*Journal homepage: www.fia.usv.ro/fiajournal*
*Journal of Faculty of Food Engineering,*
*Ştefan cel Mare University of Suceava, Romania*
*Volume XIX, Issue 4- 2020,* pag. 307 - 315

# BRIX PRODUCTION FROM CASSAVA PEELS AND OPTIMIZATION OF BIOETHANOL SYNTHESIS USING *SACCHAROMYCES CEREVISIAE*

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Received 21th August 2020, accepted 28th December 2020

**Abstract:** *The purpose of this paper is to produce and optimize brix from cassava peels for bioethanol production using Saccharomyces cerevisiae. The use of agricultural waste products in bioethanol production helps in decreasing reliance on food crops. Optimization of production medium is required to maximise metabolite yield. The capacity of Saccharomyces cerevisiae to ferment wort derived from cassava peels, an agricultural waste, in optimized conditions to produce bioethanol, was investigated. A box-behnken design of five factors (substrate weight, temperature, inoculum size, pH, incubation time) and three levels was adopted to improve production efficiency. The substrate was subjected to physical and biological pretreatments to obtain simple sugars. Alcoholic fermentation was done using S. cerevisiae for six days. Brix content was measured before and during the fermentation process, as well as alcohol content after fermentation. Response surface plots of the factors were plotted. The results showed that brix value ranged from 1.7 ºBx to 3.8 ºBx while bioethanol production ranged from 1.01g/l to 2.29g/l. At optimal conditions of pH 8, temperature of 33ºC, inoculum size of 3, substrate weight of 20g and fermentation time of 119h, predicted ethanol yield will be 3.67g/l. Cassava peel is a good substrate for bioethanol production. Higher yield of bioethanol was realised with optimization of the fermentation medium.*

**Keywords***: Optimization, Agricultural Waste Products, Brix, Bioethanol, Response Surface Methodology*

## 1. Introduction

Many economically important compounds that have application in pharmaceutical, food and chemical industry, as well as in energy production, are produced through fermentation technology. Various microorganisms have been reported to produce many primary and secondary metabolites, but in a very low quantity. Bioethanol, a product of fermentation, is an alcohol produced from organic biomass. Fermentation of any material that contains sugars can produce ethanol [1], [2].

Cellulosic materials such as agricultural wastes like corncob, cornstalk, cornhusk, sugarcane bagasse, have been used in the production of bioethanol [3-5]. The production of ethanol is achieved through fermentation, engineered by microorganisms [6-9].

The use of agricultural waste products in bioethanol production helps in decreasing reliance on food crops, forest woody biomass, hence reduction of deforestation. Crop residues that have short harvest period renders them readily available to bioethanol production [10-12]. The Fermentation processes are used from

generations but the need for production in a sustainable way as to meet the market requirements in a cost effective manner, has become a great challenge.

Media optimization is one of the phenomena seriously investigated in any large scale metabolite production. Before now, media optimization was carried out by classical methods, which were expensive, time consuming, involving series of experiments with compromised accuracy. The advent of modern mathematical/statistical techniques such as the response surface methodology (RSM); media optimization has become more prominent, effective, economical, efficient and robust-result oriented. In the design of a production process, pH, temperature, agitation speed, substrate weight, inoculum size etc. are fermentation conditions that must be recognised and optimized accordingly, to achieve maximum output [13-15].

This work focused on the optimization of brix/reducing sugar production for use in bioethanol production by *Saccharomyces cerevisiae*.

## 2. Materials and methods

### 2.1 Sample collection and processing

Fresh cassava peels were collected from different areas in Owerri, Imo state. The substrate was washed, dried for weeks, ground separately using a laboratory blending machine and sieved to obtain fine powdered stock. This was labelled and stored at room temperature in transparent polyethylene bags. Crude Fibre, ash, fat, crude protein, and carbohydrate content of the cassava peels were determined in triplicate according to the method of A.O.A.C. [16].

### 2.2 Design of experiment

The Box-Behnken design was adopted for optimization of brix conversion in a 5×3 design, that is, five factors in three levels, using Minitab 1.7. Substrate weight (10g, 15g and 20g); pH (6, 7 and 8); Inoculum size (3, 4 and 5); Temperature (30$^o$C, 35$^o$C and 40$^o$C) and Incubation time (72h, 96h and 120h) were factored.

