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# EFFECT OF YEAST (*Saccharomyces cerevisiae*) CONCENTRATION ON BIOETHANOL YIELD FROM CASSAVA (*Manihot esculenta*)

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**Abstract**
The limitation of fossil fuel reserves and environmental pollution has been a problem in the recent decades that need to be tackled. The aim of this research work was to produce bioethanol from Cassava flour as substrate through fermentation and using yeast (Saccharomyces cerevisiae) as a catalyst for sustainable energy utilization. The effect of catalyst concentration on bioethanol production was studied in order to obtain optimum yield. The bioethanol was produced from Manihot esculenta (cassava) starch using yeast catalyzed fermentation process followed by distillation. The reaction conditions were maintained at a constant fermentation time of 6 days while varying the amount of catalyst added. The results obtained show that the pre-treatment yield of bioethanol ranged from 0.5 -4.7%. The maximum bioethanol yield of 3.98% occurred at 2.5g of catalyst being used. Furthermore, regression of the production process gives a Second-Order Equation of the form:$Y = -0.116x^2 + 1.882x$ where Y is the predicted yield of bioethanol and X, the mass of Catalyst added. This study revealed that bioethanol can be produced from cassava processing plant effluent with a maximum yield obtained using Saccharomyces cerevisiae for fermentation.

**Keywords***:* Bioethanol, Brewer's Yeast, Distillation, Fermentation, Fossil fuel, Yield,

## Introduction

Bioethanol is a member of a group of organic chemicals called alcohols. Bioethanol has the chemical formula of CH3CH2OH, with the OH (hydroxyl ion) being the functional group. Because of the presence of the -OH hydrogen bonding is quite common. Bioethanol is a primary alcohol since the hydroxyl group is attached to a carbon atom which is itself attached to only one other carbon atom. Bioethanol is a clear, colourless liquid with a characteristic sweet smell; melts at –114.1°C, boils at 78.5°C, and has a density of 0.789 g/mL at 20°C (Shakhashiri, 2009). The low freezing point has made it useful as the fluid in thermometers for temperatures below –40°C, the freezing point of mercury, and for other low-temperature purposes, such as for antifreeze in automobile radiators. Bioethanol burns with a clear, hot, soot free flame and is often used as a fuel for portable stoves. Complete combustion yields carbon dioxide and water. When reacted with sodium, bioethanol produces hydrogen gas and sodium ethoxide (CH3CH2ONa). Bioethanol can be chemically oxidized using a mixture of concentrated tetraoxosulphate (VI) acid and sodium dichromate to produce the aldehyde, ethanal (also called acetaldehyde).

Further oxidation yields ethanoic acid (also called acetic acid), a carboxylic acid (Chemguide, 2012). Methods other than distillation can however be used to obtain a higher concentration of bioethanol. In the laboratory higher concentration bioethanol, with less water, can be produced by refluxing the rectified spirit with quicklime and then distilling the alcohol mixture. The product of this is absolute alcohol. Industrially the same process can be carried out by azeotropic distillation with another liquid. Benzene is added to the rectified spirit and the mixture is fractionally distilled, the third fraction produced from this is absolute alcohol. Bioethanol is highly hygroscopic and must therefore be properly stored to avoid re-absorption of water and preserve the absolute concentration (Anon, 2012b).

Bioethanol exists in nature and has been made since ancient times as a product of fermentation

of sugar and other carbohydrates such as starch by yeasts, bacteria, and molds. All beverage bioethanol (consumed by humans) is manufactured only by fermentation while industrial bioethanol is now frequently manufactured synthetically, either from acetaldehyde made from acetylene by catalytic hydration; or from ethylene made from petroleum; or by the hydrolysis of ethyl sulphates (Zaldivar *et al.*, 2001).

**Feedstocks in Bio-bioethanol Production**

Application of agricultural wastes in bio-fuel industry is the focus of many researches aimed at achieving an effective and efficient waste management scheme. Fossil fuel is a non renewable source of energy and the concern generated by its sustainability, economic and environmental impact led to the use of renewable sources for fuel (Schneider, 1989). Energy from biomass is renewable and can contribute to sustainable development. In addition, biomass resources are often available locally and processing is feasible without high capital investment. Furthermore, dependence on biomass energy can help reduce green house gas emission thereby impacting on public health (Lynd, 1996).

