



# Comparative Studies on Bioethanol Production from Cassava Peels using *Saccromyces Cerevisiae* and *Zymomonas Mobilis*

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Received: 📅 January 25, 2020

Published: 📅 February 03, 2020

## Abstract

Cassava (*Manihot esculenta*) is one of the most popular tropical food consumed worldwide. Cassava is a common term embracing a number of species and cultivated mainly for its food. The bioethanol from cassava peels were hydrolysed with *Aspergillus niger* for 5 days, the hydrolysates were fermented with *Zymomonas mobilis* at a temperature of 35°C for 5 days. The bioethanol from cassava peel produce a reducing sugar of 0.15mg/ml that of *Saccromyces cerevisiae* while that of *Zymomonas mobilis* produce the lowest reducing sugar of 0.012mg/l, the concentration obtained was 0.961mg/l from *Saccromyces cerevisiae* while 0.988g/ml from *Zymomonas mobilis*, the quantity of the bioethanol was 180g/l from *Saccromyces cerevisiae* while 175g/l from *Z. mobilis* and the density of the bioethanol was 0.988g/ml, the quantity of the bioethanol produced was 175±35. This shows the cassava peels are possibly used.

**Keywords:** Bioethanol; Cassava peels; *Saccromyces cerevisiae*; *Zymomonas mobilis*

## Introduction

Bioethanol is a renewable energy resource produced through fermentation of simple sugars by yeast. Bioethanol is widely used as partial gasoline replacement in the US and other parts of the world such as Canada, Brazil, Sweden, and China etc. It can also be used in a variety of heating, cooking and light appliance (Anuj et al. 2007). The Federal Government of Nigeria has concluded plans to invest 400 Billion Naira (3.5 Billion US dollars) in Jigawa State for ethanol production programme in order to diversify its sources of revenue (Thisday 2006). The US President George W Bush announced in his state of union speech, an agenda to develop alternative energies such as bioethanol fuel from grains and cellulose in order to terminate Americans dependence on oil (Analyst, 2006).

The use of bioethanol fuel for automobiles can significantly reduce petroleum use and existing greenhouse gas emission [1-5]. Ethanol is also a safe alternative to methyl tertiary butyl ether (MTBE), the most common additive to gasoline used to provide cleaner combustion Mani & Marko et al. [2]. Mani [2] MTBE is a toxic chemical compound and has been found to contaminate ground

water. The search for alternatives to the current, oil based, fuels is the focus around the world. One of the most attractive alternatives is the Bio-ethanol-alcohol produced from agricultural crops [6]. At present, bio ethanol derived from corncobs, sugar beets, grain and sugar cane, with the help of baker's yeast (Marko, 2006) [2]. A great number of by-products result from the cultivation of these crops, such as straw and corn husks. It would be a major step forward if this waste material, which also largely consists of sugar, could be used for the production of Bioethanol (Marko, 2006). This would allow agricultural land to be used more efficiently and at the same time prevent competition with food supplies.

The use of ethanol as a fuel was first advocated more than 100 years ago. The first extensive use of ethanol in gasoline was adopted as part of a domestic energy strategy in the 1970s. Ethanol was used as an octane, replacement in the 1980s and as a tool in the battle against air pollution in the 1990s. Today, ethanol meets a host of energy, agricultural, rural development and economic policy objectives (summer, 2007). The increasing demand for cleaner

transportation fuels creates great opportunities for biofuels – agricultural renewable fuels such as bioethanol and biodiesel. Production of ethanol in the United States, reached an historic high level in 2006. While sorghum, sugar crops and waste materials such as cassava peel, banana peel, yam peel, rice and wheat straw etc are used to produce ethanol for at least another decade. The implementation of national Renewable Fuels Standard (RFS) is a key factor in expansion of ethanol use nationally (Summer, 2007).

Ethanol is a biofuel that can also be produced from biomass by anaerobic digestion of plant materials with high sugar content. Ethanol can be made from grain such as corn, sorghum and wheat, sugar cane and cassava, (Twildell and weir, 1986). Ethanol is compatible with Premium Motor Spirit (PMS) and can be burned directly in automobile engine adapted to use this fuel, or it can be mixed with PMS up to 10 percent to be used in any normal automobile engine. A mixture of PMS and ethanol raises octane ratings and is a good substitute for lead antiknock agents, the major cause of lead pollution. It also helps to reduce carbon monoxide emission in automobile exhaust (Cunningham, 1999).

