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Comparative Assessment of Banana, Plantain and Cassava Peels as Potential Feedstock for Bioethanol Production

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Abstract:- Biofuels derived from feedstocks like lignocellulosic biomass are excellent substitutes for fossil fuel as they are more environmentally advantaged and eco-friendly than fossil fuel. The high cost of biomass conversion to biofuel is the main factor impeding large-scale biofuel production. This research project examined the production of biofuel from locally sourced materials obtained and processed at a very low cost. 40g of cassava, plantain (ripe and unripe), and banana (ripe and unripe) peels were pretreated using the physical method which involved shredding, grinding, and heating of substrates to increase the surface area for enzymatic action. Gloeophyllum Sepiarium (Polypore mushroom) and Pleurotus Ostreatus (Oyster mushroom) were used to hydrolyse the substrate at room temperature for 7 days. Saccharomyces Cerevisiae (Baker yeast) was further used to ferment the samples at room temperature for 7 days. The fermented samples were distilled at 78^{o}C and the quantity of bioethanol produced was determined. After the hydrolysis process, the amount of sugar present in the samples was determined using the DNS reagent method. The highest yield of 0.8859g/L was obtained in cassava peels, followed by 0.7440g/L in unripe banana peels, and the least was obtained in ripe plantain peels with a yield of 0.5330g/L. During fermentation of the samples using saccharomyces cerevisiae, the quantity of reducing sugar present in the samples was determined at a 24-hour interval. The value obtained showed a steady decline in the quantity of reducing sugar present in the samples indicating the conversion of sugar to bioethanol. The quantity of bioethanol distilled from the samples was highest in cassava peels with a yield of 13.2cm^3 and lowest in ripe plantain with a yield of 3.9cm^3. This research project revealed that the maximum yield of bioethanol can be obtained on a large scale from cassava peels using low-cost enzyme-producing organisms like mushrooms for hydrolysis.

Keywords:- *Cassava Peels, Banana peels, plantain peels, Bioethanol, Pleurotus Ostreatus, Gloeophyllum Sepiarium, Enzymatic Hydrolysis, Renewable Energy, Biofuels, Sustainability.*

I. INTRODUCTION

Renewable energy is presently being developed as a suitable and viable alternative to conventional fossil energy stemming from concerns about environmental factors, declining supplies of fossil fuels, increase in population and ever-increasing demand for fuels as in the case of Nigeria. Governments all over the world have encouraged the use of alternative sources of energy for the impending energy crisis in many countries of the world (Naik *et al.*, 2010). One of these recommended alternatives are biofuels from lignocellulosic biomass. According to Ogunsiyi (2015), Biofuels are considered reliable substitutes in view of their intrinsic qualities such as renewability, sustainability, biodegradability, non-toxicity, and non-emissions of greenhouse gases. The higher price of oil, which stares fiercely at most oil-demanding nations, has attracted greater attention to biofuels, especially bioethanol. Itelima *et al.*, (2013) defined bioethanol as ethanol fermented from renewable sources for fuel or fuel additives. Bioethanol has been reported as a clean form of liquid fuel that does not pose any health threats to the consumer. Presently, research efforts are being focused on generating bioethanol from biomass resources that will not compete with the food chain. Bioethanol fuel is preferred to its fossil-sourced counterparts because of its zero-carbon emission or CO_2 neutrality (European Commission, 2012).

Ethanol is a common industrial solvent used in the production of varnishes, perfumes, preservatives, essences, flavourings, and disinfectants. It is also used as a fuel additive. Due to the increase in demand in the energy sector, bioethanol production has gained exceptional attention in solving the world energy crisis. Londo *et al.*, (2010) established that the production of bioethanol is economically viable and feasible with available technologies.

Due to the feasibility of generating clean and reliable energy from biomass, the project effort focused attention on the production of bioethanol from plantain, cassava, and banana peels generally known as lignocellulosic biomass.

Plantain (botanically known as musa paradisiaca) and banana (with botanical name - Musa Sapientum) are very important and relevant staples in Nigeria and other tropical African and American Countries. In Nigeria, they are seen in almost all parts of the country especially the south due to the humid nature of the region. The chemical composition of

the peels has been reported to contain high content of carbohydrates, fibres, magnesium, and potassium. The peels' high potassium content, if taken orally aids in maintaining normal blood pressure (Leaf Television, 2016).