The various raw materials used in the manufacture of bioethanol via fermentation are classified into three main types: sugars, starches and cellulose materials (Lin and Tanaka, 2006). Sugars (from sugar cane, sugar beets, molasses and fruits) can be converted to bioethanol directly by fermentation. The most widely used sugar for the production of bioethanol via fermentation is molasses. Starches (from corn, cassava, potatoes and root crops) and cellulose (from wood, agricultural residues, waste sulphite liquor from pulp and paper mills) must first be hydrolyzed to fermentable sugars. Enzymes from microorganisms can then ferment the sugars to bioethanol.

Most agricultural biomass containing starch can be used as potential substrate for bioethanol fermentation by microbial processes. These substrates include corn (maize), wheat, oats, rice, potato and cassava. On a dry basis, corn, wheat, sorghums and other grains contain about 60 to 75% (wt/vol) of starch, hydrolysable to hexose with a significant weight increase (stoichiometrically the starch to hexose ratio is 9:10), and these offer a good resource in many fermentation processes (Lin and Tanaka, 2006). Fermentation of starch is somewhat more complex than that of sugars because starch must first be converted to sugar. Starch is hydrolysed with the aid of alpha- and glucoamylases to glucose. The process requires cooking at high temperature (140–180°C). Among many starchy materials, cassava is the least expensive fermentable source of starch. It is a tropical root crop produced in more than 80 countries (Sasson 1990). A large portion of cassava is used as human food in developing countries while about 20% each is used in animal feeds and industrial use. The rest which is lost since cassava is perishable after harvest (Lin and Tanaka, 2006), can be harnessed for bioethanol production. The sugar and starch based feedstock used in the first and second generation biofuel respectively are still currently being used. However, these raw materials may not be sufficient to meet demands for biofuel production. Moreover, being food crops, the controversy over use in biofuel production lingers (Greene, 2004). Lignocellulosic biomass (plant biomass), offers a great potential resource for the production of bio-fuels because it is largely abundant, inexpensive and its production is environmentally sound in terms of greenhouse gas emission. Cellulose materials represent the most abundant global source of biomass and have been largely unutilized. The global production of plant biomass, of which over 90% is lignocellulose, amounts to about $200\times10^9$ tons per year, where about 8 to $20\times10^9$ tons of the primary biomass remains potentially accessible (Lin and Tanaka, 2006; Saliu and Sani, 2012).

Several factors affect the fermentation process, these include catalysis conditioning of the yeast (S. cerevisiae) and processing parameters such as pH, fermentation time, reaction temperature, design of fermentation

process reactor etc. (Lichts, 2010, Abara and Rakshit, 2004). These conditions need to be adequately monitored to maintain an optimal substrate for fermentation. Yeast uses glucose to produce a high yield bio-ethanol provided the environment is ideal (Lichts, 2010). To avoid the yeast being exposed to extreme concentrations of sugar the glucose level must be kept at an optimal level. A too high or too low pH will cause the yeast to use valuable energy to balance its own internal pH while not maximizing productivity (Lichts 2010). High operating temperature will cause the yeast to die, while low operating temperature restrains the yeast and can make it inert. Hence, without active yeast, sugar will not convert into ethanol and the potential for contamination grows as the sugar is available for other micro-organisms (Lichts, 2010).

With industrial development growing rapidly, there is need for environmentally sustainable energy sources (Zaldivar *et al*., 2001). Bio-ethanol is an effective sustainable energy source. Based on the premise that fuel bioethanol can contribute to a cleaner environment and with the implementation of environmental protection laws in many countries, demand for efficient bio-ethanol production processes may increase.

