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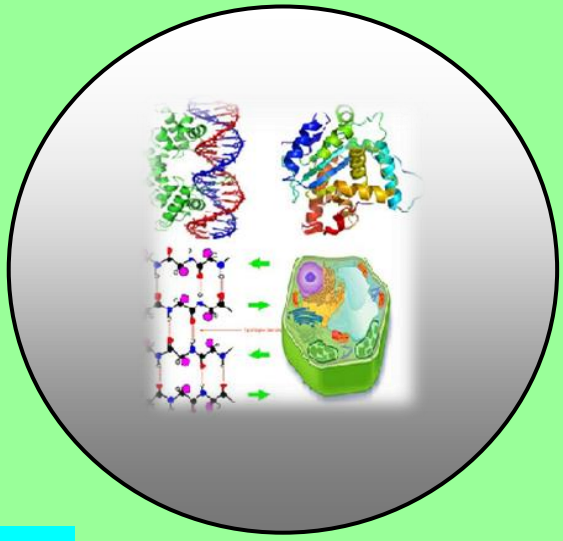

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Proximate composition and production of bioethanol from *Cassava* bagasse using Hydrochloric acid and *Saccharomyces cerevisiae* (Baker's yeast)

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ABSTRACT

Cassava bagasse is a waste material generated from the processing of fermented and non fermented cassava. It is considered to be of little or no significance and is often discarded as waste after the fufu or garri has been processed thereby constituting nuisance to the environment. This study was therefore designed to determine the proximate composition and feasibility of cassava bagasse as a raw material for the production of ethanol as a biofuel. The determinations were done using standard methods of analyses of Association of Official Analytical Chemists (AOAC) using different concentrations of HCl for acid hydrolysis. Results of the proximate analysis showed the following; moisture (3.60%), crude ash (1.52%), crude fibre (19.60%), crude fat (1.63%), protein (0.93%), carbohydrate (72.72%). Acid hydrolysis revealed that different concentrations of acid gave different amounts of reducing sugar in this order; 15.0%, 17.9%, 26.0%, 48.5% and 19.1% for 0.8M, 1.0M, 1.5M, 2.0 M and 2.5M respectively. The ethanol yield also followed this trend as follows, 8.30% v/v, 9.57%v/v, 12.55%v/v, 21.68%v/v and 10.44%v/v for 0.8M, 1.0M, 1.5M, 2.0M and 2.5M respectively.

Keywords: Proximate composition, Acid hydrolysis, Cassava bagasse and Waste management.

INTRODUCTION

The word waste may have different connotations, since what one considers waste may not be waste for another person. In other words, some wastes are not totally useless. In this sense, what are usually considered as waste may no longer hold since such can be recycled

to produce another product. This position is corroborated by what obtains in some countries of the world, where waste recycling had become an entrenched part of waste management strategies. In Nigeria for instance, quite often one sees waste scavengers with their huge sacks as they search through heaps of wastes for all manner of things, ranging from plastics, batteries, old auto parts, other metal junks etc, which are ultimately sold to the smelting industries that convert them into essential raw materials for fabricating various plastic, metal or other equivalent wares for households and/or industrial applications or uses. It is mostly in the Agricultural and food industrial sectors that wastes are mostly generated.

With the advent of the search for alternative or renewable energy sources to take over from the fast depleting finite (fossil fuel) energy sources, these wastes are being turned into raw materials. Essentially, this class of wastes is biological in nature, with reasonable components of them being made up of energy-rich photosynthetic biomass entrapped.

