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Evaluation of Bioethanol Synthesis from Cassava Roots and Peels

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Abstract— This paper evaluates the potential of fuel-grade bioethanol production from cassava roots and cassava peels; the later a material often disposed as valueless agro waste. Cassava roots and peels are sourced from Nwafor market in Nnewi-North of Anambra State. The roots and peels were both pre-treated and mechanically processed to extract starch. The starch was gelatinized and hydrolysed to prepare the starch for fermentation of bioethanol. Bioethanol was produced by anaerobic fermentation of the starch extract using cultured *Saccharomyces Cerevisiae*. The pH, absorbance and bioethanol concentration were determined. For a fixed fermentation time and equal mass of starch substrates, the result indicates that bioethanol yield from cassava roots' starch is only slightly higher (about 5.0% maximum) than that of cassava peels' starch for various fermentation times and mass of starch substrate considered in this work. We conclude therefore that the cassava peels commonly disposed as waste could be a potential source for bioethanol production. Production of bioethanol from this waste can add to the national energy mix, and consequently ease the heavy overdependence on the conventional fossil based petro-fuel.

Keywords— Renewable energy, Cassava, Starch, Bio-ethanol, Fermentation

I. INTRODUCTION

The recent acute scarcity of petroleum and petro-related products experienced in Nigeria nationwide, adversely affects the economic development of its people; primarily as a result of its concomitant hike in the price of these products. This continuous increase has seen prices of some products more than treble in some parts of the country in recent months. In addition, in other parts of the country the products are not only expensive but are not readily available due to poor roads infrastructure necessary for the distribution of the products. This incessant shortage comes as an irony though; especially when the prices of crude oil is generally on the decrease in the global market. Furthermore, scarcity of petroleum products also comes with its multiplier effects, where prices of essential commodities rise astronomically, and thereby placing undue hardship on the common people.

The consumption and overdependence on fossil based fuels also has other adverse consequences such as severe environmental pollution and emission of greenhouse gases generally believed to be responsible for global warming. Sequel to these concerns therefore, there is a reawakening to shift attention away from overdependence on fossil based sources of energy in order to dampen the effects of global warming and enhance energy security. In addition, other benefits could be derived by deescalating the use of fossil based energy sources; for instance, it could encourage concerted effort to harness alternative energy sources that could ensure safety and energy reliability.

These myriads of problems that Nigeria faces as a result of dwindling energy sector and heavy overdependence on petroleum based energy supply; are evident that the need for Nigeria to diversify its energy sources by exploiting and harnessing alternative energy resources, cannot be overemphasized. Amongst the various and prominent renewable options that are attracting interest worldwide today, is the use of biomass-based ethanol as fuel, commonly referred to as bioethanol which could offer the advantage, in addition to been renewable, of sustainability.

II. BIO-ETHANOL PRODUCTION FROM CASSAVA STARCH

Conventional ethanol is well researched fuel for the past 30 years. It is an alcohol family which could be used as an alternative to gasoline; mainly in form of ethanol/gasoline or ethanol/diesel blends in various concentration up to 100% ethanol with only minor engine modifications. Proponents of this technology argue that using alcohol as fuel proffers a number of directly derived benefits including high quality and low cost fuel for exceptional engine performances. More also, ethanol has high octane-rating thus is capable reducing air pollution, improved automobile engine performance and ethanol can serve as an antifreeze in engines during winter [1-4].

Ethanol can be synthesized from various sources including mineral oils, sugars or starches; the cheapest being starches. Common examples of starch sources are sugarcane, cassava, maize, corn, sorghum and pearl millet. Brazil produces ethanol from sugarcane, while majority in the United States of America use corn as source of starch; while pearl millet is used by the south-eastern region of the country. Countries in the tropical region of the world, like Nigeria and Ghana utilize cassava as their source of starch. It is important to note that the choice of starch source depends on the geographical location of these countries and the availability of the raw material.

Currently, Nigeria is reputed to be the leading producer of cassava in the world; with annual cassava production of 45 million tonnes [5]. Cassava plants can grow in poor soils, are drought resistant, and need a minimum temperature of 17°C. They can use solar radiation up to 300 W/m², and optimum annual rainfall requirement use is 100 to 150cm which implies that cassava can grow in nearly all regions of the country. Consequently, cassava therefore, has a very strategic advantage as a suitable raw material to be used in Nigeria to harness starch for the production of bioethanol. And many researchers [6-9] have elucidated the potentials of cassava as a viable feedstock for bioethanol production.