Bioethanol produced from renewable biomass has given considerable attention in the present years. Using ethanol as a gasoline fuel additive, as well as transportation fuel helps to reduce global warming and environmental pollution. In the last decade, most research has tended to focus in developing an economical and eco-friendly ethanol production process. Much emphasis is being given to the production of bioethanol from agricultural and forestry residues and other forms of lignocellulosic biomass (Kadam et al., 2000). In the past disposal and burning of agricultural products residues and waste such as cassava, yam peel, banana peel, wheat straws, rice straws and rice husks etc was an accepted practice. This practice is now being challenged due to concern over health effects from burning fields. Most of these residues and waste contain a reasonable amount of cellulose content which is a good source of fermentable sugars for significant use.

Bioconversion of cellulosic biomass into fermentable sugar, for production of ethanol using microorganisms, especially cellulose degrading fungi, makes bioethanol production environmentally friendly and also renewable [7]. The world's ethanol production was about 29.9 billion liters in 2000, which was less than 31.4 billion liters forecasted (Mani et al, 2002). The benefit of developing biomass-to-ethanol technology are increased national energy security, reduction in greenhouse gas emissions, use of renewable resources, foundation of a carbohydrate base chemical process industry, macro-economic benefit for rural communities and society at large (Wayman et al., 1992).

In the worldwide economy much focus has been laid on the rising oil price which has become a hot topic. The rising crude oil price has increased the interest of finding other possible ways to produce fuel. All energy sources have an impact on the environment. Concerns about the greenhouse effect and global warming, air pollution and energy security have led to increasing interest and more development in renewable energy sources such as biofuel, solar, wind, geothermal, and hydrogen. But there is need to continue to use fossil fuels and nuclear energy until new and

cleaner technologies can replace them. The aim of this research is to investigate the possibilities of using cassava peel.

This aim will be achieved through the objectives of produce Bioethanol using Cassava peel using enzyme hydrolysis, determination of the quantity, density, reducing sugar and concentration of Bioethanol produced from cassava peels and comparing the performance of *S. cerevisiae* and *Z. mobilis*.

## Materials and Methods

### Sample collection

The cassava peel to be used for the research will be collected from Dan Kure market Sokoto state. The sample will be aseptically collected in a clean polythene bag.

### Sample preparation

A cassava peel collected will be sorted and washed under running tap water to remove sand and other impurities will be sundry for about two weeks and then milled into powder form using mortar and pestle. Each powdered product is to be sieved several times with different size sieve to obtain fine powder [7].

### Preparation of culture medium

The media to be used are potato dextrose agar (PDA) which is to be used to grown *Aspergillus niger* and nutrient agar (NA) medium was to be used to grow the *Zymomonas mobilis*. Both media are to be prepared according to the manufacturer's instruction as the standard procedure [8] [oyeleke and manga 2008]. But the PDA medium is to be incorporated with chloramphenicol (antibiotic) to in hit bacterial growth.

## Isolation of the Organisms

### Isolation of *Aspergillus niger*

*Aspergillus niger* is to be obtained from stock culture in Usmanu Danfodiyo University laboratory. The culture is to be carefully picked and inoculated on a freshly prepared PDA medium. The media plate is to be incubate at 30°C for five (5) days, which will give rise to pure culture of the *Aspergillus niger*. For the further clarification, it would be stained with lactophenol cotton blue dye and observed under the microscope by taking note of the shape and colour of the conidiospores as described by [8]; oyeleke and manga (2008).

### Isolation of *Zymomonas mobilis*

**Serial dilution:** Five sterile test tubes containing 9ml of sterile distilled water were labeled for the serial dilution. 1ml (i.e. 10-1) collected using syringe and was transfer into another test tube and labeled as 10-2, 1ml from 10-2 was collected and transferred into another test tube labeled 10-3, the same procedure was used for the next test tube labeled 10-4 up to 10-5. 0.1ml was later taken from last test tube i.e. 10-5 and inoculated into fresh prepared nutrient agar medium and incubated at 37°C in an anaerobic jar for 28 to 48 hours. The fully cultured bacteria will further be characterized based on colonial morphology, cultural characteristics and biochemical test as described by [8]: oyeleke and manga (2008). The *Z. mobilis* will be confirm by comparing its characteristics

and biochemical reaction with those of known taxa using bergey's manual of determinative bacteriology [Holt et al., 1994] [9].