### 2.3 Microbial source and inoculum development

*Saccharomyces cerevisiae* was obtained from 33 Consolidated Breweries, Awo-Omanma, Imo State, Nigeria. The strain obtained was characterized to ascertain their cultural and microscopic characteristics, quality, viability, purity and fermentative capacity [17], [18]. The yeast, *Saccharomyces cerevisiae* was activated using 1% glucose solution and standardized using a spectrophotometer at wave length 600 ($A_{600}$), to optical density (OD) values of (3, 4 and 5) respectively.

### 2.4 Pretreatment of the agricultural waste material

Two stages of pretreatments were used:

**2.4.1 Heat treatment** Different weights (10g, 15g and 20g) of the substrate (cassava peels) were dissolved in 150 ml of deionized water in 46 separate Erlenmeyer flasks, according to the design of experiment. After capping, the flasks were sterilized in batches in an autoclave at 121$^o$C for 15 mins, to convert the carbohydrate into sugary liquid called wort. The samples were filtered using a filter bag [19].

**2.4.2 Enzymatic hydrolysis**

On completion of wort production, 1 ml each of commercially available enzymes, amylase and neutrase were simultaneously added to the flasks and allowed to stand for 48 hours. Neutrase was first added and the reaction was maintained at pH of 5.5-7.5 and at temperature of 30$^o$C-55$^o$C for 24h. Then amylase was added and reaction parameters maintained at 32$^o$C- 37$^o$C and

pH of 6.7-7.0 for 48 hours. Amylase further breaks down any trace of carbohydrate that was not broken down during autoclaving (boiling), while neutrase breaks down any trace of protein present in the sample [20], [4]. After 48 hours of addition of enzymes, the contents of the flasks were autoclaved to stop the action of the enzymes [19].

## 2.5 Alcoholic fermentation process

One-tenth normality (0.1 N) of NaOH and 0.1 N $H_2SO_4$ was prepared [21] and was used to adjust the pH of the contents of the flasks (wort) to pH 6, 7 and 8 respectively, to conform to design of experiment, with buffer solution introduced to the flasks to maintain the respective pH. After 24 hours, the contents of all the flasks were made up to a volume of 100mls each, to ensure uniform fermentation volume. According to the design of the experiment, 3, 4 and 5 standardized yeast (*Saccharomyces cerevisiae*) were aseptically introduced into the flasks. The content of the 46 flasks was allowed to ferment at 30ºC, 35ºC and 40ºC respectively [22]. Fermentation was stopped after 72h, 96h or 120h respectively as defined by the design and brix level as well as alcohol content of the samples in the flasks was measured using the refractometer.

**Table 1.**

**Interpretation from Experimental Design Table (Uncoded)**

| No. crt. | pH | Temp (ºC) | Time (hours) | Inoculum size (OD) | Substrate (grams) |
|---|---|---|---|---|---|
| 1 | 6 | 30 | 96 | 4 | 15 |
| 2 | 8 | 30 | 96 | 4 | 15 |
| 3 | 6 | 40 | 96 | 4 | 15 |
| 4 | 8 | 40 | 96 | 4 | 15 |
| 5 | 7 | 35 | 72 | 3 | 15 |
| 6 | 7 | 35 | 120 | 3 | 15 |
| 7 | 7 | 35 | 72 | 5 | 15 |
| 8 | 7 | 35 | 120 | 5 | 15 |
| 9 | 7 | 30 | 96 | 4 | 10 |
| 10 | 7 | 40 | 96 | 4 | 10 |
| 11 | 7 | 30 | 96 | 4 | 20 |
| 12 | 7 | 40 | 96 | 4 | 20 |
| 13 | 6 | 35 | 72 | 4 | 15 |
| 14 | 8 | 35 | 72 | 4 | 15 |
| 15 | 6 | 35 | 120 | 4 | 15 |
| 16 | 8 | 35 | 120 | 4 | 15 |
| 17 | 7 | 35 | 96 | 3 | 10 |
| 18 | 7 | 35 | 96 | 5 | 10 |
| 19 | 7 | 35 | 96 | 3 | 20 |
| 20 | 7 | 35 | 96 | 5 | 20 |
| 21 | 7 | 30 | 72 | 4 | 15 |
| 22 | 7 | 40 | 72 | 4 | 15 |
| 23 | 7 | 30 | 120 | 4 | 15 |
| 24 | 7 | 40 | 120 | 4 | 15 |
| 25 | 6 | 35 | 96 | 3 | 15 |
| 26 | 8 | 35 | 96 | 3 | 15 |
| 27 | 6 | 35 | 96 | 5 | 15 |
| 28 | 8 | 35 | 96 | 5 | 15 |
| 29 | 7 | 35 | 72 | 4 | 10 |
| 30 | 7 | 35 | 120 | 4 | 10 |
| 31 | 7 | 35 | 72 | 4 | 20 |
| 32 | 7 | 35 | 120 | 4 | 20 |
| 33 | 6 | 35 | 96 | 4 | 10 |
| 34 | 8 | 35 | 96 | 4 | 10 |
| 35 | 6 | 35 | 96 | 4 | 20 |
| 36 | 8 | 35 | 96 | 4 | 20 |
| 37 | 7 | 30 | 96 | 3 | 15 |
| 38 | 7 | 40 | 96 | 3 | 15 |
| 39 | 7 | 30 | 96 | 5 | 15 |
| 40 | 7 | 40 | 96 | 5 | 15 |
| 41 | 7 | 35 | 96 | 4 | 15 |
| 42 | 7 | 35 | 96 | 4 | 15 |
| 43 | 7 | 35 | 96 | 4 | 15 |
| 44 | 7 | 35 | 96 | 4 | 15 |
| 45 | 7 | 35 | 96 | 4 | 15 |
| 46 | 7 | 35 | 96 | 4 | 15 |

