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Science World Journal Vol 14(No 2) 2019
www.scienceworldjournal.org
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Full Length Research Article

BIO-ETHANOL PRODUCTION FROM CASSAVA (MANIHOT ESCULENTA) WASTE PEELS USING ACID HYDROLYSIS AND FERMENTATION PROCESS

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ABSTRACT
In this research study, cassava peel waste was used as a sole carbon source for ethanol production using the process of fermentation and co-culture techniques. Production of Bio-ethanol from cassava peels was examined using co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae*. Sulfuric acid solution with concentration of 2 %, 6 % and 10 %, was used to hydrolyze the substrates. *Aspergillus niger* and *Saccharomyces cerevisiae* were further used to ferment the substrates at 28 ^{o}C for 4 days. The fermented liquid was distilled at 78 ^{o}C and quantity of ethanol produced was determined. These findings proved that 10 % H_2SO_4 concentrated acid pretreated sample resulted into maximum ethanol yield (37.35 g/ml), pH 4.55, sugar content (15.5 %) and alcohol content (8.5 %) after 4 days. This study further revealed that bio-ethanol can be produced from cassava peels with maximum yield obtained using 10 % H_2S0_4 acid for hydrolysis and *Aspergillus niger* and *Saccharomyces cerevisiae* for fermentation.

Keywords: *Bio-ethanol, cassava peels, acid hydrolysis, fermentation, renewable energy*

INTRODUCTION

The quest by many countries for energy independence as well as the widespread awareness of the need to reduce green-house gas emissions have heightened the search for alternative energy sources (Farrell *et al*., 2006). Bio-fuels are expected to reduce dependence on imported petroleum with associated political and economic vulnerability, reduce greenhouse gas emissions and other pollutants, and revitalize the economy by increasing demand and prices for agricultural products (Balat, 2009). There is an increasing demand for bio-ethanol as alternative source of energy and Nigeria currently depends on the importation of ethanol to meet its local demand.

Bio-ethanol is a microbiological way of converting simple sugar into ethanol and carbon dioxide (CO_2) (Adrade *et al*., 2004). It is a principal fuel that can be used as petrol substitute for vehicle (Pakula & Pentella 2005), and also a renewable energy source produced mainly by sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam (Kroumov *et al*., 2006). The main sources of sugar required to produce ethanol come from fuel or energy crops such as maize, cassava and cassava products, wheat crops, waste straw, guinea corn husk, rice husk, millet husk, sawdust, sorghum plant, sugar cane and sweet potato etc. (Kim & Dele, 2005; Balat *et al.,* 2008). Sugar cane, as a raw material, is used for 60% of global ethanol production, while 40% of global production of ethanol comes from other crops. Cassavas are the main raw material of ethanol production in Nigeria whereas in Brazil, sugar cane is the major source (Nasidi *et al.,* 2010). *Saccharomyces cerevisiae, Zymomonas mobilis, Aspergilus niger and Schizo saccharomycespombe* are microorganisms able to convert sugars to ethanol (Sean & Johann 2015).

In Nigeria and many developing countries, there is a growing interest in the conversion of the huge biomass of organic wastes generated by the food processing sector and other human endeavors into useful products such as ethanol. A number of studies have been carried out in an attempt to optimize the yield of ethanol from cassava peel using different organisms including *Saccharomyces cerevisiae* (Adesanya *et al*., 2008; Marx & Nquma, 2013), *Zymomonas mobilis* and *S. cerevisiae* (Sulfahri *et al*., 2011), *Gloeophyllum sepiarium* with *Pleurotus ostreatus* for hydrolysis and *Z. mobilis* and *S. cerevisiae* for fermentation (Oyeleke *et al.,* 2012; Adiotomre 2015), *Aspergillus niger* for hydrolysis and *S. cerevisiae* for fermentation (Adetunji *et al*., 2015). The search is still ongoing. Odunfa & Olanbiwoninu (2012) recommended that cassava peels could be subjected to pretreatment with sulphuric acid prior to fermentation for higher ethanol content.