A major problem facing most developing nations of the World is to increase agricultural production without degrading the environment. Food is a basic human need and producing enough to feed the growing population of developing nations is one of the biggest challenges facing a large proportion of nations. Hence, there should be a greater intervention in form of environment friendly science and technology in food production (USDA, 2010). One of such environment friendly intervention is effective management of wastes, particularly as it concerns agricultural and food processing wastes. The quality of the total environment and health status of the inhabitants are related to the quality and quantity of wastes generated in those areas, as partly defined by the nature of activities carried out by the populace. This environment-health relationship dynamics are particularly evident in most tropical environments where various environmental media are laden with pollutants most of which are often furnished by wastes. In Nigeria for instance, it is not disputable that municipal waste is the most visible and serious environmental problem, given the mountainous heaps of wastes (particularly refuse) that are common sights in greater number of urban cities , doting the roads and disfiguring the landscape, with sundry public health implications (Ezejiofor et al., 2013).

Waste could exist either as solid, liquid or gas and the effect of each phase varies. The health implications of these wastes includes: breeding of flies which carry germs on their bodies and legs and deposit them on our food; mosquitoes (vector of malaria parasites and other disease agents) breed in stagnant water, in blocked drainages and in cans, tyres, etc that collect rain water; breeding of rats and other rodents which spread typhus, salmonella, leptospirosis, Lassa fever and other diseases; they also cause loses by biting and spoil millions of tons of food. The refuse workers themselves also face some hazards including parasite infestation and infected cuts resulting from skin contact with refuse; others include hazards on disposal sites e.g. injuries from glass, razor blades, syringes, tissue damage or infection through respiration, ingestion or skin contact.

At present, the transportation sector is almost entirely dependent on petroleum-based fuel, it being responsible for around 60% of the world oil consumption. Biofuels represent an alternative to petroleum-based fuel. In particular, bioethanol is the most widely used biofuel for transportation (Balat, 2008). Bioethanol can be produced from different raw materials which are commonly classified into three categories:

Sucrose-containing feedstocks (sugar cane, sugar beet, sweet sorghum), starch materials (corn, potatoes, cassava, yam wheat etc) and lignocellulosic materials (wood, grasses).

In recent years, there has been an increasing trend toward an efficient utilization of agricultural wastes such as cassava peels, sugar cane bagasse, coffe pulp/husk, apple pomace etc. Several processes have been developed that utilize these as raw materials for the production of bulk chemicals and value added fine products such as ethanol, single cell protein (SCP), mushrooms, enzymes, organic acids, amino acids, biologically active metabolites (Pandey, 1991,1992,). Using agricultural wastes in bioprocesses on the one hand provides alternative substrates and on the other hand helps in solving pollution problems, which their disposal may otherwise cause. With the advent of biotechnological innovations, mainly in the area of enzyme and fermentation technology, many new avenues have been opened for their utilization. These agricultural wastes are promising feedstock for glucose production (an energy source) and a good glucose production technology results in energy generation (Demirbas, 2008).

Cassava (*Manihot esculenta* Crantz) belonging to Euphorbiaceae is a short-lived perennial crop, 1 to 5 meters tall. According to FAO, more than 600 million people depend on the cassava in Africa, Asia and Latin America (Elkholy and Eltantawy 2000). Industrial processing of cassava tubers is mainly done to isolate flour and starch, which generates more liquid and solid residues (processing for flour generates solid residues while for starch generates more liquid residues). Solid residues include brown peel, inner peel, unusable roots, bagasse and flour refuse, among which bagasse is the main residue (Fig.1). Cassava bagasse is made up fibrous material and contains starch that physical process could not extract. Poor processing conditions may result in even higher concentrations of starch in cassava bagasse.