One important requirement is to have efferent microorganisms that are able to ferment a variety of sugars well as to tolerate stress condition (Manikandan and virurhagin, 2010). Bacterial and yeasts strains having traits for ethanol production have been constructed through metabolic engineering After several rounds of modification/evaluation, three main microbial platforms Saccharomyces cerevisia, Zymornonasmorabilis and Escherichia coli have emerged and they function well in pilot studies (Manikandan and Virutagiri, 2010). While there are on-going efforts to further enhance their properties improvement of fermentation process is just one of the second factors that need to be fully emphasized and integrated to generate competitive fuel bio-ethanol

During batch fermentation, the rate of ethanol per milligram of cell protein s maximal for a short period and declines progressively as ethanol accumulates in the surrounding broth (Moulin *et al.,* 1984, Assien *et al.,* 2010). However studies including that of Millar *et al.,* (1982) and Dombek and Ingram (1983) demonstrated that the removal of accumulated ethanol during fermentation does not immediately restore fermentative activity and provided evidence that decline in metabolic rate is not exclusively due to the presence of ethanol.

The aim of this research was to investigate the effect of yeast, pH and nutrient supplement on bioethanol from cassava.The specific Objectives are: To study the effect of fermentation duration on the yield of bioethanol produced from cassava (*Manihot esculenta*) broth using catalyst (*Saccharomyces cerevisiae)*; the effect of catalyst concentration on the yield of bioethanol produced from cassava (*Manihot esculenta*) broth; the effect of pH on the yield of Bioethanol produced from cassava (*Manihot esculenta*) broth using catalyst (*Saccharomyces Cerevisiae)* and the effect of inorganic nutrient supplement on the yield of Bioethanol produced from cassava (*Manihot esculenta*) broth using catalyst (*Saccharomyces cerevisiae)*

**Materials and Method**

**Materials**

Fresh cassava tubers were obtained from Takum Local Government market area Taraba State. These were especially collected in a polythene bag and taken to chemistry laboratory of the Taraba State University, Jalingo for further analysis. The organism used was saccharomyces *cerevisiae*; this was collected from the biology laboratory of the same institution. Other materials includes: Distilled water, 0.1M HCl, 0.1M Na0H and NPK (Nutrient).

**Preparation of Cassava Tubers for Fermentation**

Fresh cassava tubers were peeled using kitchen knife and washed with distilled water.

The starchy layer was size reduced using knife.200g of the peeled cassava was measured and 250ml of distilled water added. The cassava tubers were fermented for a period of 6 days in a conical flask by addition of yeast powder.

**Effect of Fermentation Duration on the Yield of Bioethanol**

This experiment was done to determine the residence time. The catalyst concentration that gave the optimum bioethanol yield was chosen while varying fermentation duration 2, 3, 4, 5, 6 and 7 days. Bioethanol and reducing sugar concentrations were determined. The time at which maximum bioethanol yield was obtained was noted.

**Effect of pH on Bioethanol Yield**

To evaluate the effect of pH on bioethanol yield, the pH of each hydrolysate was varied ranging from 3,4,5,6 to 8. During fermentation; the pH of each set up was monitored at interval of 30minutes for stabilization. At the expiration of fermentation duration bioethanol and reducing sugar were respectively determined and the pH that gave the highest yield of bioethanol was chosen for further experiments.

**The Effect of Inorganic Nutrient Supplementation on Bioethanol Yield**

The fermentation process of the same catalyst concentration, fermentation duration of the optimum yield and favorable pH, an inorganic supplement (nutrient i.e. NPK) was added at ratio of 0.01, 0.1, 1.0, 1.5mg (L) for optimum yield. At the end of fermentation duration, bioethanol yield concentration was determined.

**The Effect of Catalyst Concentration on the Yield of Bioethanol Production**

The effect of catalyst concentration was done by fermenting the cassava for a period of 6 days at varying catalyst concentration of 0.5,1.0,1.5,2.0,2.5,3.0,3.5,4.0g per 200g of peeled cassava and 250ml distilled water added.

**Distillation of Cassava Broth**

This was carried out using distillation apparatus (set up), figure 1. The cassava broth was decanted and transferred into round bottom flask and placed on a heating mantle fixed to a distillation column fitted with a thermometer enclosed in running tap water. Anti bumping chips were also added into the flask for even distribution of heat. Another flask was fixed to the other end of distillation column to collect the distillate at the temperature between 70 - $90^0$C.The volume of bioethanol distillate was measured and recorded. This was done for each of the fermented broth for catalyst concentration of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0g.