Cassava peels (CP) was used as the plant waste for this research work because it contain high amount of starch deposit constituting 20-35% of the tuber (Nwabueze & Otunwa, 2006), it offer numerous advantages in comparison to other crop residues such as rice straw, wheat and sugarcane bagasse and can easily be attacked by micro-organisms (Wongskeo *et al.*, 2012). However, Nigeria is the largest producer of cassava in the world with over 34 million tonnes produced in 2007, (Food and Agricultural Organization, 2007). Industrial and local processing of cassava to food and other products has led to generation of enormous wastes that are dumped in drainages rather than transforming them to useful products. These wastes end up polluting the surface and underground water (Odunfa & Olanbiwoninu 2012). For example, about 2.96 million metric tons of cassava peels are generated and discarded annually in Nigeria from about 10 million metric tonnes of cassava processed for garri alone (Aro *et al.,* 2010). Nigeria needs to explore the abundant agricultural wastes to produce enough ethanol for consumption and exportation. This will serve as a source of employment and income to the citizenry and the country at large. It will also curtail spending Nigeria's scarce resources in importation of ethanol.

This present study is aimed at contributing to this ongoing effort by using combinations of microorganisms (*Aspergillus niger* and *S. cerevisiae*) for fermentation and acid hydrolysis in the combined saccharification and fermentation process to produce ethanol from the peels of cassava. While the objectives of this research is to investigate the bio-ethanol production potential of

cassava peels using different concentration of 2%, 6% and 10% sulfuric acid by fermentation method, to investigate the ethanol yield of the samples, to determine the percentage sugar content of the filtrate, to determine the percent alcohol content of the filtrate, to determine the specific gravity of the filtrate and to check if the peaks representing ethanol bonding were present or not in the pretreated sample with H_2S0_4 acid compared to untreated sample using FTIR analysis.

MATERIALS AND METHODS

Materials
Weighing balance, conical flasks, beakers, measuring cylinder, spatula, funnels, filter paper, cotton wool, and aluminum foil, water bath, autoclave, distillation set-up, pH meter, refractormetre.

Chemicals
Sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), and potassium dichromate ($K_2Cr_2O_7$).

Samples Collection:
Cassava peels (CP) were collected from domestic wastes dump site located at Kawo area, Kaduna state, Nigeria. The samples were aseptically collected into a polythene bag and taken to chemistry laboratory of Kaduna State University (KASU) for further analysis.
The cassava peels samples were washed thoroughly with distilled water to eliminate adhering soil and dust. The peels were sun dried and then grounded into powdered form using pestle and mortar. The grounded powder were then sieve through a 1mm screen to standardized the particle size range of 1mm. The sample was kept in a tightly close container at room temperature. The organisms used were *Aspergilus niger* and *Saccharomyces cerevisiae* and were collected from stock cultures of Microbiology Laboratory at Kaduna state University (KASU). The cultures were characterized and confirmed using morphological and biochemical methods described by (Holts *et al.,* 2009; Oyeleke *et al*., 2012).

Bio-ethanol Production
The methods used for Bio-ethanol production includes; acid hydrolysis, filtration, neutralization of the filtrate, fermentation and distillation process.

Acid hydrolysis:
20 g each of the cassava peel samples was weighed and was poured in a 500cm^3 conical flasks, then distilled water, and 2 %, 6 % and 10 % of sulphuric acid were added separately to respective conical flasks. Sterile distilled water was added to make up to 200 cm^3 mark and the flasks were plunged with sterile cotton wool wrapped in aluminium foil to avoid contamination, the samples were then heated for 2 h in a water bath at 98 ^oC, followed by sterilization in an autoclave at 121 ^oC for 15 minutes and the samples were allowed to cool and were filtered through a No 1 Whatman filter paper. The pH of the filtrate sample was adjusted to pH of 4.5 using 10 % NaOH. The residual samples was washed with distilled water to obtain a neutral pH for all treatment. The 4 samples were oven dried at 90 ^oC overnight (12 h) and were subjected to further analysis. The samples were labeled as follows;

C1 = Untreated cassava peels samples (control)
C2 = Pretreated cassava peels sample with 2 % H_2SO_4
C6 = Pretreated cassava peels sample with 6 % H_2SO_4
C10 = Pretreated cassava peels samples with 10 % H_2SO_4