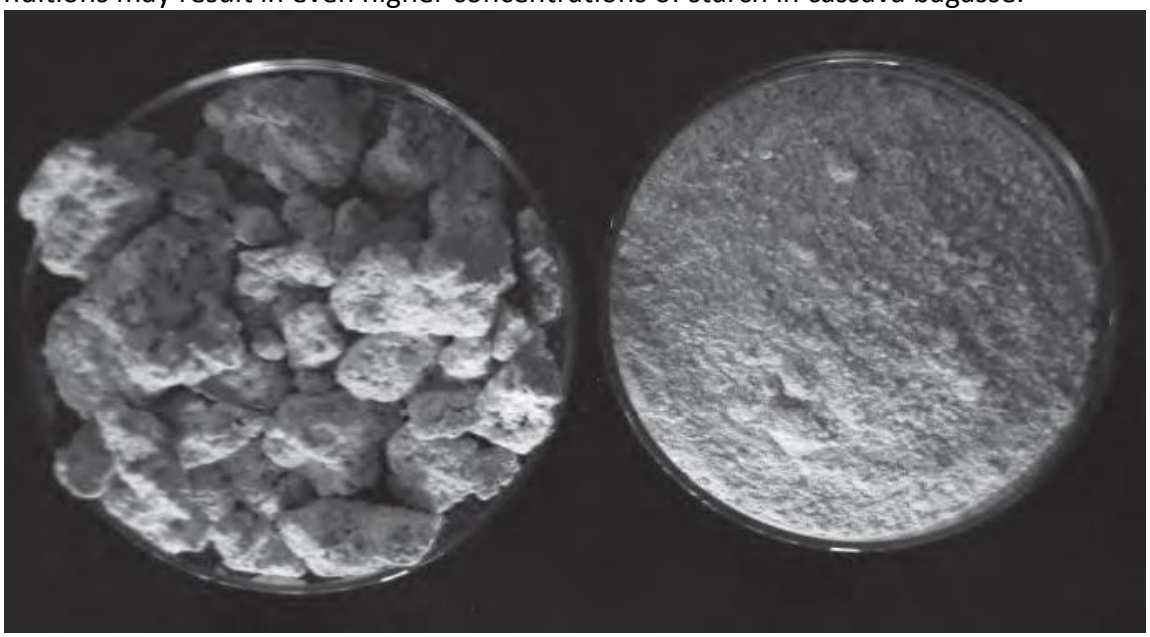

Fig 1. Cassava bagasse.

The molds such as *Rhizopus stolonifer, Neurospora sitophila* and lactic acid bacteria, *Leuconostoc pseudomesenteroides, Leuconostoc mesenteroides, Enterococcus faecium, Weissella cibaria, Lactobacillus plantarum, L. manihotivorans* etc. were identified as the natural microflora in cassava.

There is a restriction of the growth of a wide variety microorganism in cassava due to its cyanogenic content. As there is no cyanogenic content in cassava bagasse, it was used as a suitable substrate for many microorganisms to produce the value added products. Fibrous starchy cassava waste could be utilized to produce many value added products by biotechnological, chemical and physical processes.

MATERIAL AND METHODS

Sample collection and processing

Cassava bagasse was collected after processing the cassava for garri, sorted to remove sticks and dirts, and sun dried. The sample was sun-dried for two weeks with constant turning to ensure proper drying. It was then milled into powder using an electric blender. After milling, the sample was then stored in a properly washed, dried and labeled container for further analyses.

Proximate Analysis

Proximate analysis was carried out on the sample to determine its actual nutrient composition. The proximate composition was determined using the standard methods of analysis of Association of Official Analytical Chemists (AOAC, 1990). Moisture content of the sample was determined by air oven (Gallenkamp) method at 105°C. The crude protein of the sample was determined using micro-Kjeldahl method. Crude lipid was determined by Soxhlet extraction method using hexane as extracting solvent. The ash content was determined using a muffle furnace set at 500°C for 4hours until constant weight of ash is obtained. Crude fibre was determined using the method of Saura-Calixto et al., (1983). The carbohydrate content was obtained by difference.

