



## Optimization of Bioethanol Production from Unripe Plantain Peels Using *Saccharomyces cerevisiae*

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### Abstract

This study investigated the production of bioethanol from unripe plantain peels in an optimized condition. Optimization of production medium maximises metabolite yield. Reliance on food crops is reduced when agricultural waste products are used to produce bioethanol. The capacity of *Saccharomyces cerevisiae* to ferment wort derived from unripe plantain peels, an agricultural waste, in optimized conditions to produce bioethanol, was studied. 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 (unripe plantain peels) was subjected to physical and biological pretreatments to obtain simple sugars. Cellulase enzyme was used to breakdown the substrate to simpler 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 0.5 °Bx to 2.0 °Bx while bioethanol production ranged from 0.2g/l to 1.1g/l. At optimal conditions of pH 6, temperature of 40°C, inoculum size of 5, substrate weight of 20g and fermentation time of 75h, predicted ethanol yield will be 1.3g/l with maximum concentration of brix as 2.2 °Bx. 1.3g/l of bioethanol was realized with optimization of the fermentation medium. The peel of unripe plantains is a good substrate for the synthesis of bioethanol.

**Keywords:** Unripe plantain peels, Bioethanol, Brix, Optimization, Response Surface Methodology.

### Introduction

The rising global demand for sustainable and renewable energy sources has increased research efforts into bioethanol production from agro-industrial wastes (Sun & Cheng, 2002). Unripe plantain peels are rich in starch, cellulose, and hemicellulose. This make them a viable substrate for bioethanol production (Adeeyo et al., 2015). Unripe plantain peels provide good biomass resource due to their high carbohydrate content, especially starch and non-structural polysaccharides (Akinyemi et al., 2022). Adding value to such wastes does not only address waste management issues but also supports economy strategies; conversion of waste to wealth. Bioethanol production involves pretreatment, hydrolysis, fermentation, and distillation processes (Kumar et al., 2009; Braide et al., 2010). *Saccharomyces cerevisiae* has high fermentative capacity, ethanol tolerance, and generally recognized as safe (GRAS) status; this makes it a good microorganism for industrial bioethanol production (Cheng et al., 2023).

To maximize ethanol yield from unconventional substrates, process optimization is critical. Optimizing bioethanol synthesis from unripe plantain peels includes determining the ideal conditions for hydrolysis to release fermentable sugars, as well as refining fermentation parameters such as temperature, pH, inoculum size, and fermentation time (Chandel et al., 2012). This involves pretreatment methods to enhance fermentable sugar release, enzymatic

hydrolysis efficiency, and fermentation parameters such as pH, temperature, inoculum size, and substrate weight (Adeboye et al., 2023). These pretreatment techniques such as acid hydrolysis or enzymatic saccharification helps to minimize inhibitory byproducts while maximizing sugar recovery (Ogbuagu & Okonko, 2024). Statistical optimization approaches like Response Surface Methodology (RSM) and Artificial Neural Networks (ANN) have been increasingly applied to regulate the complex interactions between fermentation variables, thereby significantly boosting bioethanol yield (Alabi et al., 2024). Recent studies have demonstrated that with proper optimization, agro-wastes like plantain peels can achieve competitive ethanol yields, contributing to waste valorization and bioenergy development (Sornvoraweat & Kitpreechavanich, 2008). The optimization of brix for use in bioethanol production using *Saccharomyces cerevisiae*, was the main focus of this study.

## Materials and Methods

### Sample collection and processing

A significant amount of fresh, unripe plantain peels was gathered from several locations in Owerri, Imo state. To create finely powdered stock, the substrate was cleaned, allowed to dry for weeks, then ground separately using a lab blending machine and sieved. This was labeled and kept in clear plastic bags at room temperature. The A.O.A.C. technique was used to determine the unripe plantain peels' crude fiber, ash, fat, crude protein, and carbohydrate content in triplicate. (AOAC, 2000).

### Design of experiment

Using Minitab 1.7, the Box-Behnken design was used to optimize brix conversion in a 5×3 design, or five factors in three levels. Temperature (30°C, 35°C, and 40°C), incubation length (72, 96, and 120 hours), substrate weight (10g, 15g, and 20g), pH (6, 7, and 8), and inoculum size (3, 4, and 5) were all taken into consideration.