Fermentation Process
The fermentation was carried out along with simultaneous saccharification and fermentation process (SSF), as described by (Kroumov *et al.,* 2006; Oghgren *et al.,* 2006). The conical flask containing the hydrolyzed samples were covered with cotton wool, wrapped in aluminium foil, and autoclaved at 121^0C for 15 minutes, and the samples were allowed to cool at room temperature. Co-cultures of *Aspergillus niger* and *Saccharomyces cerevisiae* was aseptically inoculated into each flasks containing the hydrolyzed samples while the control set still served as control. The flasks were corked using cotton wool, shake and incubated at room temperature (28 ^oC ±2 ^oC) for three days. The flasks were shaken at interval to produce a homogenous solution and even distribution of the organisms in the substrates mixture.

Distillation Process
Distillation was carried out by using distillation apparatus setup. The fermented liquid was transferred into round bottom flask and placed on a heating mantle fixed to a distillation column enclosed to a running tap water. Another flask was fixed to the other end of distillation column to collect the distillate at 78 ^oC (standard temperature for ethanol production). This was done for each of the fermented broth according to the method described by (Oyeleke *et al.,* 2012).

Analytical methods for bio-ethanol production
Different analytical methods were used for further analysis of bio-ethanol after distillation such as pH test, determination of quantity of ethanol produced, determination of percentage sugar content, determination of specific gravity of the filtrate, determination of the percentage alcohol of the filtrate, FTIR analysis of treated and raw samples, and confirmatory test for bio-ethanol produced.

pH Test
pH meter was first calibrated and was inserted separately into each of the filtrate. The readings was then taken as described by (Ademiluy *et al.,* 2013).

Determination of Quantity of Ethanol Produced:
The distillate collected from C1, C2, C6 and C10 were measured using a measuring cylinder and expressed as quantity of ethanol produced in g/l by multiplying the volume of the distillate by the density of ethanol (0.8033 g/cm^3) (Humphrey & Okafoagu, 2007).

Determination of percentage sugar content
Refractometer was used to determine the percentage of total sugar content of the cassava hydrolysate after hydrolysis. This was carried out by placing a drop of cassava hydrolysate on the graduated hand refractometer glass slide and expressing the brix reading in percentage. The brix (%) was determined using a hand refractometer according to AOAC (2000).

Determination of specific gravity of the filtrate
The brix table was used to determine the specific gravity of the filtrate according to AOAC (2000).

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www.scienceworldjournal.org
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Determination of the percentage alcohol of the filtrate
The brix table was used to determine the percent alcohol of the filtrate according to AOAC (2000).

FTIR analysis of treated and raw samples
Evaluation of chemico-structural changes that occurred with the different treatment was carried out on a BUK scientific FTIR model 530, NARICT Zaria. 2mg of the untreated and treated samples was mixed with 250mg of dried KBr, pressed to pellets and scanned over the range 4000-600 cm^{-1} spectral resolution. The spectra (from KBr pellets) was used to evaluate the chemico-structural changes that occurred with different treatment (Himmelsbach *et al.,* 2002; Pavia *et al.,* 2005).

Confirmatory Test for Bio-ethanol Produced
Confirmatory test was carried out on the extracted bio-ethanol sample using potassium dichromate test as indicated by Caputi *et al.* (1959). 5 mL of the distillate sample was taken and 2 drops of potassium dichromate was added into the distillate, heated in a water bath for 30 minutes.

RESULTS AND DISCUSSIONS

Macroscopic and Microscopic Observation of *Aspergillus niger*
The macroscopic and microscopic identification of the organism was based on colony pigmentation and the structure of the conidial head as described by (Verweji *et al*., 2007). Also Verweji *et al*. (2007) reported that colonies of *A. niger* are carbon black with a dark globular conidial head. *Aspergillus niger* strain was found to have good amylase production potential which is important in the hydrolysis of starch, this was in agreement with the work of (Omemu *et al.,* 2005) who reported that A. *niger* can be used for industrial production of ethanol, citric acid and gluconic acid because of it hydrolytic capacities in amylase production and it ability to have high tolerance to acidity thereby enabling it to prevent bacterial contamination. While *Saccharomyces cerevisiae,* on the other hand is a yeast that is capable of withstanding stressful conditions and have high fermentation efficiency, effective sugar used, tolerance to high ethanol concentration, which are fundamental for industrial used (Andrietta *et al.,* 2007).