Acid Hydrolysis

The hydrolysis was carried out according to the method of Teerapatr et al., (2007). The waste (Cassava bagasse) was hydrolysed with 100ml of five different concentrations of Hydrochloric acid (HCl). The concentrations are 0.8M, 1.0m, 1.5M, 2.0M and 2.5M. The hydrolysis was done at 100^{O}C after pretreatment with 50ml of 0.1M HCl at 50^{o}C for 20minutes. The waste was hydrolysed with different concentrations of acid to determine the concentration of acid that will give the maximum yield of reducing sugar for fermentation. The hydrolysis involved boiling the sample with 100ml each of the concentrations of acid at 100^{0}C. The boiling samples were reacted at intervals on a white tile with Iodine solution to monitor the progress of hydrolysis. This gave a blue black colouration which kept reducing in intensity until the blue black colour totally disappeared to give an orange colour, this indicated complete hydrolysis. The boiled samples were then allowed to cool and neutralized with equal concentrations of NaOH to a pH of 5.0. This was followed by filteration using whatman filter paper. The solution was thereafter subjected to Benedict's test for the presence of reducing sugar. The quantity of reducing sugar produced after hydrolysis with different concentrations of acid was then measured with a refractometer.

Preparation of yeast culture

10g of *Sacharomyces cerevisiae* (Bakers' yeast) was added to 50ml of distilled water at room temperature. The solution was stirred for five minutes and allowed to stand for two hours before it was added to the hydrolysates (Ogbuneke et al., 2012).

Fermentation

The activated yeast was asceptically incoculated into the hydrolysates. The solutions were properly mixed before covering the flasks with aluminium foil and left at room temperature.

The flasks were shaken on daily basis till the seventh day. The pH of the solutions were also monitored on daily basis until the seventh day. This was to ensure that they remained within the pH range of fermentation.

Distillation

After fermentation, distillation was carried out on the various hydolysastes. Distillation involved removing ethanol from the mixture of ethanol, water and other impurities. Ethanol was boiled off from the mixture of water and other impurities in distillation column where it was monitored from a temperature of 78^{0}C. The bioethanol produced from distillation were assessed for quality with the following parameters - colour, odour, boiling point, volatility, and specific gravity.

Distinguishing Test for Ethanol

Five drops of the distillate was added to 5ml of iodine solution in a test tube, sodium hydroxide was carefully added until the colour of the iodine disappeared. The test tube was then placed in a water bath at 70^{o}C for 3minutes. It was removed and allowed to cool. Yellow crystals of Iodoform were formed and the smell was reminiscent of an antiseptic. Determination of quantity of ethanol-mixture produced was done by measuring the distillate collected with a 100ml pyrex measuring cylinder.

Specific gravity of the distillate was measured using the specific gravity (density) bottle. The specific gravity bottle was filled with ethanol sample, weighed and recorded. The bottle was also filled with distilled water, weighed and recorded. Specific gravity was calculated thus:

$$\text{Specific gravity} = \frac{\text{Weight of ethanol sample}}{\text{Weight of equal volume of water}}$$

Percentage by volume of the alcohol (ethanol) corresponding to apparent specific gravity at 30^{o}C was read from the AOAC table (1990).

Statistical analysis

Statistical tools used in this study include descriptive statistics e.g standard deviation and coefficient of simple determinant.

RESULTS AND DISCUSSION

Analysis of proximate composition provides information on the basic chemical composition of the agricultural waste. The compositions are moisture, ash, crude fat, protein, crude fibre, and carbohydrate. These components are crucial to the assessment of the nutritive quality of the food being analysed. Table 1 showed the proximate compositions of cassava bagasse. The results obtained from the proximate analysis showed that cassava bagasse is rich in carbohydrate and low in other components.

Table 1. Proximate composition of Cassava bagasse.

Constituents	**% composition**
Moisture Content	3.60 ± 0.04
Crude Ash	1.52 ±0.16
Crude Fibre	19.60 ±0.16
Crude Fat	1.63 ±0.24
Protein	0.93 ±0.43
Carbohyrate	72.72 ±0.06

From the result, moisture content of the sample was very low- 3.60± 0.04%, this was lower than 28.47% and 29.93% reported by Akpabio et al., (2012) as the moisture contents of fresh peels of two cassava cultivars. This may be due to the fact that the moisture contents were determined with fresh peels. The moisture content of foods or its processed products gives an indication of its freshness and shelf life, high moisture contents subjects food items to increased microbial spoilage and deterioration (Adepoju and Onasanya, 2008). In the case of fruits, moisture content is related to its dry matter content (Warner, 2002).