**Pre-treatment of cassava peel using acid hydrolysis:** Pretreatment of cassava peel was done using dilute H<sub>2</sub>SO<sub>4</sub>. 30g of cassava peel was weighed and placed into separate 500ml conical flask and 250ml of dilute H<sub>2</sub>SO<sub>4</sub> was added to a conical flask containing the sample. It was then autoclaved at 121°C and was filtered using Whitman filter paper. The residue was washed for 30 minutes until neutral PH and oven dried at 100°C -105°C (Nicholas and wayman, 2012).

**Enzyme hydrolysis:** A the *Aspergillus niger* will be used to hydrolyzed the cassava peel powered. Thirty grams (30g) of cassava peel powder would be weighted into 500ml sterile conical flask, and 250ml of distilled water would be added to the conical flask to suspend the substrate. A sterilized wire loop was used to pick the growth of the Angier and aseptically inoculated into the solution in the conical flask and stirred very well with a sterile glass rod, so as to enable the microorganism get access to the substrate. The flask would be covered with cotton wool wrapped in aluminum foil to avoid contamination and kept on the bench at room temperature for seven (7) days. During which on daily basis it was shaken. After seven (7) days, the hydrolysate (solution) would be filtered using the Whitman filter paper No.1. The filtrate will be collected and sterilized for further use as fermentable sugar (Humphrey et al.,2006).

**Fermentation:** Fermentation of the hydrolyzed sample was carried out according to the methods described by oyeleke and jibrin (2009). One hundred milliliter (100ml) of cassava peel hydro slates were suspended into 500ml capacity conical flask and covered with cotton wool and wrapped with aluminum foil and it was then autoclaved at 121°C for 15minutes. The conical flask was allowed to cool at room temperature and were then inoculated with fermentative organism. The conical flask was then inoculated with *Zymomonas mobilis*. The flask was then incubated anaerobically at 35°C for 5 days. The hydroslated were then distilled according to standard method [7].

**Fractional distillation:** The ethanol produced from fermentation processes contains a significant quantity of water which was removed. This was achieved by dispensing the mixture into the round bottom flask fixed to the other end of distillation column to collect distillate. The temperature of heating mantle was adjusted to 78°C which was used to heat the round bottomed flask

containing the ethanol-water-mixture, since ethanol can only be distillate at 78°C [7].

**Determination of quantity of bioethanol produced:** The distillates collected would be measured using a measuring cylinder and expressed as the quantity of bioethanol produced in g/l by multiplying the volume of the distillates collected by the density of ethanol (0.8033g/ml). it should be noted that g/l is equivalent to the yield of 200g of dried substrate (Humphrey and okafor, 2007).

**Determination of reducing sugar:** The reducing sugar content of hydrolyzed cassava peels will be determined using dinitrosalicylic acid calorimeter method (Miller, 1959) with glucose as standard. It would be assayed by adding 2ml of 3,5-DNS reagent to 1ml of sample. The mixture will be heated in boiling water for 5minute to develop the red brown color. Then 1ml of 40% potassium sodium nitrate solution will be added to stabilize the color, it will then be cooled to room temperature under running tap water. The absorbance of the sample will be measured at 540nm using ultraviolet (UV -VIS) spectrophotometer. The reducing sugar content will be determined by making reference to a standard curve of known glucose (Miller, 1959).

Concentration of reducing sugar =

**Determination of density of ethanol:** Measured 10ml from each sample, weighed it and divide by volume measured.

Density =

**Determination of percentage ethanol concentration:** 5ml of each sample would be measured into different test tubes and then 2ml of the prepared potassium dichromate solution was added to each and shaken thoroughly and allowed to stay for 20 minutes. The solution labelled in each test tube was poured into labelled cuticle in the ultraviolet (UV) visible spectrophotometer and analyzed to determine the wavelength and percentage of ethanol concentration which was extrapolated from the standard ethanol curve [7].

## Results

The result obtained in this study revealed bioethanol production using *Zymomonas mobilis* and *Aspergillusniger*. Isolation and identification of *Zymomonas mobilis* from roselle juice is presented in Table 1. The organism was identified base on cultural and morphological characterization: a motile, gram negative rod bacteria, catalase and glucose positive, Urease, Oxidase, and Lactose negative.