Substrate Fermentation for Ethanol Production
Aspergillus niger and *Saccharomyces cerevisiae* were used to carry out fermentation of cassava peels at 28 ^{o}C, pH 4.5 and 20 g substrate for four days. From the results obtained, there was a gradual increase in ethanol yield as a result of higher concentration of sulfuric acid but a decrease in yield was observed with lower concentration of sulphuric acid indicating that under the condition of temperature (28 ^{o}C), pH (4.5) and substrate concentration (20 g); the maximum ethanol yield was obtained with 10 % H_2SO_4, followed by 6 % and 2 % sulphuric acid treatment as shown in table 1.

Table 1: showing quantity of ethanol produced, sugar content, specific gravity and percent alcohol of the filtrate

Serial no.	Samples	Quantity of ethanol (g/ml)	Sugar Content (%)	Specific Gravity	Percent Alcohol (%)
1	20g CP + distilled H_2O	20.40	4.0	1.0166	2.0
2	20g CP + 2 % H_2SO_4	23.45	8.3	1.0330	4.3
3	20g CP + 6 % H_2SO_4	29.80	11.6	1.0467	6.1
4	20g CP + 10 % H_2SO_4	37.35	15.5	1.0633	8.5

Quantity of Ethanol Produced
The distillate collected was measured using a measuring cylinder, and expressed as the quantity of ethanol produced. The result proved that the quantity of ethanol increased with concentration. The sample with maximum ethanol was that treated with 10 % H_2SO_4.

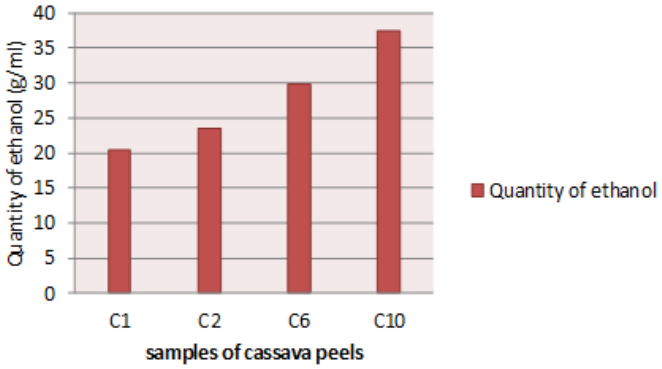

Figure I: Ethanol yielded after distillation at 96 h fermentation time.

Percentage Sugar Content of Filtrate
Brix refractormeter was used to estimate the percentage sugar content produced after pretreatment of sample with water and acid of different concentration. It was observed that the sugar content increased with increased in concentration of acid. The maximum sugar content utilized during the process was found in 10 % H_2SO_4.

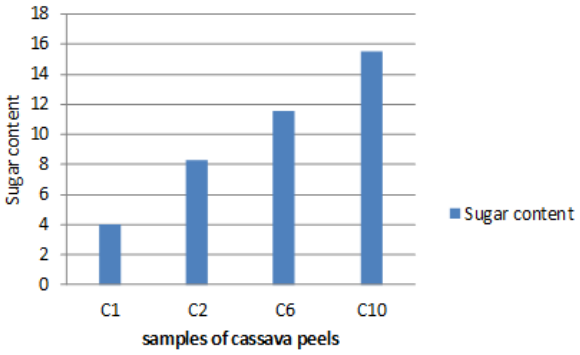

Figure II: Estimation of percentage sugar content after hydrolysis.

Specific gravity of filtrate
The brix table was used to determine the specific gravity of the filtrate. It was observed that the specific gravity increased slightly with increased in acid concentration

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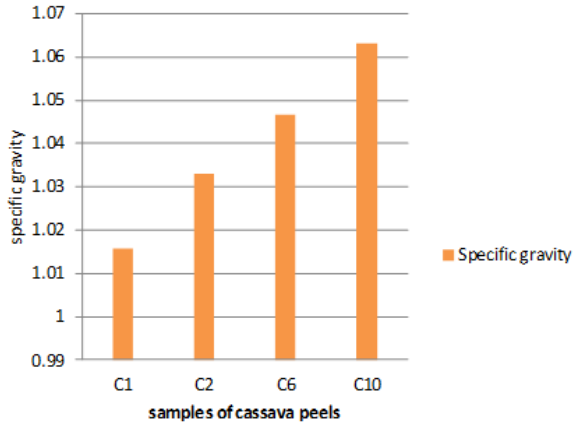

Figure III: Estimation of Specific gravity after hydrolysis

Percentage Alcohol of the Filtrate

The brix calculator was used to determine the percentage alcohol of the filtrate. It has been observed that the percent alcohol increased with increased in concentration of acid. The maximum alcohol content utilized during the process was found in 10 % H_2SO_4.