### 2.6 Optimization of parameters for alcohol production

Minitab 17 software was used to produce surface plots of the interactions of the parameters that affect the production of brix and alcohol. Response Optimizer (Minitab$^R$ 17) was used to optimize them to give the maximum yield of alcohol.

## 3. Results and discussion

The table shows the chemical composition of cassava peels. The values for the crude protein, fat, fibre, ash content and carbohydrate content were 2.90%, 0.38%, 5.45%, 3.02% and 74.30%. carbohydrate content had the highest value while fat content had the lesst value.

**Table 2.**
**Chemical composition of cassava peels (%)**

| | Crude protein | Fat | Fibre | Ash | Carbohy-drates |
|---|---|---|---|---|---|
| **Mean** | 2.90 | 0.38 | 5.45 | 3.02 | 74.30 |
| **SD** | 0.48 | 0.07 | 0.47 | 0.07 | 0.14 |

### 3.1 Determination of brix and alcohol content

The highest yield of ethanol was 2.29g/l with brix value of 3.8 from flask 36 at conditions of pH 8, temperature 35$^o$C, fermentation time of 96h, inoculum size of 4 and substrate weight of 20g while the lowest yield was 1.01g/l with brix value of 1.7 from flask 12 at conditions of pH 7, temperature 40$^o$C, fermentation time of 96h, inoculum size of 4 and substrate weight of 20g. At optimal conditions, the predicted ethanol yield will be 3.67g/l. This is quite higher than the alcohol content of the other set up operated under different combination of parameters. These are shown in figures 1 and 2.

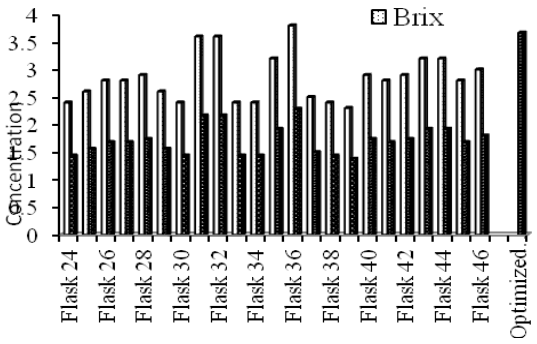


**Fig. 1. Brix and ethanol content of each flask**

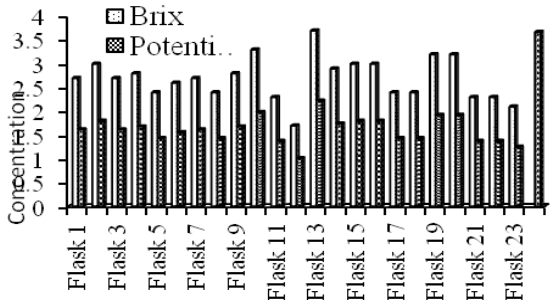


**Fig. 2. Brix and ethanol content of each flask**

Main effect plot of the five factors indicated at pH 6, response was almost 3.1. The yield dropped as the pH was increased to 7 but gave its maximum yield at pH 8. Temperature of 35$^o$C was seen as the best temperature for highest yield. Fermentation time of 100 days, inoculum size of 1.2×10$^9$cfu/ml and substrate weight of 20, gave the highest yield as shown in figure 3.