**Table 1:** Morphological and Biochemical Characterization of *Zymomonas mobilis* isolated from Roselle Juice.

Isolate	Gram Reaction	Motility	Catalase	Glucose	Urease	Oxidase	Lactose	Orgabism
1	-ve Rod	+	+	+	-	-	-	<i>Zymomonas mobilis</i>

Key: (Positive+); (Negative-); Gram positive: -Rod; Motility: (+); Catalase: (+); Glucose: (+); Urease: (-); Oxidase: (-); Lactose: (-); Organism: *Zymomonas mobilis*

**Table 2:** Morphological Characterization of *Aspergillus niger* isolated from cassava peels Sample.

Isolate	Colony characterization	Cell shape	Organism
1	black, dotted surface as conidia	Filamentous, with Septed hyphae	<i>Aspergillus niger</i>

*Aspergillus niger* was also identified in Table 2: it was found to be Haploid filamentous fungi with septed hyphae, produced colonies that are composed of yellow felt, covered by dark asexually produced fungal spores.

The Concentration of the reducing sugar produced from the treated cassava peel is also presented in Table 3. The results show that the highest reducing sugar yield of 0.156 mg/L was obtained from *Saccharomyces cerevisiae* while the lowest reducing sugar was obtained from (0.0121 mg/L) of *Zymomonas mobilis*.

**Table 3:** Analyzed result for Bioethanol produced from *Zymomonas mobilis*.

Parameters	Results
Reducing sugar (mg/ml)	0.012
Quantity (ml/l)	175± 35.
Viscosity (milli pasca)	11.667 ± 0.33
Density(g/ml)	0.988
Concentration(mg/l)	1.3±0.75

Quantity of bioethanol produced was represented in Table 4 which shows that the quantity of bioethanol produced from *Saccharomyces cerevisiae* of 180 ml/L has the highest yield as compare to the quantity of bioethanol yield by *Zymomonas mobilis* which has 175 ml/L. The densities of bioethanol produced from cassava peels is presented in Table 4. The results revealed that samples with low densities tend to produce more bioethanol and the density should be less than the density of water which is one (1). The results further revealed that bioethanol produce through fermentation with *Saccharomyces cerevisiae* has the lowest density of 0.961g/ml, whereas bioethanol produce through fermentation with *Zymomonas mobilis* has the highest density of 0.988 g/ml.

**Table 4:** Analyzed result for Bioethanol produced from *Saccharomyces cerevisiae*.

Parameters	Results
1 Reducing sugar (mg/ml)	0.156
2 Quantity (g/l)	180± 40
4 Viscosity (millipasca)	14.667± 0.33
5 Density(g/ml)	0.961
6 Concentration(mg/l)	2.8±0.37

The viscosities of bioethanol produced from cassava peel is presented in Table 1-4. the result revealed that viscosity of bioethanol produces with *saccharomyces cerevisiae* yield the highest viscosity as a viscosity of (14.667± 0.330 which is greater than that of *Zymomonas mobilis* (11.667 ± 0.33) (Table 1 & Table 2).

## Discussion

Bioethanol production is receiving great attention worldwide; it was widely studied due to its immense importance and usage, as a member class of biofuels. Different researches have been conducted with various raw materials (biomass) and different

methods for bioethanol production. Recently it was being observed that lignocelluloses and celluloses are the main biomass source for bioethanol production, in this research work cassava peel was used as a chosen substrate.

The results showed that *Aspergillus niger* have a black mycelium on the agar medium, it had septate hyphae, long and smooth conidiospores, and long unbranched sporangiospores with a large and round head. The results obtain in this present study on morphological of *Aspergillus niger* and is similar to the findings of [7].

*Zymomonas mobilis* was found to be Gram-negative short rod, catalase-positive, oxidase and urease- negative, motile and hetero-fermentative, producing gas from glucose, fructose and sucrose. This finding is in conformity with that of [9], who reported the isolation of *Zymomonas mobilis* from fresh wine-saps. This therefore reveals that bioethanol can be produced any time *Zymomonas mobilis* is used. This agree with Rabah et al. [10] who reported the microbial pretreatment of rice husk and groundnut shell for bioethanol production using *Zymomonas mobilis* (Negative rod, motility, catalase, glucose, fructose and sucrose positive. Maltose, Arabinose, Urease, Oxidase, Lactose negative) and another ruminant microorganism.

The highest reducing sugar yield of 0.15mg/L was obtained after the treated cassava peels was hydrolyzed with *Aspergillus niger*. This might be because the hydrolyzing microorganisms use the sample as their source of carbon and at the same time produce enzymes that hydrolyse the cassava peel into glucose during the process of feeding. The 0.15mg/L reducing sugar obtained is almost in agreement with the 0.16mg/L reducing sugar obtain by [11] and lower than 0.18mg/L reported by [6]. The studied further showed that hydrolyzing the cassava peel with *Aspergillus niger* produce more reducing sugar.