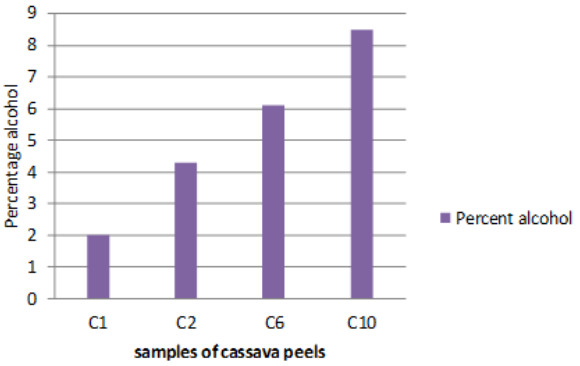

Figure IV: Estimation of percent alcohol after hydrolysis

The ethanol yield from Figure I shows that there was increase in yield and this may be because ethanol yield increase with increase in concentration but begins to decrease when the organism becomes starved and die thereby leading to a decrease in the metabolic activity and subsequent decrease in ethanol yield. This research is in agreement with the work of Shilpa *et al.* (2013) and Zainal *et al.* (2014) who carried out a similar research using cassava peels and banana peels respectively, where the optimum day of ethanol yield was the fourth day.

The effect of substrate concentration on ethanol yield was carried out using varying substrate concentration of 2 %, 6%, and 10 %, of H_2SO_4. From the results obtained from this research analysis, the ethanol yield increased with increase in substrate concentration which was maximized at 10 % (37.35 g/ml) as presented in Figure I. The increase in ethanol yield may be because, at low substrate concentrations the yeast tends to starve and productivity decreased. Also, increase in ethanol yield could be due to the presence of substrates that can readily be hydrolyzed to sugar by the amylolytic activity of *A. niger* and subsequent sugar conversion to ethanol by the yeast cells in the medium (Stanberg *et al.,* 2001). This work is in agreement with the work of Wen *et al.* (2004) and Ado *et al.* (2009) who carried a similar work using cassava starch. This work was also in agreement with the work of Jimoh *et al.* (2009) and Ajay *et al.* (2014) who reported that ethanol yield increased with increase in substrate concentration where the optimum concentration for ethanol yield was recorded to be 10 % and 12 % H_2SO_4 respectively using banana peels.

FTIR results after pretreatment with acid

FTIR analysis of the residue were done to determine the effect of pretreatment on various bonding present in the sample compared to the untreated samples (Kim *et al.,* 2013).