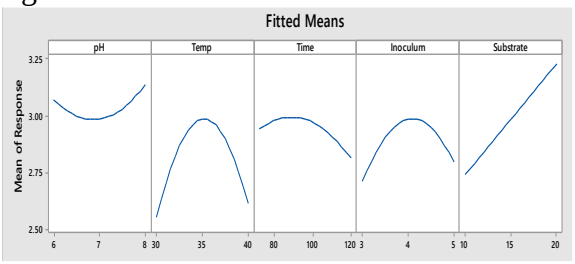


**Fig. 3. Main effect plot for carbohydrate (sugar) converted to ethanol**

Response Surface plots which showed the interactions between the factors that affected the production of bioethanol are shown in figure 4.

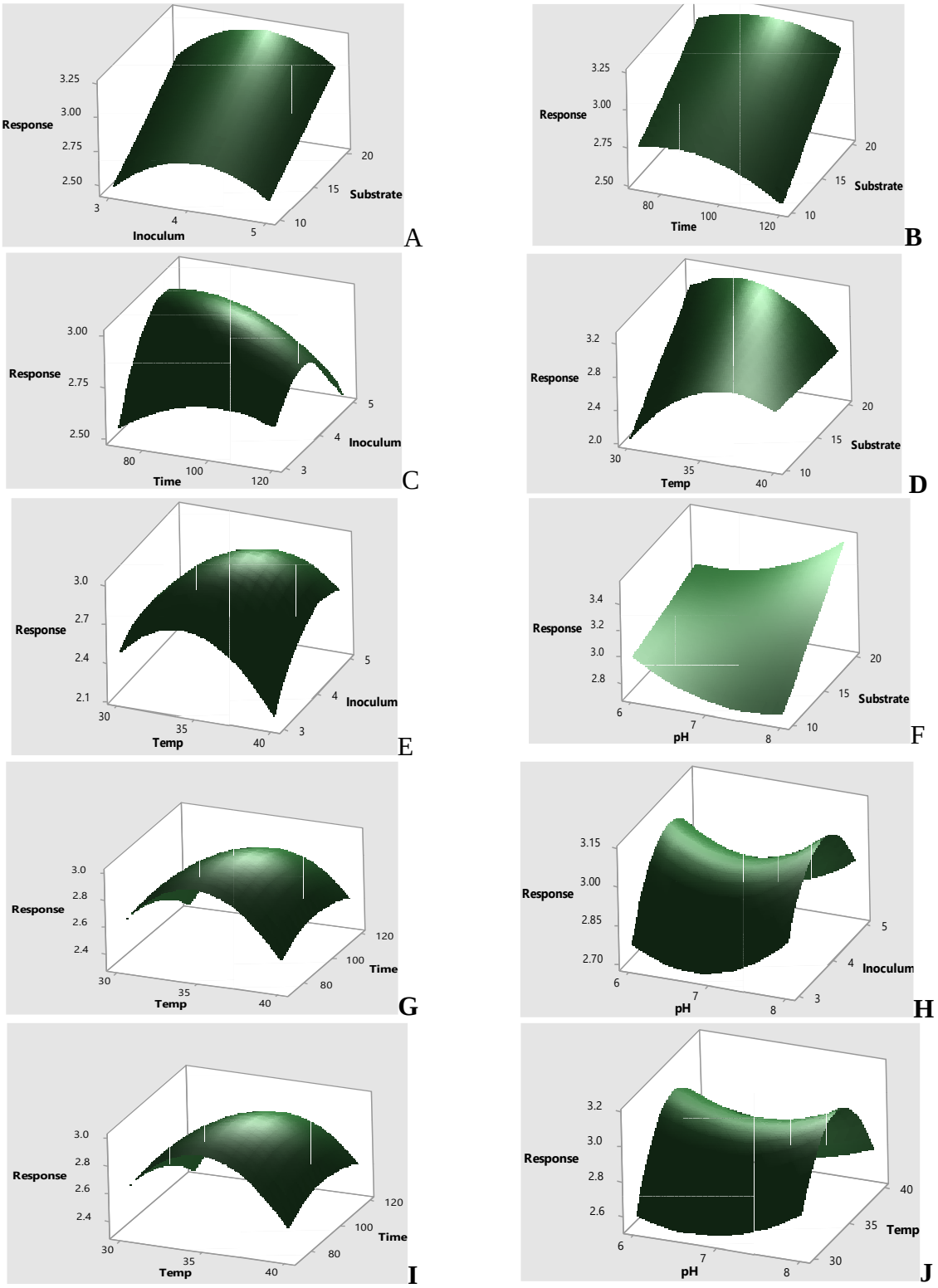


**Fig. 4. Surface plots of carbohydrate converted.** (a) Against substrate weight and inoculum size, (b) Against substrate weight and time of fermentation, (c) Against inoculum size and time of fermentation, (d) Against substrate weight and temperature of fermentation, (e) Against inoculum size and temperature of fermentation, (f) Against time of fermentation and temperature of fermentation, (g) Against substrate weight and pH of fermentation, (h) Against inoculum size and pH of fermentation, (i) Against time of fermentation and pH of fermentation, (j) Against temperature of fermentation and pH of fermentation.

In (a), Inoculum size and substrate weight interacted, while pH, temperature and time were the hold values. Inoculum size of 4 and substrate weight of 20 gave the highest yield. Fermentation time and substrate weight interacted in (b) while inoculum size, pH and temperature were kept on hold. Highest yield was seen at fermentation time of 100h and substrate weight of 20. In (c), fermentation time andinoculum size interacted while substrate weight, pH and temperature were kept on hold. At fermentation time of about 100h and inoculum size of 4, the best yield was realised. Substrate weight of 20 and temperature of 35$^{o}$C gave the highest yield in (d), while the inoculum size, fermentation time and pH kept as hold values. In (e) temperature and inoculum size interacted. As the temperature was increased from 30$^{o}$C to 35$^{o}$C, increased yield was seen. The inoculum size of 4 gave the highest yield. pH, inoculum size and substrate weight were the factors kept on hold while fermentation time and temperature interacted in (f). Best yield was seen at fermentation time of 100h and temperature of 35$^{o}$C. In (g), Substrate weight of 20 and pH of 6 gave the highest yield when the two factors interacted, while keeping inoculum size, time and temperature