These fruits are inexpensive in areas where they are cultivated and it is used in different local food formulations which have high calories and great taste. The use of Plantain for local food formulation includes but is not limited to flour, chips, dodo, and even roasted with fried fish and/or coconut. Banana also serves many purposes as it is eaten either as fruit or food. Unripe banana is crushed and used to prepare banana porridge in some local communities in Nigeria while some eat it exclusively as fruit. The increase in the consumption level of these crops has placed them among the most consumed food crops in Nigeria. This increase as noted by Akubor and Ishiwu (2012), necessitates the determination of the potential of plantain peel utilization in human and /or animal diets. In livestock farming, farmers use fresh plantain peel as parts of the feed for livestock. Ogazi (1990) reported the use of plantain peel in biogas (methanol) production and soap making, indicating the utilization of waste to wealth via the production of valuable materials.

Cassava botanically known as Manihot esculenta is the third largest source of carbohydrates for human consumption in the world. With an estimated annual world production of over 314 million tonnes as at 2021, Africa, which is the largest centre of cassava production in the world accounts for over 63% of the global production volume. Kuiper *et al.*, (2007) noted that this cassava can maintains a reasonable yield on infertile land where the cultivation of other crops is difficult. However, if the situation of food deficit occurs today, the volume of cassava currently produced may not be enough to meet mitigate food insecurity. Therefore, channeling the available volume into ethanol production to meet energy demand will create a deficit in the food supply thus increasing food insecurity. Thus, it is imperative to exploit alternative feedstocks for ethanol production. Peels and other root residues which are not edible are excellent alternative feedstock that can be assessed for suitability in energy production taking into account the fact that this does not interfere with the food supply. Kosugi *et al.* (2009) noted that non-food parts of cassava may play a very significant role in the production of energy since they produce relatively high amounts of biomass, are easily hydrolyzable, and also contain high amounts of dry matter. Furthermore, Akpan *et al.* (2008) mentioned that starch extraction industries produce lignitic and cellulosic materials that may be used for generating ethanol.

Cassava is cultivated in commercial quantities in almost all the states in Nigeria due to the suitability of the soil for the growth of the crop. This crop requires good well-drained soil and well-distributed rainfall. Although production in some local communities is on a subsistent level which is mainly caused by low yield, improved varieties and mechanized practice have shown to have a higher result. Thus, it is one of the most promising sources of starch for alcohol.

Huge quantities of waste are generated in plantain and banana processing factories which are mainly peels containing about 25-35% of the entire crop. These peels can also be gotten from the locals in the communities, restaurants, and plantain roasters on the street. Cassava on the other hand has a promising quantity of waste since it is processed in very large quantities in its processing plant and in local communities for garri which happens to be one of the most consumed commodities.

II. RESEARCH PROBLEM

Waste treatment and disposal are major issues for the majority of countries, regardless of how developed or economically advanced they are. To meet hygienic requirements, wastes could be treated in a variety of ways, such as by decreasing their weight or by recovering and reprocessing them into valuable chemicals. In Nigeria, wastes from banana, plantain, and cassava peels are in abundance and they do not interfere with food security however, they tend to pose serious environmental threats associated with their disposal. There are a plethora of food crops that have been specifically grown for the production of biofuels. However, bio-ethanol production from waste materials is very rare (Itelima *et al.*, 2013). The country is facing several challenges in the area of production of ethanol due to the shortage in food supply and high dependency on imported food. However, the waste generated from the consumption of food (mostly agricultural produce) has always been under-utilized. Agricultural produce such as cassava, plantain, banana, etc. are common food crops readily available, and waste generated from these crops daily is very high; thus to help keep the environment green and increase ethanol supply, these wastes can be utilized to produce bioethanol. Okorondu et al. (2009) mentioned that various investigations have been carried out, aimed at improving the processes employed in the production of ethanol from different feedstock (sugar, starch, and cellulose-based raw materials).

III. RESEARCH OBJECTIVES

- To produce bioethanol from lignocellulosic feedstock using low-cost enzyme-producing organisms
- To compare the yield with the data from literature
- To compare the yield of the different feedstock

IV. LITERATURE REVIEW

A. Bioethanol: An Alternative to Fossil Fuel

Parry (2007) noted that in developing countries with a growing population, increased fossil fuel use and competition for limited resources such as fertile land and water, coupled with rising demand for vehicle fuels, are contributing to an escalation in the excessive use of petrol and diesel. This, in turn, leads to a rise in atmospheric CO_2 concentration and the potential for significant and severe greenhouse gas-mediated climate change. Apart from the depletion of fossil fuel reserves, its abrasive impacts on the environment necessitate extensive research on renewable energy sources such as biofuels to preserve and promote a green ecosystem and fill in the energy demand gap. The

International Energy Agency (IEA, 2010) expects that biofuels will contribute 7% of total fuel use by 2030.