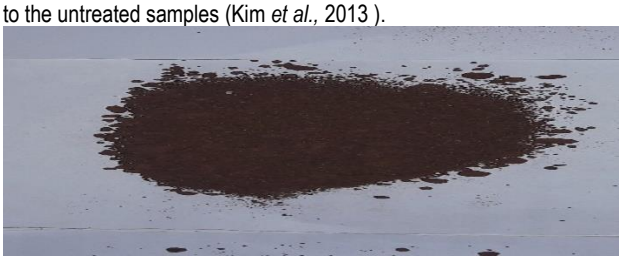

Figure V: Residual sample of untreated cassava peels

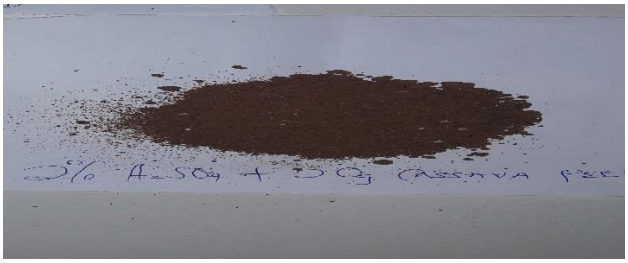

Figure VI: Residual samples after pretreatment with 2% H_2SO_4 cassava peels

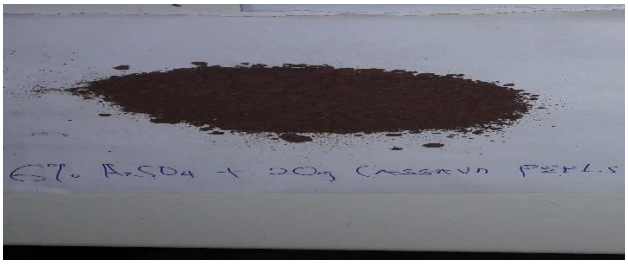

Figure VII: Residual samples after pretreatment with 6 % H_2SO_4 cassava peels

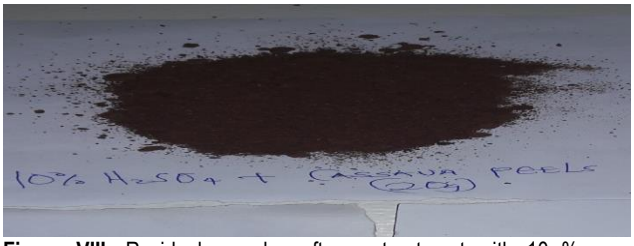

Figure VIII: Residual samples after pretreatment with 10 % H_2SO_4 cassava peels

The effect of acid was determined compared to the untreated sample in (Figures IX-XII).

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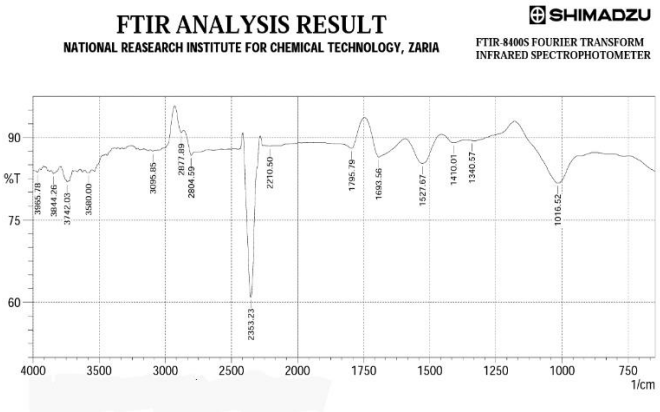

Figure IX: FTIR analysis for untreated cassava peels sample

This finding reveals that Lignin remains in the structure of untreated sample (control) with the help of various chemical bonds such as ester bonds, phenyl glycosidic bonds, acetal linkages. The band width of 1527 cm-1 and 1410 cm-1 signifies the range of aromatic rings by which lignin has bound. From the spectrum, it is obvious that various peaks lies within the range of aromatic rings as it is with the untreated or control sample.

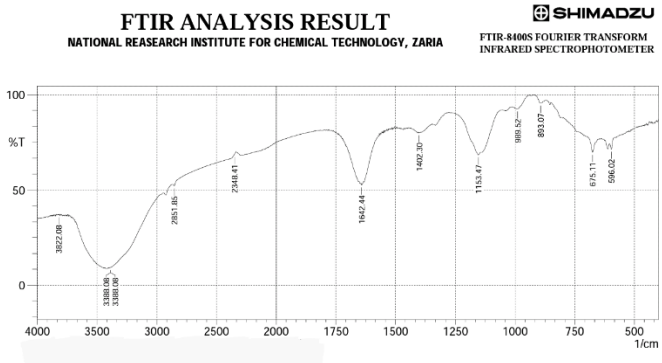

Figure X: FTIR analysis for acidic pretreated (2% H_2SO_4) cassava peels sample

It can be observed that the stretch peak at 3388.08 cm-1 represents the stretching of O-H group. These results represents that partial degradation of cellulose has occur. The C-H stretch at 2851.85 cm-1 represents that various ester bond have also been disrupted.