The problem with using cassava as starch source for bioethanol production however, is that cassava is the third largest source of carbohydrates for human food in the world, with Africa as the largest center of production. Cassava is also known as manioc, manihot, yucca, mandioca, sweet potato tree, and tapioca plant. It is an important food crop in the tropics where it is grown for its starchy, tuberous roots [5]. Cassava is not only the most important staple food in Nigeria; it is also one of the most commercialized agricultural product Nigeria produce. But the good news is that the edible portion is the root which is very rich in starch - starch constituting about 30% of the entire root. A mature root possess three distinct regions: a central vascular core, the cortex (flesh) and the phelloderm (peel). Cassava roots are normally peeled to rid them of two outer coverings; the thin brown outer covering and a thicker leathery parenchymatous inner covering.

In line with the huge cassava production and consumption in Nigeria, correspondingly, large amount of cassava peel is generated annually which inevitably constitutes one of the major agricultural biomass wastes derived from the production of cassava tuber for human consumption, starch production and industrial uses. For instance, it is reported that an estimated 450 000 tonnes [9] of the peel is generated annually in Nigeria, and this trend is projected to increase in the future. This huge volume of cassava peel generated annually also pose a serious environmental challenges. Usually it is discarded and left open to rot often emitting obnoxious smell and badly polluting the environment.

The peels are 1- 4mm thick and may often account for 10-12% of total dry matter [10] and about 20-35% of the dry weight [9] of the root tuber. The peel is a lignocellulolytic material and as such is pertinent to exploit its potential as an alternative starch substrate for bioethanol production [11, 12]. In contrast to the cassava roots, these discarded peels could be used to harness starch for bioethanol production without competing for food security.

This work is aimed to conduct a comparative evaluation of bioethanol production of from cassava root's starch and cassavas peel's starch.

III. Materials And Method

A. Materials

Freshly collected cassava roots and peels, salivary amylase 1500Ukg-l, potassium phosphate buffer, Baker's yeast, glucose, iodine solution, 0.1N HCl solution, Benedict's reagent, filter paper, cotton wool and foil paper. UV spectrophotometer, autoclave, stirrer, oven, shaker, water bath, weighing balance, 200ml conical flasks, measuring cylinders, beakers, pipettes, test tubes and thermometer:

B. Methods

To synthesize bioethanol from cassava root or cassava peel starch, four major unit operations are involved; namely pre-treatment stage, gelatinization of the starch, hydrolysis to obtain reducing sugars and, fermentation of the syrup to bioethanol.

C. Substrate Preparation

Fresh cassava tubers were obtained from Nwafor market, Nnewi –North of Anambra State, Nigeria. The cassava roots were manually peeled and grated into small pieces. The chipped cassava roots were thoroughly washed in tap water to remove dirt and other impurities. Similarly, the peels from the roots and additional extra peels sourced separately, were de-skinned, chipped and washed in tap water. The grated and washed samples of cassava roots and peels were separately milled in a blender machine batch wise, to obtain a homogeneous pulp paste. The paste samples were soaked further in water separately for 1 hour to enable release of starch granules. The soaked samples were then diluted with water forming a slurry. The slurry was mechanically sieved using muslin cloth. The starch solution was collected from the bottom of the sieve while the pulp was removed from the top of the sieve. The starch solutions for both cassava roots and peels were put separately into settling tanks and allowed to stand for about 24 hours to allow for a clear separation of the starch from the water. The supernatant was decanted off to recover the starch. The 'wet' starch samples for both cassava roots and peels were dried overnight in a hot air oven at 50ºC, the moisture content was found to be 10.5% and 11.3% for the root and peel starches respectively. The starch granules were grinded in a ball mill to obtain a fine powder of the starch so as to increase the surface area of the particles to enhance reaction with the enzymes. The fine starch powder samples were weighed to determine the amount of starch present in the cassava roots and cassava peels. The block flow diagram of the steps involved in the extraction of the starch from cassava roots and peels is shown in Fig. 1.