The ash content can provide an estimate of the quality of the product. The result obtained in this study showed that cassava bagasse has a very low ash content (1.52±0.16%). This indicates that cassava bagasse may not have high mineral content especially the macro minerals. The result obtained for ash content in this study (1.52±0.16%) was lower than 4.1 to 6.6% reported by Adebowale and Bayer (2002) from agricultural hulls and husks. It is also lower than that reported by Akpabio (2012) who reported 5.89% and 4.86% ash in the bitter and sweet varieties of cassava respectively. When compared with the result of Devendra (1977), who reported ash content of 4.2% in cassava peels, the result is also lower.

Crude fibre measures the cellulose, hemicellulose and lignin contents of food samples and their processed products. Lignin comprises polymers of phenolic acids and hemicellose is made up of hetero-polymers of polysaccharides (Zakpaa et al., 2010). The result obtained for crude fibre (19.60 ±0.16%) showed that cassava bagasse has high crude fibre content. The result was comparably higher than 8.47% reported by Adeolu and Enesi (2013) for fresh plantain bract. it was also high when compared with the results of Akpabio et al., (2012) who reported 16.42% and 10.78% for two varieties of cassava peels. The result could however compared with the result of Devendra (1977) of 21.1% from fresh cassava peels. The result obtained for crude fibre (19.60 ±0.16%) in this study indicated that the sample used in this study contain enough dietary fibre which has been reported to result in increased removal of carcinogens, potential mutagenes, steroids, bile acids and xenobiotics by binding or absorbing to dietary fibre components and be rapidly excreted (Ayoola and Adeyeye, 2009). Inadequate dietary fibre has been implicated as causative factor of various diseases such as biventricular disease, colon cancer, hyperlipedemia etc. Fibre plays an important role in human and animal nutrition.

Crude fat (lipid content) determines the free fatty lipids of a product. This property can be used as the basis in determining processing temperatures as well as auto-oxidation which can lead to rancidity (affect food flavor). The lipid content of the sample used in this study (1.63 ±0.24) was slightly lower than the results of Adeolu and Enesi (2012) who reported a low lipid content of 1.83% for fresh plantain bract. The result was also very low when compared with the results of Mkharty (2007) who reported 15.5%, 22.3% and 25.6% as the lipid contents of some agricultural wastes.

The protein content of the sample was very low – 0.93±0.43%. The low protein content might be due to the high carbohydrate content of the sample (Akpabio et al., 2012). The result was close to those obtained by Akpabio et al., (2012) who reported 1.40% and 1.98% as the crude protein content of two cultivars of cassava. Protein is an essential component of diet needed for survival of animals and human beings, their basic function in nutrition is to supply adequate amount of required amino acids. Protein deficiency causes growth retardation, muscle wasting, oedema, abnormal swelling of the belly and collection of fluids in the body (Mounts, 2000).

The carbohydrate content of the sample was quite high- 72.72±0.06%, The value may have been so because cassava is a carbohydrate-rich food. The carbohydrate content of was higher than that of Adeolu and Enesi (2013) who reported a carbohydrate content of 60.87% in plantain bract waste. Adegbola and Asaolu (1986) reported that cassava peel is high in soluble carbohydrates. The result obtained for carbohydrate in this study (72.72±0.06%),was also higher than the results reported by Akpabio et al., (2012), they reported a carbohydrate content of 17.40% and 18.86% for two cultivars of cassava peels. The difference may be as a result of difference in dry matter basis. The high carbohydrate contents of cassava bagasse, explains why it yielded a good quantity of reducing sugar and bioethanol.