**Determination of Quantity of Bioethanol Produced**

The distillate collected was measured using measuring cylinder and expressed as quantity of bioethanol produced in g/L was calculated using

$$Y = \frac{Ve}{Vm} \times 100\% \quad (1)$$

Were Y=yield of bioethanol, $V_e$ and $V_m$=volume of bioethanol distillate and fermented mixture distilled respectively.

Figure 1: Distillation Set-Up for the production of bioethanol

## Results and Discussion

Table 1 gave the reaction conditions and the pretreatment yield of bioethanol from the fermented cassava tubers. The data indicate that the pre-treatment yield of bioethanol ranged from 0.5 – 4.7% in the reactions conducted.

**Table 1: Reaction Conditions and Yield of Bioethanol**

| Sample No. | Quantity of Yeast Added(g) | Fermentation time (day) | Volume of Fermented Mixture Distilled($cm^3$) | Volume of Ethanol Distillate($cm^3$) | Bioethanol Yield (%) |
|---|---|---|---|---|---|
| 1 | 0.5 | 6 | 200 | 1.0 | 0.5 |
| 2 | 1.0 | 6 | 200 | 1.2 | 1.3 |
| 3 | 1.5 | 6 | 200 | 2.6 | 2.2 |
| 4 | 2.0 | 6 | 200 | 4.4 | 3.4 |
| 5 | 2.5 | 6 | 200 | 5.4 | 4.7 |
| 6 | 3.0 | 6 | 200 | 3.8 | 3.9 |
| 7 | 3.5 | 6 | 200 | 4.2 | 3.1 |
| 8 | 4.0 | 6 | 200 | 3.8 | 2.9 |

## Effect of Catalyst Concentration

The effect of quantity of yeast (catalyst) added during fermentation on the yield of bioethanol was shown in figure 2 at a varying catalyst concentration of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0g. During the fermentation, 2.5g of yeast gave an optimal bioethanol yield of 4.7%.Further increase in yeast concentration beyond 2.5g resulted in decrease of bioethanol yield. This may be as a result of more yeast consuming the limited glucose for self-sustenance, thereby resulting in low yield of bioethanol. Other conditions were identical to the normal fermentation conditions; bioethanol concentration was measured with time.

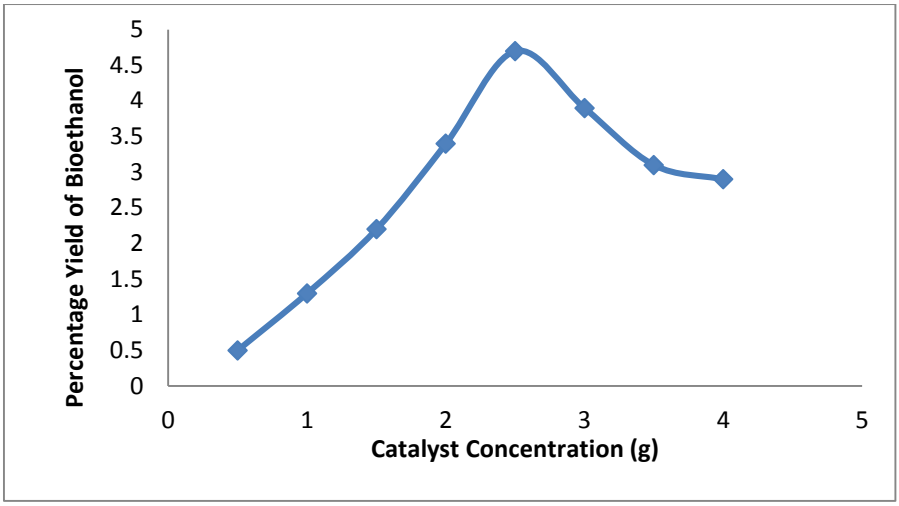


Figure 2:.Effect of Catalyst concentration on Bioethanol Yield.

**Effect of fermentation duration on bioethanol yield**

This experiment was done to determine the residence time and the dynamics for optimum bioethanol yield by the test isolate. The standardized inoculum was added to 200ml of each hydrolysate contained in 500ml conical flask. These were then incubated at $30^0$C for 144hours. However at interval of 6, 24, 72 and 144 hours bioethanol and reducing sugar concentration were determined. The time at which maximum bioethanol yield was obtained was noted and subsequent experiments on pH and supplements were therefore used at this duration.