The highest quantity of 180 ml/L was obtained after the pre-treated sample with *Saccharomyces cerevisiae*, which is greater than that of *Zymomonas mobilis* (175 ml/L). This result reveals a higher production through *Saccharomyces cerevisiae* which may be because *Saccharomyces cerevisiae* contains enzymes and it can reduce a carbonyl group into a hydroxyl group in properly high yields reported by Shang Xueying, (2011) which tends to facilitate the breaking down of the sugars in to alcohol. All these might be responsible for high bioethanol produced from the hydrolysates.

The fermentation of the hydrolysates through *Saccharomyces cerevisiae* had a maximum yield of 2.8 bioethanol after 24 hours of fermentation. The highest bioethanol yield obtained was lower than 3.0 reported by Fish et al. (2009). This may be associated to environmental factors and differences in methods of fermentation. Statistical analysis using T-test reveals that there is significant difference at  $p < 0.05$  in the percentage mean concentration of the bioethanol produced after fermentation with *Zymomonas mobilis* and *Saccharomyces cerevisiae*.

These studies revealed that Bioethanol can be produced from cassava peel with maximum yield obtained using *Saccharomyces*

cerevisiae. This is because *Saccharymyces cerevisiae* can ferment the glucose more efficiently to break down the sugar into minor metabolite such as Alcohol and CO<sub>2</sub> to produce ethanol.

The highest density obtained from *Zymomonas mobilis* is (0.988g/ml) which is greater than the highest density obtained from *Saccharomyces cerevisiae* which is (0.980 g/ml). These results disagree with the result obtained by [12], which in his work got 0.8035 from Cassava peel substrate and 0.8023 from Potato peel substrate. This might be as result of difference in the nature of substrate, there was no pretreatment in [12], findings while there was pretreatment in this work, the hydrolysis in [12], work was done using *Gloeophyllum sepiarium* (0.8035g/ml) and *Pleurotus ostreatus* (0.8023g/ml) from sweet potato peel and cassava peels. While in this work *Aspergillus niger* was used in hydrolysis, and 5% H<sub>2</sub>SO<sub>4</sub> was also used for pretreatment of the cassava peel substrate [13-18].

The scientific implication in this study shows that, among the biofuels, bioethanol is very impressive and leading fuel produce in the different part of the world (Mudale, 2010). The literature records that the bioethanol usage causes low emissions of greenhouse gases (GHG) (Lee and shah, 2012) and can be produced by utilizing the biomass, molasses, or any lignocellulosic such as cassava peel, with the help of microorganism [19-25]. This agreed with the "Comparative study of bioethanol production from sugarcane molasses by using *Zymomonas mobilis* and *Saccharomyces cerevisiae*" by as if et al., (2015). The use of microorganisms is usually considered as environment friendly and also renewable. The efficiency and specificity of the microorganism are an advantageous aspect to targeted products like bioethanol. Cheap materials, low-cost processing and high productivity are the main consideration for most ethanol fermentation (Tao et al., 2005) [26-30].

## Conclusion

Fermentation using *Saccharomyces cerevisiae* has the highest concentration of Bioethanol 2.8 mg/L than *Zymomonas mobilis* with (1.3 mg/L). This is because *Saccharymyces cerevisiae* can ferment the glucose more efficiently to break down the sugar into minor metabolite such as Alcohol and CO<sub>2</sub>. Furthermore, the fermentation waste is found to conserve nutrients and mineral elements that could serve as animal feedstock or fertilizer to increase soil fertility. Bioethanol production with concentration 1.3. viscosity is good for raw material for bioethanol using cassava peels [31-35].

## Recommendation

In spite of laboratory-based bioethanol success stories, the production of fuel i.e. ethanol at plant scale still remains a challenging issue. A positive solution to this issue could bring economic advantage not only for fuel and power industry, but also benefit the environment rehabilitation and balance issues and cause. Therefore, for a flourishing bioethanol industry, it requires the following:

- a. Optimizing the conditions for the production of simple sugars from cassava peel and to isolate *Saccharomyces cerevisiae* strains that may result in improved yield of ethanol
- b. Advancement in pre-treatment by acid catalyzed hemicelluloses hydrolysis or employing an integrated approach in the form of consolidated bio processing with application of novel, tailored cocktails of enzymes for the cellulose breakdown of substrate
- c. Government support which is critical in correcting tax anomalies, exemption from excise and sales tax, deregulation of feed stock and its pricing, and encouraging pilot project and rural development work on bioethanol.

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