Table 2. Effect of different concentrations of HCl on Bioethanol production from cassava bagasse by *Saccharomyces cerevisiae.*

Acid Conc. (M)	Glucose Conc. % (after hydrolysis)	Quantity of ethanol mixture(cm^3)	Ethanol conc. (% v/v)	Specific gravity at 30^o C	Mass of ethanol produced (g)	Boiling Point
0.8	15.0±1.00	21.0±1.00	8.20±0.33	0.9884	16.87±0.22	83 ±1^{o}C
1.0	17.9±0.40	24.0±1.84	9.43±1.23	0.9868	19.28±1.93	82 ±2 ^{o}C
1.5	26.0±1.00	19.0±1.80	12.30±0.18	0.9831	15.26±1.08	81±2^{o}C
2.0	48.5±0.60	45.0±0.35	20.97±0.20	0.9725	36.15±0.15	80 ±1^o C
2.5	19.1±0.20	25.0±0.26	10.28±0.65	0.9857	20.08±0.06	82 ±1^{o}C

From the results obtained for acid hydrolysis, the maximum reducing sugar which was 48.5±0.60% at 2.0M HCl gave an ethanol-mixture of 45mls and the concentration of ethanol in the mixture was 20.97±0.20% which is the highest yield. At 0.8M HCl, the quantity of reducing sugar (15.0±1.00) and ethanol yield (8.20±0.33) were low, the same thing was also observed for 1.0M HCl, which gave 17.9±0.40% of reducing sugar and 9.43±1.23% of ethanol. This may be due to the fact that these acid concentrations were not able to complete the breaking down of the cellulose and hemicellulose in this waste at 100^{o}C. It was observed that the solution obtained after hydrolyzing with these concentrations (0.8M and 1.0M) were browner in colour when compared with the other concentrations. At 2.5M concentration of HCl, there was a decrease in reducing sugar concentration. This could be attributed to the fact that at a higher concentration of acid, there is a decrease in the rate of hydrolysis resulting in browning and charring of the solution. Delegenes et al (1990) reported that at increase in concentration of acid, other chemical reactions may occur, like formation of furfural from xylose. Furfural is reported to inhibit activities of some glycolytic enzymes, particularly dehydrogenases in *Saccharomyces cerevisiae* for ethanol production (Banerjee et al 1981). The highest ethanol concentration obtained with cassava bagasse (20.97±0.20%) is lower than 36.02% obtained by Okolie (2012) as the highest ethanol yield from cassava peel when hydrolysed with 0.8M H_2SO_4. The yield was also higher than 10.36g/cm3 obtained by Oyeleke et al (2012) from cassava peels. When compared with the report of Berry (1996), the result was slightly higher. Berry (1996) reported 15 -16% as the maximum ethanol yield in anaerobic fermentation by yeast. The result might be due to more starch remnants trapped in cassava bagasse after casaava processing.

The boiling points of the bioethanol samples obtained for the different concentrations of acid were 83±1°C, 82±2°C, 81±2°C, 80±1°C, and 82±1°C for 0.8, 1.0, 1.5, 2.0 and 2.5M respectively. All the liquid obtained after distillations were colourless, volatile and had a characteristic odour. However that of 2.0M concentration of acid was more volatile than others and had a stronger odour.