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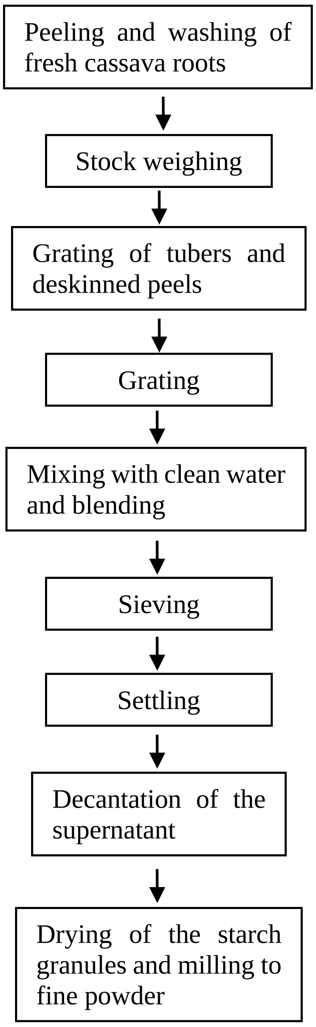

Fig. 1. *Steps in the extraction of starch from cassava roots and peels*

D. *Gelatinization of the starch samples*

For both milled starch samples of cassava root and peel; four (4) batches of 1g, 2g, 3g and 4g respectively were weighed and put in 500ml conical flask, to each flask 0.004g of $CaCl_2$ was added to activate and stabilize the enzymes used for hydrolysis [14]. The mixture in each flask was dissolved in 10ml of distilled water and mixed thoroughly.

The solution mixtures were gelatinized by heating each of the mixture solution in the flasks in a water bath at 105°C for about 5 minutes. The starch was observed to swell due to the temperature rise and constant agitation ensured throughout the period.

E. *Hydrolysis of the starch*

After the process of gelatinization, the prepared starch samples in the flasks were allowed to cool to room temperature. The samples were hydrolysed by adding 2ml of salivary amylase to each flask. The pH was adjusted to 6.5 using phosphate buffer and the hydrolysis was carried out 50°C for 2 hours in the shaker at a speed of 200 rpm. The presence of reducing sugar syrup was tested using Benedict's solution.

F. *Fermentation*

Yeast (*Saccharomyces cerevisiae*) was obtained from the Department of Crop Protection, University of Maiduguri, Nigeria. The microorganism was grown on YPD agar slant at 30°C for 4 days and was stored at 4°C with regular sub-culturing.

The inoculum was prepared by using mineral salts basal medium solution -which is a modification of the synthetic medium developed by previous workers [13]; 25 ml of the medium was measured into each of the three flasks, the flasks were cotton plugged and sterilized in an autoclaved at 121°C for 15 minutes. The sterilized medium was allowed to cool to room temperature and large amounts of the microorganism *S. cerevisiae* was scraped from the cultured slant and added to each of the medium in the flask aseptically using a flame and an inoculating loop. The flasks were transferred to the gyratory incubator shaker and growth was achieved at a temperature of 30°C and an agitation rate of 200rpm for a period of 24 hours. This period was sufficient to allow the microorganism reach its exponential growth phase.

The fermentation medium was prepared by scaling up the amount of the semi-synthetic medium mineral salts. The appropriate quantities of the medium composition were calculated based on 200ml of glucose syrup in each flask. The flasks were cotton plugged and sterilized in an autoclaved at 121°C for 15 minutes. The flasks were allowed to cool to room temperature and then inoculated aseptically with the microorganism grown in the basal medium. The content of the flasks were poured into each of the reducing syrup and anaerobic fermentation was carried out in a shaker at a temperature of 30°C and an agitation rate of 200 rpm for a period of 48 hours. Samples were collected at 12-hours intervals and analysed for ethanol concentration using UV spectrophotometer.

IV. Results And Discussion

A. *Bioethanol Yield from Cassava Roots' Starch*

Table 1 to Table 4 show the results of bioethanol production from cassava root starch for the various substrate levels investigated at 12 hours intervals up to 48 hours.

TABLE I. CONCENTRATION OF BIOETHANOL FROM CASSAVA ROOT STARCH AFTER 12 HOURS

Mass of Sample, g	pH	Absorbance	Conc. (mmol/l)
1.0	5.7	1.681	1.675×10^{-3}
2.0	5.6	1.739	1.739×10^{-3}
3.0	5.4	1.790	1.786×10^{-3}
4.0	5.2	1.844	1.843×10^{-3}

TABLE II. CONCENTRATION OF BIOETHANOL FROM CASSAVA ROOT STARCH AFTER 24 HOURS

Mass of Sample, g	pH	Absorbance	Conc. (mmol/l)
1.0	5.3	1.764	1.752×10^{-3}
2.0	5.1	1.778	1.786×10^{-3}
3.0	4.9	1.834	1.834×10^{-3}
4.0	4.7	1.882	1.887×10^{-3}