First-generation biofuels are produced directly from food crops like sugarcane, cassava, maize, peanut, and soybean. However, maize and sugarcane are the most commonly used first-generation feedstock. Second-generation biofuels widely known as lignocellulosic fuels include but are not limited to inedible parts of woody grass plants, stalks of sweet sorghum plants, and agricultural residues examples peels, saw dust, stalks, etc. (Kasthuraiah and Kishore, 2016). Second-generation feedstocks are regarded as the most promising renewable resources for biofuel production as they do not interfere with food supply. This is the chief distinguishing factor between these two-generation feedstocks. Second-generation bioethanol production fulfills the impractical gap of first-generation since it employs non-edible feedstock sourced from agriculture and forestry wastes (Aditiya et al., 2016). Important to note that the properties of biofuel itself do not change between generations, but rather the source from which the fuel is derived changes (Kasthuraiah and Kishore, 2016).

B. Characteristics of Lignocellulosic Biomass

Lignocellulosic biomass from several studies has been shown to exhibit great potential for biofuel production. They consist of complex structural polysaccharides, cellulose, hemicelluloses, and lignin (Otulugbu, 2012). Several second-generation biofuel crops like sweet sorghum feedstock consist of cellulose (45%), hemicelluloses (27%), and lignin (21%) (Kim and Day, 2011). Cellulose is regarded as the most abundant component of all plant matter and is responsible for the mechanical strength and chemical stability of plants. While hemicellulose consists of repeated polymers of pentose, and hexose sugars, and depending upon the source their structural composition varies (Iqbal *et al.*, 2013), lignin contains three aromatic alcohols (coniferyl, sinapyl, and p-coumaryl alcohol) produced through a biosynthetic process and act as a protective seal around cellulose and hemicelluloses. Lignin physically obstructs enzymatic bioconversion as it plays a negative role in converting biomass to biofuels (Zeng *et al*, 2014). Thus, by removing the lignin, the cellulose becomes vulnerable to enzymes and allows the yeast to convert the glucose into bioethanol during fermentation (Lalitha and Sivaraj, 2011). Delocalization of lignin is crucial and essential to enable the complete digestion of secondary cell walls of plants by cellulose and hemicellulose.

C. Production of Bioethanol from Lignocellulosic Biomass

For a feasible industrial production of biofuels, and other derivatives from lignocellulosic materials, Wyman (2003) noted that both cellulose and hemicellulose need to be hydrolysed to sugars that in the proceeding steps can be further converted. This conversion is difficult due to:

- The resistive nature of lignocellulosic biomass to degrade or break down.
- The variety of sugars released when the polymers of cellulose and hemicellulose are broken, thus necessitating the need for genetically engineered organisms to efficiently ferment the released sugars.
- The collection and storage cost of low-density lignocellulosic feedstocks.

The conversion of lignocellulose into bioethanol involves basically four steps:

- Pre-treatment
- Hydrolysis
- Fermentation
- Product separation/Distillation.

D. Pre-Treatment

Pre-treatment, according to Tivana, (2012) is to enhance the release of carbohydrates from the biomass for easy conversion to reducing sugar by hydrolysis. Having known that the lignocellulose (peels) especially cassava contain high cyanide concentrations, Tivana (2012) further mentioned that it is imperative to pre-treat the peels before hydrolysis to remove the cyanide content that could hinder microbial and enzyme activities and invariably affect the final reducing sugar yield. This pretreatment process, according to Olabiwoninu and Odunfa (2012) increases the porosity of the lignocellulosic materials and also reduces the formation of by-products that are inhibitory to the enzymatic hydrolysis, thus, reducing the possibilities of loss of carbohydrates.

Primarily, pre-treatment of lignocellulosic materials is done with the aim of removing the hardest part of the lignocellulosic biomasses - lignin, reducing the structural crystallinity, and increasing the surface area of cellulose thereby exposing the cellulose for enzymatic activities. This process as noted by Taherzadeh and Karimi (2008) can be done by various methods which are categorized into three main groups which are; chemical, physical, or physic-chemical and biological.

Aside from the obvious economic impact, another drawback of the pretreatment process as Chandra et al. (2007) mentioned is the generation of inhibitory compounds that can negatively, to a greater degree, influence the action of enzymes and microorganisms. These Inhibitors are produced as a consequence of extreme pH and high-temperature treatment of lignocellulosic materials (Olsson et al., 2005).