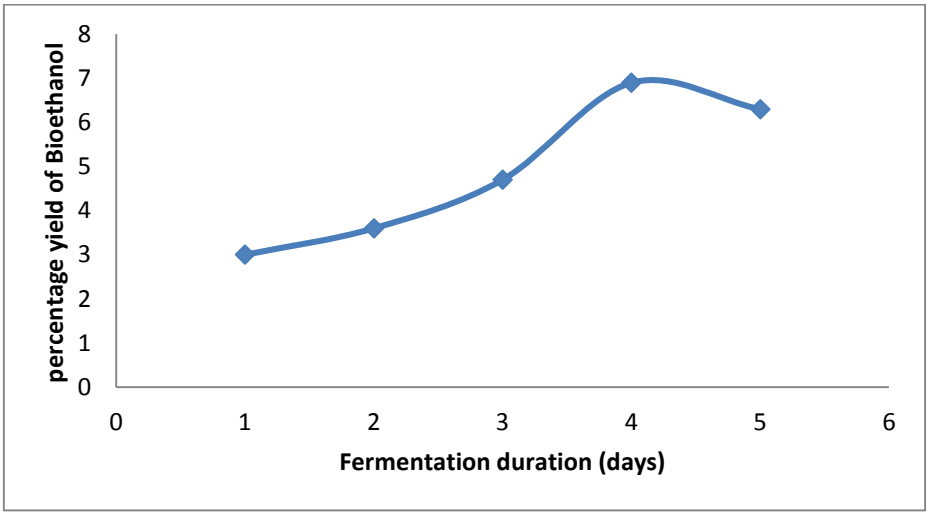


Figure 3: Effect of Fermentation Duration on Bioethanol Yield.

**Effect of pH on Bioethanol Yield**

To evaluate the effect of pH on bioethanol yield, the pH of each hydrolysate was varied ranging from 3, 4, 5, 6 and 8. The standardized inoculums were then introduced into various flasks and fermented for the stated days to determine the day that resulted in maximum bioethanol production. During fermentation, the pH of each set-up was monitored at intervals of 30 minutes for stabilization with appropriate buffer when necessary. At the expiration of the fermentation duration bioethanol and glucose concentrations were respectively determined and the pH that gave highest yield in bioethanol was chosen for further experiments as presented in figure 4.

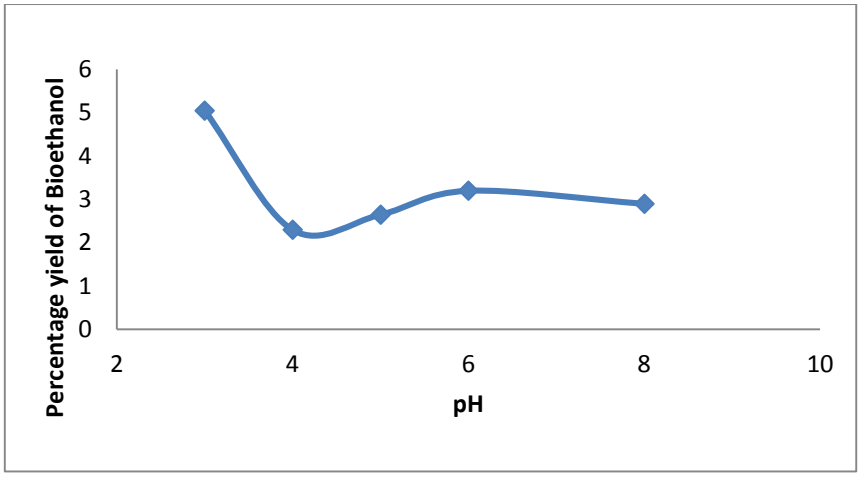


Figure 4: Effect of pH on Bioethanol Yield.

**Effect of Inorganic Supplement on Bioethanol Yield**

Concentration of 0.01, 0.1, 1.0, 1.5 (mg/L) of NPK was prepared with each hydrolysate. The pH of various hydrolysate was adjusted appropriately and 3 days old standard inocula was introduced and fermentation was done for 6 days (144h). At the end of fermentation duration bioethanol yield and glucose concentration were determine and results presented in Figure 5.