TABLE III. CONCENTRATION OF BIOETHANOL FROM CASSAVA ROOT STARCH AFTER 36 HOURS

Mass of Sample, g	pH	Absorbance	Conc. (mmol/l)
1.0	4.6	1.851	1.857×10^{-3}
2.0	4.5	1.879	1.883×10^{-3}
3.0	4.3	1.914	1.916×10^{-3}
4.0	4.1	1.958	1.951×10^{-3}

TABLE IV. CONCENTRATION OF BIOETHANOL FROM CASSAVA ROOT STARCH AFTER 48 HOURS

Mass of Sample, g	pH	Absorbance	Conc. (mmol/l)
1.0	4.2	1.934	1.934×10^{-3}
2.0	4.0	1.951	1.945×10^{-3}
3.0	3.8	1.993	2.036×10^{-3}
4.0	3.7	1.999	2.118×10^{-3}

B. *Bioethanol Yield from Cassava Peels' Starch*

Table 5 to Table 9 show the results of bioethanol production from cassava peel starch for the various substrate levels investigated at 12 hours intervals up to 48 hours

TABLE V. CONCENTRATION OF BIOETHANOL FROM CASSAVA PEELS' STARCH AFTER 12 HOURS

Mass of Sample, g	pH	Absorbance	Conc. (mmol/l)
1.0	5.9	1.639	1.647×10^{-3}
2.0	5.8	1.718	1.713×10^{-3}
3.0	5.5	1.740	1.732×10^{-3}
4.0	5.3	1.756	1.751×10^{-3}

TABLE VI. CONCENTRATION OF BIOETHANOL FROM CASSAVA PEELS' STARCH AFTER 24 HOURS

Mass of Sample, g	pH	Absorbance	Conc. (mmol/l)
1.0	5.6	1.715	1.706×10^{-3}
2.0	5.4	1.729	1.737×10^{-3}
3.0	5.1	1.819	1.812×10^{-3}
4.0	5.0	1.834	1.834×10^{-3}

TABLE VII. CONCENTRATION OF BIOETHANOL FROM CASSAVA PEELS' STARCH AFTER 36 HOURS

Mass of Sample, g	pH	Absorbance	Conc. (mmol/l)
1.0	4.8	1.841	1.838×10^{-3}
2.0	4.6	1.829	1.827×10^{-3}
3.0	4.4	1.873	1.873×10^{-3}
4.0	4.3	1.938	1.932×10^{-3}

TABLE VIII. CONCENTRATION OF BIOETHANOL FROM CASSAVA PEELS' STARCH AFTER 48 HOURS

Mass of Sample, g	pH	Absorbance	Conc. (mmol/l)
1.0	4.4	1.905	1.912×10^{-3}
2.0	4.3	1.918	1.928×10^{-3}
3.0	4.2	1.990	2.011×10^{-3}
4.0	3.9	1.996	2.064×10^{-3}

C. Comaprison of Bioethanol Yield from Cassava Roots and Peel

Fig. 1. to Fig. 5. illustrate the concentration of bioethanol derived from the fermentation of the hydrolyzed cassava root and peel starches. As expected, the concentration of bioethanol was found to increase steadily with amount of substrate and the fermentation times. This implies that that the sugar is being fermented by the activity of the selected enzyme (yeast; *saccharomyces cerevisae*). The trends show that the bioethanol concentration for all the substrates levels considered was lowest in the first 12 hours and highest after 48 hours of fermentation.

We observed that for all fermentation runs, it could be observed that the cassava root's starch gave higher bioethanol yield relative to cassava peel's starch. The peel's bioethanol yield is only about 5% lower than the cassava root's starch at most. This indicates that cassava peels have high potential as a feedstock for bioethanol production.