Physical pre-treatment methods break down the lignocellulose and reduce the particle size, which increases the surface area for acid or enzymatic attack (Fan et al., 1982). Besides the three main groups of pre-treatment (that is; chemical, physical, or physic-chemical and biological), pretreatment can also be classified as mechanical and non-mechanical (which includes irradiation, high-pressure steaming, and pyrolysis). A common physical pre-treatment is steam with or without explosion. This method eliminates the majority of the solubilised hemicelluloses from the material, making cellulose more amenable to chemical or enzymatic digestion. According to Galbe and Zacchi, (2002) and Ruiz et al., (2006), Steam explosion is initiated at a temperature of 160-206°C from several seconds to a few minutes before the material is exposed to atmospheric pressure. On a commercial and industrial scale, steam explosion is the most preferred pre-treatment process

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because of its low energy cost. The effect of steam pre-treatment, which makes a material suitable for enzymatic hydrolysis, has been debated by several authors to be attributable to acid hydrolysis of the hemicellulose rather than the "explosive" action when the pressure is released. Despite the obvious economic benefit, Hendriks and Zeeman (2009) believe that steam pre-treatment causes partial hemicellulose degradation, the generation of enzymes and fermentation inhibitors, and the incomplete separation of lignin and cellulose.

Another effective pretreatment process is the chemical pre-treatment method which alters the crystal structure of not just the hemicellulose but also the glucose. Galbe and Zacchi, (2007) mentioned the common chemicals used for this method and they include alkaline, dilute acid, concentrated acid, and ammonia.

Using alkaline for pretreatment involves soaking and heating the material in an alkaline solution, such as NaOH. These processes result in the swelling of the material which increases the internal surface area while decreasing the degree of polymerisation and crystallinity. During this process, a major portion of lignin is solubilised with part of the hemicellulose which is recovered primarily as an oligomer. Alkaline pre-treatment as noted by Galbe and Zacchi, (2007) has proven to be more effective on agricultural residues and herbaceous crops than on woody materials.

Using dilute acid like sulphuric acid for pretreatment involves soaking the material in a dilute acid solution at a temperature below 4%, and then heating up the mixture to 120 - 200°C for a few hours typically between 1- 3 hours. This process hydrolyses the hemicellulose and converts it to monomer sugars. Dilute acid pretreatment results in high glucose yield with minimal formation of inhibitors relative to concentrated acid treatment. However, common drawbacks of this method as noted by Larsson et al., (1999) are the difficulty in fermentation because of the inhibitors formed during the treatment, the corrosion of equipment, and the cost of chemical waste disposal.

Green solvents like ionic liquids have been receiving attention lately as a viable alternative to acid and alkaline in chemical pretreatment. Dadi et al., (2006) define ionic liquids as salts with minimum volatility and high thermal stability of up to 300°C. These liquids have the ability to dissolve carbohydrates and lignin to form hydrogen bonds between the non-hydrated chloride ions and the hydrogen atoms of the hydroxyl groups while reducing the formation of degradation products. Examples of ionic liquids include 1-allyl-3-methylimidazolium chloride, 3-methyl-N-butylpyridinium chloride, and 1-n-butyl-3-methylimidazolium chloride. Yang and Wyman (2008), however, expressed skepticism about the future of ionic liquids as the feasible option in full-scale pre-treatment of lignocelluloses.

Another viable pretreatment method is the biological pretreatment which involves using wood-attacking microorganisms like white-rot fungi, and sometimes soft and brown-rot fungi to degrade the lignin and hemicellulose. According to Ander and Eriksson, (1977), soft and brown-rot fungi primarily attack the hemicellulose, while white-rot fungi attack the lignin fraction.

Conclusively, it is pertinent to note that due to the variation in reactivity of cellulose, hemicellulose, and lignin towards different pre-treatment technologies, it is very difficult to generalize; and adopting a certain pre-treatment method for biofuel production depends on the type of lignocellulose as noted by Taherzdeh and Karimi (2008).

According to Silverstein et al., (2007), a successful pre-treatment must meet the following benchmarks;

- Improve sugar formation.
- Avoid the degradation of carbohydrates
- Avoid the formation of inhibitory by-products.
- Be cost-effective.

E. Hydrolysis

The hydrolysis of lignocelluloses followed by fermentation is far more difficult than simple sugar fermentation. The cellulosic portion of the biomass is converted to sugars during hydrolysis, and these sugars are fermented to produce bioethanol. There are two types of lignocellulosic biomass hydrolysis: Enzymatic and acid hydrolysis. Acid hydrolysis is one of the oldest and most applied technologies for converting lignocellulose into fermentable sugars (Jeffries and Jin, 2000). The hydrolysate formed in this process is then used for fermentation by microorganisms. Since the lignocellulose hydrolysate contains in addition to glucose various monosaccharides, such as xylose, galactose, mannose, arabinose, and oligosaccharides, Katahira *et al.*, (2006) opined that microorganisms are required to efficiently ferment these sugars for the successful industrial production of bioethanol.