### Microbial source and inoculum development

33 Consolidated Breweries, located in Awo Omanma, Imo State, Nigeria, provided the *Saccharomyces cerevisiae*. The strain was examined to determine its quality, viability, purity, fermentative potential, and cultural and microscopic traits. (Scholar & Benedikte, 1999; Suh et al., 2007). A spectrophotometer set to wave length 600 (A600) was used to standardize the yeast, *Saccharomyces cerevisiae*, to optical density (OD) values of 3, 4, and 5, respectively. The yeast was activated using a 1 percent glucose solution.

### Pretreatment of the agricultural waste material

Two stages of pretreatments were used:

#### Heat treatment

The experiment design called for dissolving different weights (10g, 15g, and 20g) of the substrate (unripe plantain peels) in 150 ml of deionized water in 46 different Erlenmeyer flasks. Following capping, the flasks were autoclaved in batches for 15 minutes at 121 degrees Celsius to turn the carbohydrate into wort, a sugary liquid. A filter bag was used to filter the samples. (Yu & Zhang, 2004).

#### Enzymatic hydrolysis

Following the completion of wort production, 1 milliliter of commercially available amylase and neutrase enzymes were added to each flask at the same time, and the flasks were left to stand for 48 hours. The reaction was kept at a pH of 5.5 to 7.5 and at a temperature of 30 to 55 degrees Celsius for 24 hours after the addition of the neutrase. After that, amylase was introduced, and for 48 hours, the reaction conditions were kept at 32–37 degrees Celsius and pH 6.7–7.0. Neutrase reduces any remaining protein in the sample, whereas amylase further breaks down any remaining carbohydrates that were not broken down during autoclaving (boiling). To halt the enzymes' activity, the contents of the flasks were autoclaved 48 hours after the enzymes were added. (Yu & Zhang, 2004, Martin et al., 2022).

#### Alcoholic fermentation process

In accordance with the experiment's design, a tenth normality (0.1 N) of NaOH and 0.1 N H<sub>2</sub>SO<sub>4</sub> were made and used to bring the wort's pH down to 6, 7, and 8 correspondingly, the flasks' pH was then maintained by adding buffer solution. After 24 hours, a uniform fermentation volume was attained by filling each flask to a capacity of

100 milliliters. Standardized yeast (*Saccharomyces cerevisiae*) was aseptically added to flasks 3, 4, and 5 in accordance with the experiment's design. At 30, 35, and 40 degrees Celsius, respectively, the contents of the 46 flasks were permitted to ferment. (Abouzeid & Reddy, 1986). According to the design, fermentation was halted after 72, 96, or 120 hours, respectively. The refractometer was used to assess the alcohol content and brix level of the samples in the flasks.

Table 1:1 Analysis of the Experimental Design Table (Uncoded)

Std Order	pH	Temp (°C)	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

### Alcoholic Fermentation Process

One-tenth normality (0.1 N) of NaOH and 0.1 N H<sub>2</sub>SO<sub>4</sub> were prepared (Haynes, 2011) and used to adjust the pH of the contents of the flasks to pH 6, 7 and 8 respectively to conform to design of experiment. Buffer solution was introduced to the flasks to maintain the respective pH. The content of all the flasks were made up to a volume of 100ml each, to ensure uniform fermentation volume. According to the design of the experiment, 3, 4 and 5 MacFaland's standards of the yeast (*Saccharomyces cerevisiae*) were aseptically introduced into the flasks. The content of the 46 flasks was allowed to ferment according to the parameters in the table of design (Abouzeid & Reddy, 1986). Fermentation was stopped after 72h, 96h or 120h respectively as defined by the design and brix/liquid mixture of the samples in the flasks was measured using the refractometer. The alcohol was determined by distillation method. The entire process was done in triplicate to avoid error.

### Optimization of Parameters for Alcohol Production

Various concentrations of bioethanol produced under the specified conditions by each run were fed into Response Optimizer (Minitab 17) and used to derive optimal factors for maximum bioethanol production. Using the optimum, fermentation of substrate was carried out and resulting concentration of bioethanol was compared to the predicted value (Offor-Emenike et al., 2020).

