*



(a)



(b)

Fig. 1. Total Reducing Sugar (TRS), (a) and glucose yield (b), from substrate hydrolysates at different acid concentrations

Bars represent means ± standard deviations (n=3)

Acid hydrolysis largely depends on the concentration of acid solution used. The comparison of the different concentrations of acid revealed that 9 M of H_2SO_4 yielded highest concentrations of reducing sugar and glucose. At higher concentration (>9 M), decrease in production was observed which was accompanied by a lot of charring or dehydrating reaction. The decrease in production might be due to the fact that the substrate could not withstand the higher concentration of acid and the condition thus suppressed the production of glucose and reducing sugars. It is also possible that at higher concentration, secondary reaction that promoted conversion of part of produced sugars to furfural and other complexes, inhibitors of fermentation process in the subsequent step [31], occurred. The decrease in reducing sugar at >9M of H_2SO_4 was expected. Although harsh conditions (concentrated H_2SO_4 solution and high temperature) have been reported to yield significantly high amounts of fermentable sugars [15], they also result in secondary reaction that could reduce the quantity of recoverable sugar [32].

3.3 Ethanol Yield

The hydrolysates from 9M treatment, being the treatment with highest reducing sugar yield, were used for batch fermentation experiments. The ethanol yields for the three substrates during 72 hour-fermentation period are shown in Fig. 2. Generally, ethanol yield increased with increase in fermentation duration during 48 hour-fermentation, with steeper increase in the first 24 hours. Notably, highest yield in all substrates was achieved at 48 hours. There were appreciable reductions in ethanol yield from all the substrates at 72 hours when the fermentation experiments were terminated, suggesting that optimal fermentation period for ethanol production from the tested feedstock using *Saccharomyces cerevisiae* is 48 hours. Interestingly, as also seen in reducing sugar yield, office paper waste yielded highest amount of ethanol at all sampling times, with highest yield of 20.2 g/L at 48 hours. The ethanol produced did not show large differences between corn cobs and *Thaumatococcus daniellii* at all sampling times. The highest yields obtained at 48 hours from corn cobs and *Thaumatococcus daniellii* were 16.8 and 15.9 g/L, respectively. To examine relationship between the intermediate products and the bioethanol yield, a correlation analysis was carried out for substrate with highest ethanol yield (office paper waste). The result (Table 2) obtained showed a statistical significance between the intermediate yield and the bioethanol yield.

Table 2. Relationship between intermediate products and ethanol yield from office paper waste

	Reducing sugar	Glucose	Ethanol
Reducing sugar	1		
Glucose	0.958**	1	
Ethanol	0.928**	0.942**	1

***. Correlation is significant at the 0.01 level (1-tailed), (P = .05)*

The results obtained revealed that the rate of ethanol production during fermentation was affected by duration of fermentation. The reductions in ethanol production observed after 48 hours fermentation, where maximum yields were achieved, might be due to inhibitory activities of accumulated ethanol and build-up of other toxic by-products in fermentation medium as previously observed in other studies [16,33]. A high concentration of ethanol in fermentation broth affects the metabolism of yeast and consequently reduces its efficiency [34], which probably explains the observed trend in the results after 48 hours. The removal of accumulated ethanol has been suggested as the solution to the inhibitory effect. However, results of previous study have shown that inhibitory effect might not necessarily be a result of ethanol accumulation alone but also nutritional and other environmental factors, which should be adjusted appropriately [33].

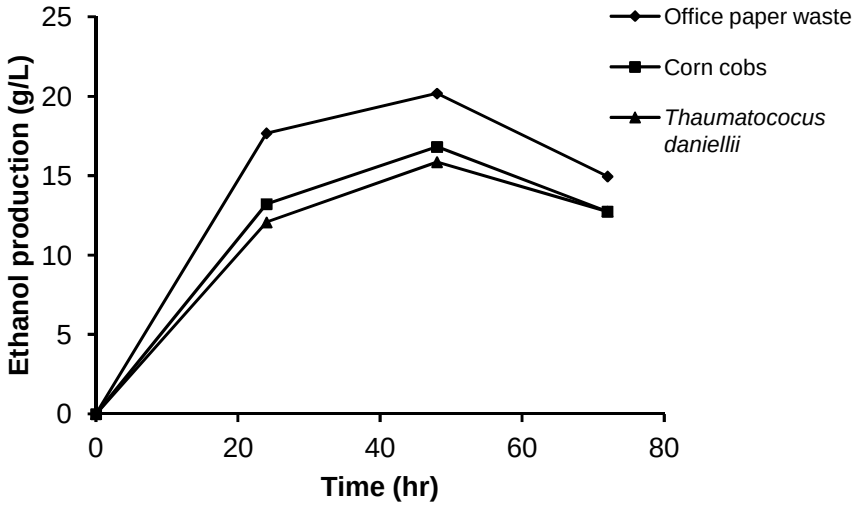


Fig. 2. Ethanol yield from fermentation of substrate hydrolysates at 9.2 M acid concentration