In enzymatic hydrolysis, viable enzymes are added to the feedstock to catalyze the degradation of the glycosidic bonds of the polysaccharides. The mechanism involved in the enzymatic degradation of lignocellulosic materials depends on the chemical nature and physical structure of the substrate (Taherzdeh and Karimi, 2008). The efficiency of this process depends on the number of enzymes as several enzymes are required to synergistically degrade the various polysaccharides. On an industrial scale, the economic feasibility of a process depends on the cost of enzymes, thus Barta *et al.*, (2010) opined that the cost of enzymes needs to be lowered since it is estimated to contribute 10-20% of the total cost of bioethanol production.

F. Fermentation

Alcohol fermentation is a biochemical process that is brought about by the action of yeast through a process that transforms the natural sugar present in any starchy material into alcohol with the evolution of carbon dioxide (CO_2) under controlled environmental conditions (Miller and Litsky, 1976; Saucedo *et al.*, 1992; Dubey, 2005; Okorondu *et al.*, 2009). A portion of the sugar is absorbed by the yeast cells and converted into lactic acid, acetaldehydes, and glycerol throughout the fermentation process. For the purpose of producing ethanol, the fermentable sugar content

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should be between 8 and 20%, with 13% being the ideal number. According to Joslyn, (1970), the optimum temperature for the production of desirable ethanol is maintained at 23.9 - 26.7°C; while Adams (1978) noted that pH, temperature, and the nutrient level of the fermentation substrate influence the alcoholic fermentation process.

Fermentation is an energy-generation process with no electron transport mechanism (Shuler and Kargi, 2008). Different paths exist, such as the Embden-Meyerhof and Entner-Doudoroff pathways, which may vary from one microbe to the next. According to Shuler and Kargi, (2008), The Embden-Meyerhof pathway is used by yeast to convert glucose to ethanol under anaerobic conditions during fermentation, whereas the bacterium *Zymomonas mobilis* follows the Entner-Doudoroff pathway.

Saccharomyces cerevisiae (*S. cerevisiae*) has generally been recognized as safe (GRAS) and is the most commonly used microorganism in the fermentation industry (Kunz, 2008). The two primary functions of S. cerevisiae are the production of alcoholic beverages and the rise of bread dough. Sugar is converted to energy to produce alcohol, and at the same time, S. cerevisiae gets the energy it requires for metabolism. Yeast uses anaerobic conditions to ferment glucose; the Embden-Meyerhof (EM) route produces carbon dioxide and ethanol as byproducts. Although fermentation is basically carried out in an anaerobic environment, Sanchez and Cardona, (2008) noted that *S. cerevisiae* needs small amounts of oxygen to synthesize fatty acids and sterols.

G. Distillation

The fermented biomass is distilled in a distillation set-up. Ethanol is obtained at a temperature of 78°C.

H. Prospects of Bioethanol

The present annual ethanol production capacity of Nigeria is grossly inadequate. According to Foraminifera (2021), Nigeria only produces 9 million litres of ethanol per year which is 3-4% of the country's requirement. The remaining 96-97% deficit is still being imported from Brazil and India among other countries. Therefore it is imperative that Nigeria explore the abundant agricultural wastes to produce enough bioethanol to meet domestic demand.

The United States is reported to produce about three billion gallons of ethanol from corn in a year. Although fermented agricultural products, which are enormous renewable resources found worldwide, can be used to make ethanol, the efficient synthesis of bioethanol from agricultural wastes is severely constrained. These constraints include the tight chemical and physical bonds that exist between lignin and the polysaccharides found in plant cell walls, as well as the crystallinity of cellulose.

V. METHODS AND MATERIALS

The following materials were used in carrying out this research work; Plantain peels, Banana peels, Cassava peels, Distilled water, Gloeophyllum Sepiarium, Pleurotus Ostreatus, Iodine Solution, Dinitrosalicylic acid (DNS), Sodium potassium tartrate, Saccharomyces Cerevisiae (Industrial Yeast), Sodium hydroxide.

The equipment used are Grinder, Oven, Shaker, Autoclave, pH meter, Knife, 500mL Erlenmeyer flasks, Aluminium foil, Cotton wool, Filter paper, Sample bottles, Test tubes, Syringes (2, 5 and 10mL), Beakers (100mL, 250mL), Spatula, Water bath (Electric bath), Electric heater, Stirrer, Thermometer, Watch, Distillation setup, pycnometer, Digital weighing balance, Cuvettes, Spectrophotometer, micro-Kjeldahl distillation apparatus.