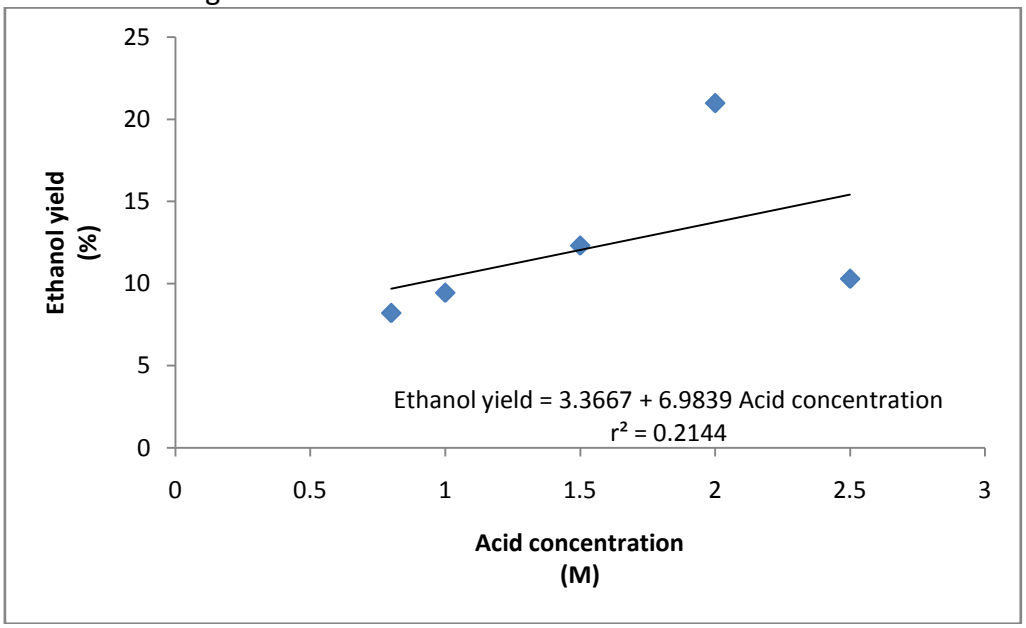

Figure 1. Shows *Plot of Ethanol conc. (%) vs HCl concentration (M) for Cassava Bagasse*
From the regression graph in fig.1, 21.4% variation on the ethanol yield of cassava bagasse was due to the different acid concentrations.

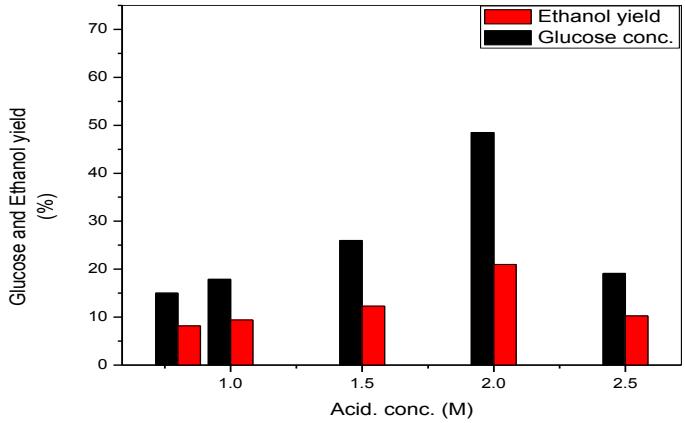

Figure 2. Glucose and ethanol yield of cassava bagasse at different concentrations of acid.

CONCLUSION

This study focused on the proximate analysis and production of ethanol from cassava bagasse using different concentrations of Hydrochloric acid.

The proximate analysis revealed that the waste contain little quantities of other nutrients but rich in carbohydrate because it is a core energy crop.

For bioethanol, the result obtained equally revealed that the concentration of acid used in hydrolysis can affect the concentration of reducing sugar and yield of ethanol. From this study, cassava bagasse yielded a high quantity and concentration of ethanol. From this study also, the bioethanol obtained from 2.0M HCl for cassava bagasse came out as the best in terms of the parameters used in assessing the quality of ethanol. The volatility was higher and its odour was stronger when compared with the other samples. This means that cassava bagasse is a good substrate for the production of biofuel. With Nigeria ranking very high in terms of production of energy crops such as cassava, corn, sorghum, soybean, yam etc, it has immense potential for the production and utilization of bioethanol, biodiesel and other biofuels and formulation of animal feed. It therefore cannot be overemphasized that the adoption of biofuel such as bioethanol has several advantages in a country like Nigeria. It can ease the financial strain relating to the heavy burden of fossil fuel subsidy and enhance livelihood within the production chains. Utilizing these wastes will also result in a healthy environment since discarding them in the open cause environmental degradation. Cassava bagasse is therefore strongly recommended for use in the production of bioethanol. Though other wastes are also good sources of energy, this study revealed that cassava bagassse contains a quantity of energy that is sufficient for the production of ethanol.

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