The results also revealed that optimal fermentation period for ethanol production from the tested feedstocks using *Saccharomyces cerevisiae* was 48 hours. The optimal fermentation period (fermentation period for production of highest concentration or yield of ethanol) that has been reported for different feedstocks using *Saccharomyces cerevisiae* varies widely. Optimal period of 24 hours was reported for cassava waste water [33] while it was 144 hours for guineacorn husk [35]. The differences between the present study and other studies thus indicate that optimal fermentation period is not only determined by the type of organism used but also by other factors such as type of hydrolysis employed and should be determined on case by case basis.

Obtaining a sufficient amount of reducing sugars during hydrolysis is a precursor to efficient ethanol production from any feedstock. As expected, the ethanol yield was highest in office paper waste. Compared to office paper waste, lower yields obtained in other feedstocks were due to lower sugar contents obtained during hydrolysis, which were previously attributed to compositional differences of the feedstocks. The ash, lipid and protein contents of feedstocks have been reported to negatively affect ethanol production; specifically influencing the environmental conditions that determine yeast growth regimes in the fermentation broth [6]. However in the present study, it appears that ash contents of feedstocks did not negatively affect ethanol production as evidenced by the comparison of results in Table 1 and Fig. 2. Similarly, the protein content of *Thaumatococcus daniellii* leaves, which has been reported to be considerably high [30], seems not to have negatively affected their ethanol production. Corn cobs were expected to give higher ethanol yield because of their higher reducing sugars content and high level of proteinaceous and fat matter in *Thaumatococcus daniellii* leaves. However, the results revealed that there were no large differences between ethanol yields from the two feedtsocks. The reason for the observed phenomenon is unclear. The lack of large differences in ethanol yields from the two feedstocks could be as a result of some characteristics of the leaves of *Thaumatococcus daniellii*, which may have enhanced their ethanol production and this phenomenon should be investigated further.

The use of agricultural residues or waste materials as an inexpensive feedstock for bioethanol production in preference to food crops, as demonstrated in the present study, provides an interesting option for biofuel production in countries where agro-industrial residues or municipal wastes are very abundant. The results of the present study suggest that in Nigeria where an estimated value of 1.9 million tonnes of paper waste (office paper inclusive) are generated annually [11,12], sustainable ethanol production could be realized from paper waste. Similarly corn cobs which at present are disposed of indiscriminately at any available space could serve as sustainable source of ethanol production. The implication of these is that using feedstocks that hitherto have little or no market value will provide additional income for farmers, waste collectors and others who are involved in activities related to the tested feedstocks. One major drawback that needs to be brought up regarding experimental results reported in this study is the analytical procedures used for determination of ethanol content, which might be less accurate than analytical instrument such as gas chromatography. Nevertheless, this does not mean the results reported here are not of any importance. It only requires further study on the feedstocks using more accurate analytical instrument. Further investigation should also be made to screening several lignocellulosic wastes that are readily found in our environment to select ideal feedstock for bioethanol production.

4. CONCLUSION

The present study has demonstrated a potential for utilizing corn cobs, office paper waste and *Thaumatococcus daniellii* leaves as fermentation feedstock for bioethanol production. The results of the present study showed that utilization of the tested feedstocks, which are continuously accumulated in the environment, could lead to large scale production, thereby promoting safe and economic waste management in the environment and improve energy security. Concentrated acid treatment with 9 M of H_2SO_4 at 100℃ for 60 minutes is recommended as an ideal treatment method for obtaining the maximum reducing sugars yield from hydrolysis of the tested feedstocks. In addition, fermentation period of 48 hours is recommended for batch fermentation of tested feedstocks using *Saccharomyces cerevisiae*. Future work should explore optimization of and continuous ethanol production from the tested feedstocks.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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