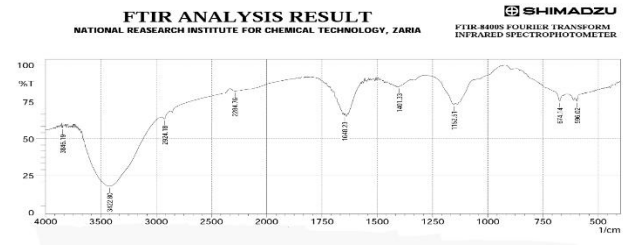

Figure X1: FTIR analysis for acidic pretreated (6 % H_2SO_4) cassava peels sample.

In figure 11; The O-H stretch at 3422.80 cm-1 shows reduction in cellulose linkages and peak at 2924.18 cm-1 represents stretching of C-H linkages. These type of reductions in various chemical bonds ensures the exposure of enzymes for higher yield of ethanol.

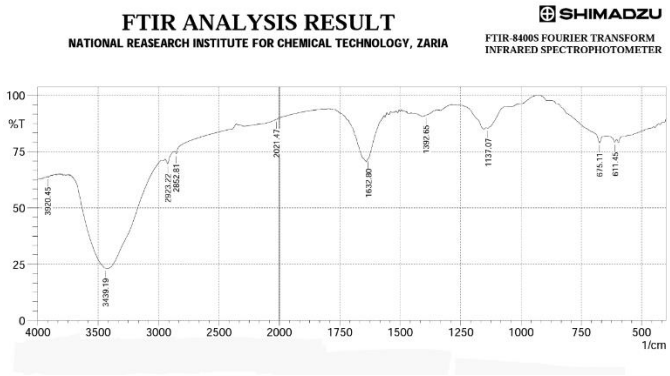

Figure XII: FTIR analysis for acidic pretreated (10 % H_2SO_4) cassava peels sample

From the figures (fig. IX-XII), it can be observed that the peaks became sharper and clear after the pretreatment with acid at higher concentration of H_2SO_4 which indicates that the substrate became more pure after treatment. The lignin peaks also represents weaker hydrogen bonding compared to the untreated samples. In figure XII; the stretch peak at 3449.19 cm-1 represents the stretching of O-H group while 2923.22 cm-1 and 2852.81 cm-1 represent the C-H stretch peaks. This results agrees with the results of Rawinder, *et al*. (2017), who performed similar work using rice husk.

Final Confirmatory Test for Ethanol Produced By the Co-Culture Using Cassava Peels

The bio-ethanol sample produced was further confirmed using potassium dichromate test as indicated by (Caputi *et al.,* 1959). The color of the crude distillate changed from pink (dichromate color) to green. The formation of green color is strong evidence for existence of ethanol in crude primary distillate.

Table 2: confirmatory test for bio-ethanol produced

TEST	OBSERVATION	INFERENCE
5 ml of distillate + 2 drops of potassium dichromate, heated in a water bath for 30mins.	The formation of green color	Ethanol present

Conclusion

The result of this study confirmed that ethanol can be produced from cassava peels which are agricultural wastes. Despite the ability to use cassava peels for ethanol production, the yield can be influenced by several factors especially temperature, pH, time and substrate concentration. The maximum ethanol yield (37.35 g/ml) obtained from the fermentation process using co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae* was carried out at 28 ^oC, pH 4.55 and 10 % substrate concentration for four (4) days.

One of the objectives of this work was to find out the effect of different concentrations of H_2SO_4 in the optimized pretreatment method, as the pretreatment is the major challenge for the production of second generation fuels due to the presence of

lignin which needs to be degraded for the exposure of cellulose to the enzymes and hence for the high yield of ethanol. From the results obtained it can be concluded that the highest percentage i.e. 10 % H_2SO_4 is the most effective concentrations for pretreatment of cassava peels. It was also confirmed that acidic treated sample with 10 % H_2SO_4 has major effect on the bonding of various groups and maximum ethanol content was also found in 10 % H_2SO_4 treated sample compared to the untreated samples of cassava peels. Hence, acidic pretreatment at particular concentration can be considered as the optimized and economical pretreatment for simultaneous saccharification and fermentation (SSF) as it resulted into highest yield of ethanol.

The use of cassava peels is a worthwhile venture for ethanol production, considering their cost and because it is a means of controlling environmental pollution, thus making bio-ethanol production economical and environmentally friendly and also renewable.

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