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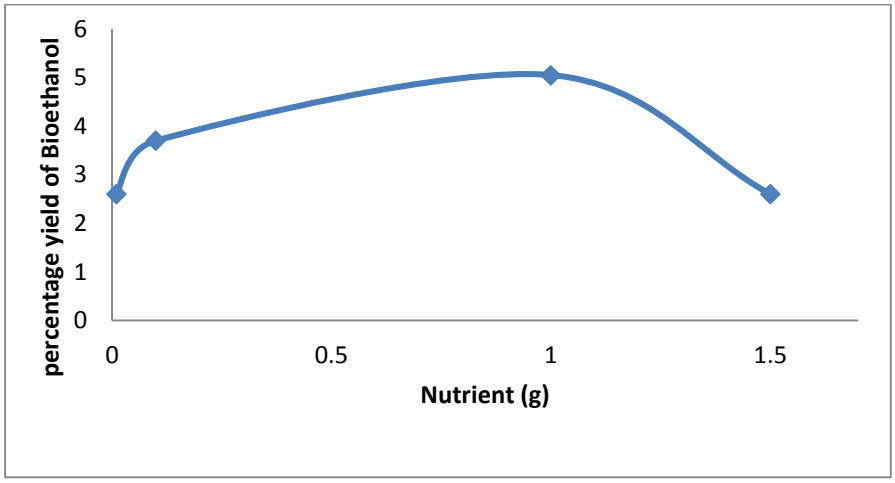


Figure 5: Effect of Nutrient Concentration on Bioethanol Yield.

The result obtained on the effect of fermentation duration on the rate of bioethanol yield when Saccharomyces cerevisiae was exposed to each hydrolysate showed that bioethanol yield reduced progressively with increase in fermentation duration. Optimum bioethanol yield obtained was 6.9% at the 6 days of fermentation as shown in figure 3. Analysis of variance at 95% confidence limit indicates that there was a significance difference in bioethanol produced at the various fermentation periods. Therefore 6 days was chosen as duration for optimum yield. Also it was noticed that reducing sugar (glucose), decreased progressively as fermentation duration increases. High amounts of glucose were consumed within 6 days of fermentation after which, utilization rate reduced. Statistical analysis at $P>0.05$ showed that there was a significance different in the amount of bioethanol produced at the various pH tested. Maximum bioethanol yield was 5.05% and this was achieve at the pH of 3 as shown in figure 4 this value was also significantly higher than those obtained from initial experiment of effect of fermentation duration on bioethanol production. Sugar consumption was also observed to increase as pH increased. PH at which highest utilization occurred corresponded to pH at which optimum bioethanol yield was recorded figure 4.

The result of the effects of nutrients on the generation of bioethanol and glucose utilization was represented in figure 5. Supplementation with various sources of nitrogen and phosphorus resulted in additional increase in the amount of bioethanol generated in each hydrolysate. However results obtained reveal that additional bioethanol generation was nutrient concentration dependent. Nutrient concentration that resulted in maximal bioethanol production was 1.0g of NPK. Bioethanol produced at this respective concentration was 5.05%. Thus nutrient in form of NPK facilitated bioethanol production.

Also, results obtained revealed that the addition of nitrate and phosphate from exogenous sources improved substrate utilization and bioethanol yield. Generally, it was observe that low concentration of each nutrient sources stimulated higher fermentation rates and bioethanol production than higher nutrient concentration. Probably this might be as a result of toxic effect of higher concentration of the nutrient. The tolerable nutrient concentration might be useful in synthesis/repair of damaged DNA and cell membrane in addition to enhancing cell growth. Introduction of nutrient must have corrected the nutritional imbalance by supplying the limiting nitrogen and phosphorus. Microorganisms require phosphorus as phospholipids in synthesing cell membranes as components of nucleic acid and for sugar phosphorylation (Andrew and Jackson 1996). They also exploit nitrate source to meet their protein and nucleic acid requirement. NPK fertilizer contains these two inorganic nutrients and hence when it was added to the cassava effluent. It was better in establishing the nutritional balance than $NaNO_3$ and $K_2HPO_4$. Thus, it is important that all bioethanol fermentation programmed to monitor nutritional variables that could influence resultant bioethanol production and to adjust same appropriately. The abrupt final decline in activity reflects the near complete exhaustion of the substrate glucose. Thus optimum combinations of pH, nutrient concentration plays a major role in getting maximum bioconversion of cassava substrate to bioethanol.