on hold. The inoculum size and pH interaction in (h) showed that at pH of 8 and inoculum size of 4, maximum yield was realised, with temperature, time and substrate weight kept as hold values. In (i), substrate weight, temperature and inoculum size were the hold values. The interaction between pH and fermentation time gave the best yield with pH of 6 and fermentation time at 120h. Temperature and pH were the interacting factors in (j) while the inoculum size, fermentation time and substrate weight were the hold values. At pH of 8 and temperature of 35$^{o}$C, the highest yield was realised.

There was increase in yield as the pH increased from 6 to 8. At the temperature was increased from 30$^{o}$C to 35$^{o}$C, the yield increased but dropped with further increase of the temperature to 40$^{o}$C. The yield increased as the time of fermentation increased from 72h to 96h and to 120h. Increase in the inoculum size from optical density 3 to optical density of 4 gave a high yield which dropped as the inoculum size was increased to optical density 5. The substrate weight showed a linear increase in yield, the yield increased as substrate weight was increased. Optimization plot shows that at pH 8, temperature of 33$^{o}$C, fermentation time of 119h, inoculum size of $9.0\times10^{8}$cfu/ml and substrate weight of 20g, predicted ethanol yield will be 3.67g/l, as shown in figure 5.

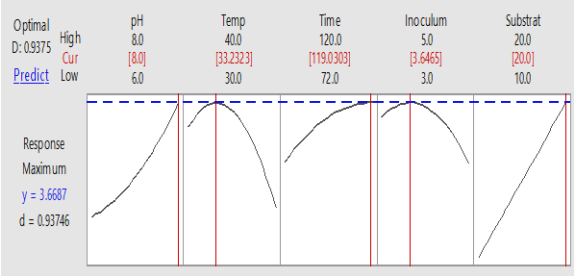


**Fig. 5. Optimization of alcohol production**

Brix optimization and production of bioethanol from agro wastes involve the pretreatment of the agro wastes to expose the simple sugars which the yeast can utilize to produce ethanol [23]. Fermentation is brought about by the yeast which converts the sugars in the substrates to ethanol [24]. *S. cerevisiae* have been used in alcohol production especially in wine making and in the brewing industries. *S. cerevisiae* was able to produce alcohol from cassava peels. This is in line with [25] who reported that the microorganism gives a high ethanol yield at a low distillation cost and can withstand high ethanol concentration. Yeasts are used to generate fuel ethanol from renewable energy sources [26].