- **Collection of Sample:** The peels were collected around the University of Uyo main campus community, specifically the Use Offot community and its environs into a plastic bag. They were transported to the laboratory where they were washed thoroughly to remove dust, organic matter, and debris.
- **Feed Preparation:** With a kitchen knife, the peels were sliced into pieces that were between 2-4 cm long. The chopped pieces were then sun-dried under mild sunlight for two days and then dried at 60°C in an oven for one day to eradicate the moisture content. The samples were taken out of the oven once they were dry enough to be crushed. The dried pieces of peels were ground in a grinder. The milled samples were then sieved in a 500µm mesh sieve to obtain fine flour and packed in a polythene bag prior to usage.
- **Pretreatment/Gelatinization of Sample:** A slurry was prepared for each sample by adding 100 ml of distilled water to 40g of each of the samples in a 500cm^3 conical flask, after which the flasks were capped with aluminium foil. The samples were heated in a boiling water bath for 1 hour to break down the lignin bond on the biomass, distilled water was added to the flasks to make up to the mark. The samples were sterilized in an autoclave at 120°C for 20 minutes, allowed to cool, and sterilized distilled water was aseptically added to make up to mark again. The flasks were plunged with sterile cotton wool wrapped in aluminium foil to avoid contamination. The sample becomes gelatinized by this treatment.
- **Enzyme Hydrolysis:** *Gloeophyllum Sepiarium* (inedible mushroom) was obtained from a dead tree along Nwaniba road in Nsukara Offot, Uyo Local Government Area, Akwa Ibom State into a black polyethylene bag. They were transported to the laboratory where it was washed thoroughly with sterile distilled water to remove dirt, tiny inserts, and organic matter. They were weighed in an electronic balance and blended with 80%w/v of water in a blender to obtain a slurry. The slurry was filtered in a sterilized 250µm mesh sieve and the filtrate was centrifuged and cultured in a petri dish under aseptic condition. The cells were harvested using a sterile 2ml syringe and inoculated under aseptic conditions into each conical flask containing substrate mixture.
- ***Pleurotus Ostreatus*** (oyster Mushroom) was obtained from an agricultural farm, at the University of Port Harcourt, Rivers State. It was stored tightly in a polyethylene bag and transported to the University of Uyo, Uyo, Akwa Ibom State. The mushroom was washed on arrival using mild sterile distilled water to eradicate any form of dirt and tiny insects. It was blended in a blender with 80%w/v of sterile distilled water and filtered in a 250µm mesh sieve. The filtrate was centrifuged in a centrifuge and cultured in a petri

dish under aseptic conditions. 0.5ml of the cells were harvested using a sterile 2ml syringe and inoculated into each set of substrate mixture. A control was set aside for result comparison. The flasks were covered and incubated at room temperature (28ºC ±2ºC) for seven days. The flasks were shaken daily in an electronic shaker to enable the organisms in the substrate mixture to be distributed evenly and to create a homogeneous solution. The rate of hydrolysis was determined daily using an iodine solution. 0.5ml of each of the samples was pipetted into different test tubes with iodine solution and the colour change was observed. The mixtures were separately filtered after seven days using No. 1 Whatman filter paper.

- **Fermentation:** The supernatants from the above enzyme hydrolysis process were transferred into another set of conical flasks correctly labelled, covered tightly, autoclaved at 120ºC for 20 minutes, and allowed to cool.

Activated free yeast cells were aseptically added into the fermentation flask containing the hydrolysate. The flasks were corked, shaken, and incubated at room temperature (28ºC±2ºC) for seven (7) days. To ensure a uniform dispersion of the organisms in the substrate mixture and a homogenous solution, the flasks were shaken every day throughout the 7 days using an electronic shaker. Fermentation was monitored for seven (7) days during which a decrease in reducing sugar (using the DNS method) and bioethanol produced (using specific gravity method) were determined at a 24-hour interval.

- **Quantitative Analysis of Reducing Sugar Present in the Samples:** The quantitative analysis of reducing sugar present in the samples was carried out using dinitrosalicylic acid (DNS) reagent. 1g of Dinitrosalicylic acid was added to 20ml of 2M NaOH. 30g of potassium sodium tartrate was then added to the solution and diluted to 100ml using distilled water. The solution was then boiled to completely dissolve the content in the solution.

4ml of distilled water was pipetted out into different test tubes, and 0.5ml of DNS reagent was added to each of the test tubes. 0.5ml of each of the samples was then added to the test tubes. The contents were heated in a boiling water bath for 10 minutes. Cooling and reading of absorbance at 540nm (using a spectrophotometer) were done correspondingly. Calculation of the amount of reducing sugar present in the sample was done using the standard glucose graph.

- **Distillation of the Fermented Broth:** The fermented samples at the end of the seven days were transferred into round bottom flasks and each flask was placed on a heating mantle fixed to a distillation column enclosed in running tap water. Another flask was fixed to the other end of the distillation column to collect the distillate at 78ºC which is the standard temperature for ethanol production. This was done for each of the fermented broths. The distillate collected was measured in a measuring cylinder and expressed as the quantity of bioethanol produced in g/L by multiplying the volume of the distillate by the density of ethanol (0.8033g/L). The concentration was determined by comparing the density of the bioethanol produced with the standard ethanol density curv.