Bioethanol production from fresh cassava roots using a low-temperature process was evaluated on a pilot-plant scale. The application of low temperature to cassava starch followed by a dual enzyme action resulted in bioethanol yield comparable to that of a traditional high temperature process. Pressurized distillation gave a satisfactory recovery efficiency of bioethanol but was slightly lower than that obtained from atmospheric pressure distillation. Over 40% of the normal steam consumption was saved by adopting the low-temperature and pressurized distillation systems in the cassava broth to bioethanol process.

The effect of Catalyst Concentration added during fermentation on yield of bioethanol is shown in Figure 6. Regression analysis of the yield of bioethanol as a function of fermentation duration by taking all data points on Figure 6 gives a quadratic equation of the form $Y = -0.116x^2 + 1.882x$, where Y is the yield of bioethanol and x is the amount of catalyst added. Optimization gives an optimum catalyst mass of 2.5g giving an optimal bioethanol yield of 3.98% with a Correlation Coefficient ($R^2$) of 0.923 signifying that most of the substrate were converted to reducing sugar and subsequently into bioethanol.

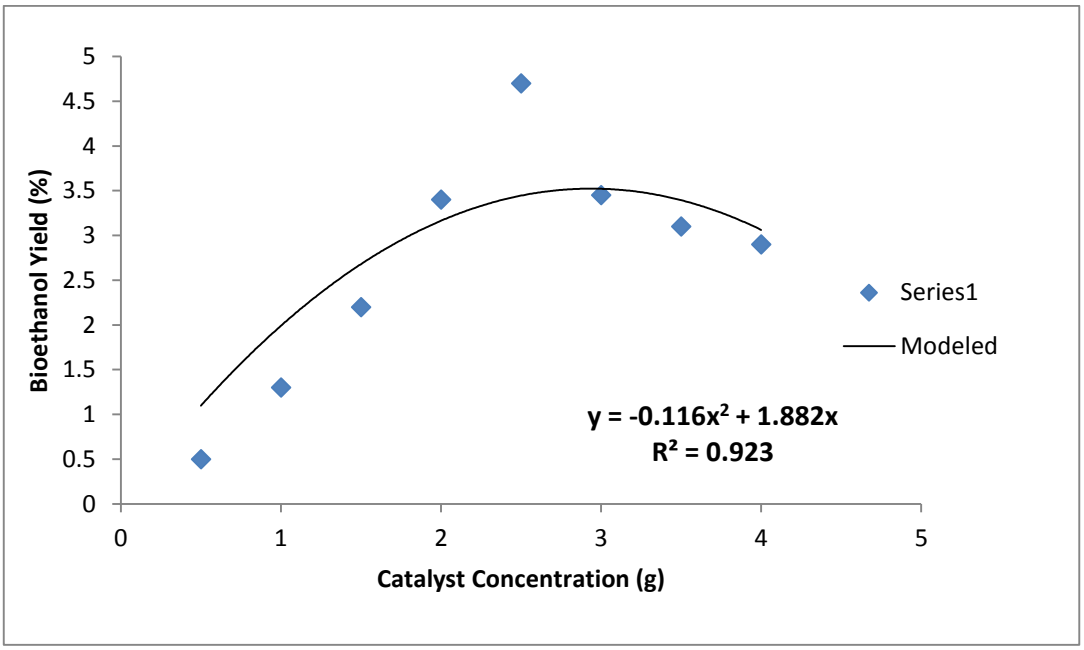


Figure 6: Bioethanol Yield against Catalyst Concentration

## Conclusion

This research study has determined that bioethanol can be produced from cassava biomass, which is the starchy content. The production of bioethanol from cassava flour is a means of controlling environmental pollution due to cassava processing. Also it mitigates the human effects on climate change by producing efficient, clean and renewable energy that can reduce the dependence of Nigeria on fossil fuels. Furthermore, catalysis was vital in the fermentation process. An optimal catalyst concentration of 2.5g with a corresponding maximum bioethanol yield of 3.98% was established while the production model followed the quadratic model.

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