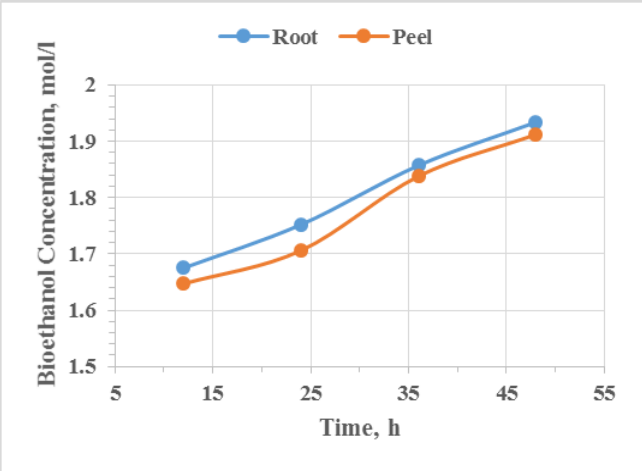

Fig. 2. *Bioethanol concentration for 1g substrare starch sample*

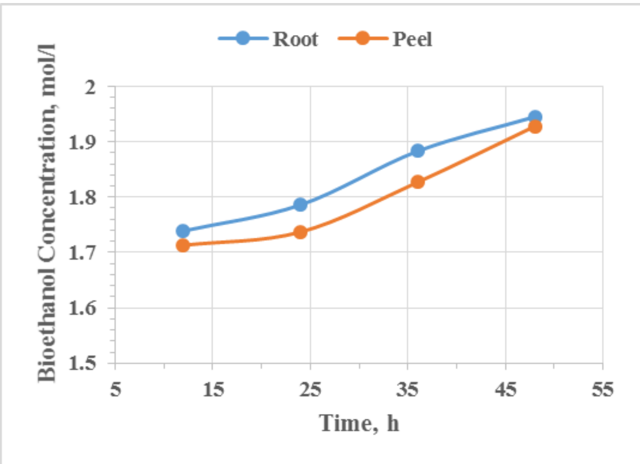

Fig. 3. *Bioethanol concentration for 2g substrare starch sample*

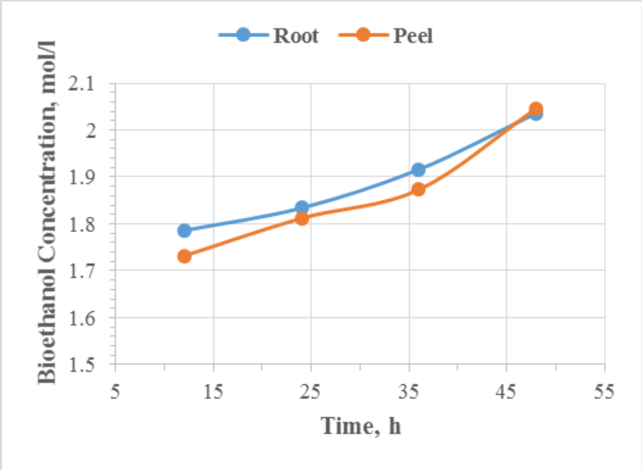

Fig. 4. *Bioethanol concentration for 3g substrare starch sample*

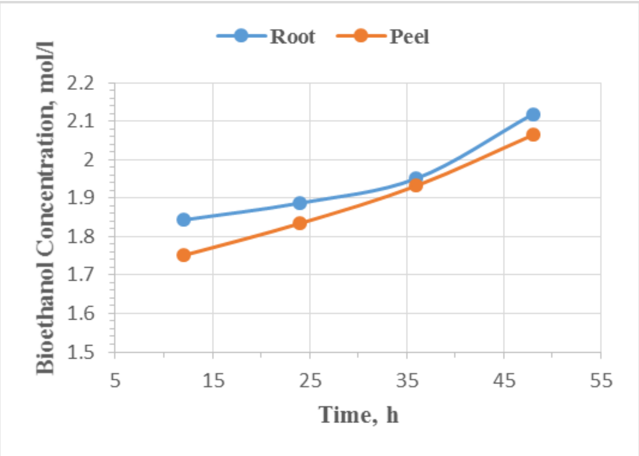

Fig. 5. *Bioethanol concentration for 4g substrare starch sample*

V. CONCLUSION

From the foregone results and discussions, it was concluded that cassava peels have approximately 30% starch content compared to the cassava roots. The respective starch yields of the roots and the peels were 0.215g/g and 0.073g/g respectively. However, both starches successfully fermented to bioethanol using *saccharomyces cerevisiae* as the fermenting micro-organism, and the bioethanol recovery from both starches increases with the amount starch substrate fermented as well as fermentation time. The highest percent difference observed in the concentration of bioethanol yield from cassava roots' starch compared to the bioethanol yield from cassava peels' starch was only about 5.0% using 4g substrate mass for both starches.

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