VI. RESULTS

Table 1: Absorbance and Concentration of Reducing Sugar of Cassava Peels

Period of Fermentation (hours)	Time (days)	Absorbance	Reducing Sugar(g/L)
0	0	2.995	0.8859
24	1	2.009	0.5974
48	2	1.558	0.4654
72	3	0.967	0.2925
96	4	0.328	0.1055
120	5	0.118	0.0440
144	6	0.103	0.0396
168	7	0.063	0.0279

Table 2: Absorbance and Concentration of Reducing Sugar of Banana Peels

Period of Fermentation (hours)	Time (Days)	Ripe Banana		Unripe Banana	
		Absorbance	Reducing Sugar(g/L)	Absorbance	Reducing Sugar (g/L)
0	0	2.225	0.6606	2.510	0.7440
24	1	1.865	0.5552	.1.803	0.5371
48	2	0.927	0.2808	1.020	0.3080
72	3	0.681	0.2088	0.983	0.2971
96	4	0.549	0.1702	0.325	0.1046
120	5	0.424	0.1336	0.198	0.0674
144	6	0.126	0.0464	0.082	0.0335
168	7	0.081	0.0332	0.072	0.0306

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Table 3: Absorbance and Concentration of Reducing Sugar of Plantain Peels

Period of Fermentation (hours)	Time (Days)	Ripe Plantain		Unripe Plantain	
		Absorbance	Reducing Sugar(g/L)	Absorbance	Reducing Sugar (g/L)
0	0	1.789	0.5330	2.020	0.6006
24	1	0.995	0.3007	1.450	0.4338
48	2	0.590	0.1822	0.935	0.2831
72	3	0.561	0.1737	0.775	0.2363
96	4	0.309	0.0999	0.665	0.2041
120	5	0.119	0.0443	0.210	0.0710
144	6	0.060	0.0271	0.108	0.0411
168	7	0.031	0.0186	0.076	0.03175

Table 4: Bioethanol produced from the different peels.

Qty(g)	Feedstock (Peels)	Volume of Bioethanol Produced (cm^3)	Quantity Produced (g/L)
40	Cassava	13.2	10.60
40	Ripe Banana	6.8	5.46
40	Unripe Banana	6.9	5.54
40	Unripe Plantain	4.5	3.61
40	Ripe Plantain	3.9	3.13

VII. DISCUSSION

A. Evidence of declining levels of reducing sugar in cassava, banana, and plantain peels:

The milled peels were pretreated using the physical pre-treatment method which employs mechanical comminution of the peels to powder form and subsequent heating to reduce the lignin bond on the hemicellulose and cellulose and help in improving the enzyme accessibility to the polysaccharides present in the peels. The pretreatment step was crucial for the enzymatic hydrolysis process as the process aided the removal of the lignin layer and the decrystallization of the cellulose for easy accessibility of the biopolymer by the hydrolytic enzymes. As noted by Balat et al., (2008), the pre-treatment is an essential step in the cellulosic bioethanol technology because it affects the quality and the cost of the carbohydrates-containing streams. The samples were inoculated with edible mushrooms (Pleurotus Ostreatus) and non-edible mushrooms (Gloeophyllum Sepiarium), and after 7 days, the samples were hydrolysed.

To determine the state of hydrolysis in the samples, an iodine solution test was carried out on the sample on a daily basis. On the first day of hydrolysis (immediately after pre-treatment), a blue-black colouration was observed on the different test tubes; however, this colour faded away slightly after each day of hydrolysis. At the end of the seven days, there was no colour change in the test tubes indicating the complete hydrolysis of the samples which is the conversion of polysaccharides to monomer sugars such as glucose and xylose.

Because enzymes are extremely selective and can function under mild process conditions, using them to hydrolyze lignocellulosic biomass is preferable to using chemicals. However, Alessandra et al., (2012) opined that despite these advantages, the use of enzymes in industrial applications is still limited by several factors: the costs of enzyme isolation and purification are high; the specific activity of enzyme is low compared to the corresponding starch degrading enzymes. But this research project proved otherwise as locally sourced, readily available, and inexpensive enzyme-producing organisms were used, thus the disadvantage of enzymatic hydrolysis due to the cost of enzymes is eliminated. The mushroom used for the hydrolysis also aids in the degradation of the lignin bond due to the secretion of lignin-degrading enzymes like lignin peroxidase, laccase, and manganese peroxidase. According to Adebayo et al. (2012), Pleurotus Ostreatus is one of the most effective lignin-degrading organisms that produce laccase, lignin peroxidase, and manganese peroxidase. da Luz et al. (2012) also noted that cellulose-hydrolysing enzymes (that is cellulase) and hemicellulose-hydrolysing enzymes (xylanase) have been reported to be produced by species of Pleurotus and gloeophyllum.