Results from the study showed that optimum temperature for alcohol production was between 30ºC and 35ºC. This could be attributed to how the growth rate of the microorganisms is directly affected by the temperature [27]. According to [28], high temperature above 40ºC is unfavorable for cells growth and it is a stress factor for microorganisms.

The ideal temperature for bioethanol production depends on the ideal temperature of the yeasts. The ideal temperature range for fermentation is between 20ºC and 35°C. This is line with the works of [29], who recorded an ethanol concentration of 78.6 g/l at 30ºC. [30] also observed a reduction in ethanol yield as temperature increased beyond 35ºC.

Fermentation time has an effect on the growth of microorganisms. Brix conversion works with the fermentation time. Higher yield of ethanol was seen at fermentation time of almost 120h when compared with the yield at 96h and 72h. Shorter fermentation time causes inefficient fermentation due to inadequate growth of microorganisms [31]. The longer the fermentation time, the toxic the microbial growth becomes, especially in batch mode. This is as a result of high concentration of ethanol in the fermented broth [32].

The result also showed that 20g of the substrate gave the highest yield of brix conversion. The lowest yield was found with substrate weight of 10g. [33] stated that high ethanol productivity and yield in batch fermentation can be obtained by using higher initial sugar concentration; the maximum rate of ethanol production is achieved when using sugars at the concentration of 150 g/L. However, it needs longer fermentation time and higher recovery cost. High substrate loading for industrial fermentation is feasible and hence always desired [32].

Although inoculum size of $9.0\times10^8$ cfu/ml gave a higher yield compared with inoculum size of $1.5\times10^9$cfu/ml, increase in the inoculum size did not really have a great effect in the yield of brix converted. This result corroborates with the work of [33], which reported that the final ethanol concentration is not significantly affected by the concentration of inoculum, even though it affects the consumption rate of sugar and production of ethanol.

From the result, highest yield of ethanol was obtained at an alkaline pH of 8. [34] stated that a wide range of optimum pH 4.0-5.0 is required for the activity of *S. cerevisiae* at temperature of 35ºC. This agrees with the work of [35] who reported that when ethanol is continuously produced from the glucose fermenting culture, other acids like carbonic acid and acetic acid are continuously generated making the system more acidic and low pH could trigger the production of ethanol.

This work has shown that the importance of optimization cannot be over mphasized. The maximum ethanol yield was 2.29g/l but with the optimal conditions of pH 8, temperature of 33ºC, inoculum size of 3, substrate weight of 20g and fermentation time of 120h., maximum ethanol yield of 3.67g/l was predicted.

## 4. Conclusion

In conclusion, we can remark that the utilization of agricultural residues and wastes that are generated from a number of agricultural activities for bioethanol production is a cost-effective and environmental-friendly approach for sustainable development. From this study, cassava peels which causes nuisance to the environment, was converted to wealth, and served as a good substrate for bioethanol production. This approach will help to mitigate the stern competitive demand of agricultural products for alcohol production. Techniques and statistical approaches used in medium optimization process have potential to save

experimental time for process development and reduction of overall product cost. From the response surface plots, prediction of optimal conditions for maximum yield of bioethanol were pH 8, temperature of 33$^{o}$C, inoculum size of $9.0\times10^{8}$ cfu/ml, substrate weight of 20g and fermentation time of 120h. This is significant in industrial fermentation systems. Proper utilization of waste products will help in developing our agricultural sector by providing viable biofuel resource.

## 5. Acknowledgement

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Ihuoma OFFOR-EMENIKE, Campbell AKUJOBI and Wesley BRAIDE designed the study and analyzed the data; Ihuoma OFFOR-EMENIKE and Wesley BRAIDE conducted the experimental work; Vincent IBEKWE supervised the study; Ihuoma OFFOR-EMENIKE and Campbell AKUJOBI wrote the manuscript. All the authors read and approved the final manuscript.

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