The sugars were fermented to bioethanol by S. Cerevisiae. The reducing sugar concentration was obtained by determining the absorbance of the samples at 540 nm. The sugar hydrolysate was fermented for a period of 168 hours (7 days).

After enzyme hydrolysis of the sample, before the fermentation of the samples, the concentration of fermentable reducing sugar was found to be highest in cassava peels (0.88g/L) and lowest in ripe plantain (0.53g/L) as shown in Tables 1, 2, and 3 above. The fermentation was monitored for 168 hours (7 days) at 24-hour intervals and the concentration of the reducing sugar in each sample after each day was determined. The data as shown in Tables 1, 2, and 3 show a gradual decrease in the concentration of reducing sugar present in the samples indicating the consumption of sugar by S. Cerevisiae in the samples as the fermentation period increases.

B. Comparative bioethanol production on all three peels:

Distillation of samples was carried out at the end of the fermentation period. The highest volume of bioethanol was obtained from cassava peels (13.2cm3) followed by unripe banana (6.9cm3) and the least was ripe plantain (3.9cm3) as shown in Table 4.4 above.

Comparing the volume of bioethanol produced from this research with data from other research papers, while Adetunji et al., (2015), got an average of 8.5% of bioethanol for a 20g cassava peels using Aspergillus Niger, Acheampong et al., (2022) when experimenting the production of bioethanol from cassava peels using on-site and off-site enzymes for hydrolysis got a bioethanol yield of 28.8g/100g of reducing sugar. These figures, although quantitatively measured on different scales, when compared are less than what was obtainable in this research using edible mushrooms (Pleurotus Ostreatus) and non-edible mushrooms (Gloeophyllum Sepiarium) for hydrolysis.

In comparing the result of the bioethanol produced from banana peels with the data from other papers, Sarkar et al., (2022) obtained a total yield of 0.137g of bioethanol per gram of dried banana peels when a study was carried out on the feasibility of banana peels as substrate for bioethanol production using Klebsiella sp. SWET4. Extrapolating this value to align with the basis of this research, it translates to 7.05cm3 of bioethanol per 40g of dried peels. This difference can be attributed to the potency of Klebsiella sp. SWET4 for bioethanol production from peels.

Stanley et al., (2018) investigated the yield of ripe and unripe plantain using different set-ups. The study showed a yield of 7.80ml of bioethanol from 50g of ripe plantain which is higher than the value of 3.90ml of bioethanol from 40g of ripe plantain obtained from this research.

In comparing the yield of bioethanol from the different feedstocks, it can be deduced that bioethanol production using mushrooms for hydrolysis is only feasible with cassava peels as the yields obtained from other feedstocks were significantly lower than those from other researchers.

VIII. CONCLUSION/RECOMMENDATIONS

Agricultural wastes, for example, crop peels which are classified as lignocellulosic biomass are potential feedstock for the production of biofuel. These fuels called lignocellulosic biofuels are gaining wide attention as a possible solution to decrease dependency on fossil fuels and produce a cleaner burning fuel yet not significantly affecting the price of agricultural commodities.

The result of this study confirmed that bioethanol can be produced from cassava banana and plantain peels which are agricultural wastes. A larger quantity of bioethanol was produced from cassava peels followed by unripe banana and the least was ripe plantain peels, thus making cassava peel a better alternative to others. When comparing the results with data from other authors, it was discovered that the yield was only higher in cassava peels and significantly lower in other peels, thus, further experiment on the use of mushroom for hydrolysing plantain and banana peels for bioethanol production is recommended to ascertain the mechanism responsible for the lower yield. Notwithstanding, the use of cassava peels is a worthwhile venture for bioethanol production; considering their cost and because it is a means of controlling environmental pollution since bioconversion of cellulosic biomass into fermentable sugar to produce bioethanol was done using cellulose degrading microorganisms, thus making bioethanol production economical and environmentally friendly and also renewable.

Bioethanol production from these feedstocks could be an attractive alternative for the disposal of these residues. Lignocellulosic feedstock does not interfere with food security and is important for both rural and urban areas in terms of energy security reasons, environmental concerns, employment opportunities, agricultural development, foreign exchange savings, etc.

This environmental and resource-advantaged fuel can be produced to meet global demand by focusing attention on its prospects through the initiation of programmes and organization of research aimed at improving the production method and increasing the volume of biofuel produced per volume of lignocellulosic wastes (peels).

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