## Results

### Chemical composition of unripe plantain peels

Plantain peels are the outer layer of the plantain fruit, composed of approximately crude protein 6-8%, crude fibre 10-15%, moisture content 6-10%, crude fat 3-6%, Ash 8-10%, carbohydrates 55-70%. (Akinwunmi et al., 2022).

Table 1.2: Composition of unripe plantain peels

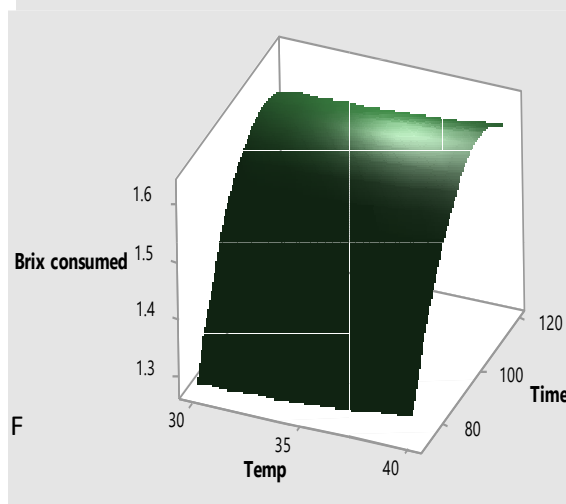
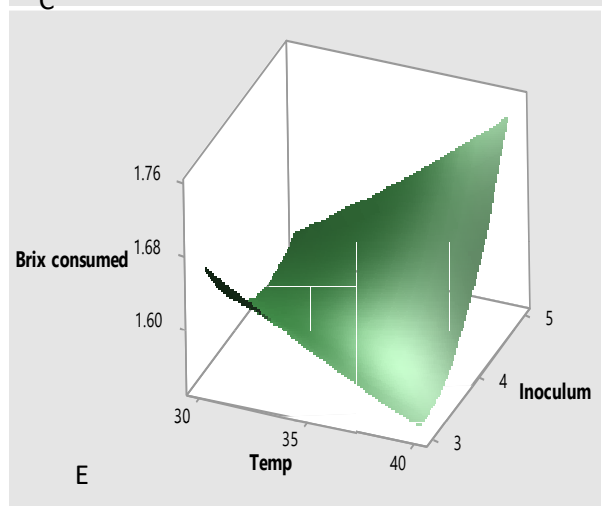
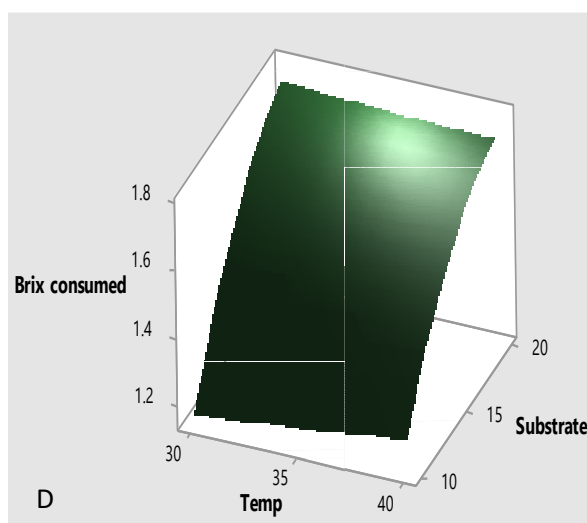
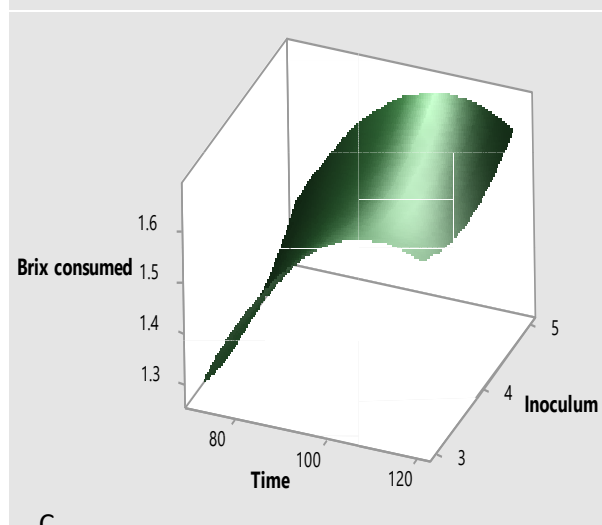
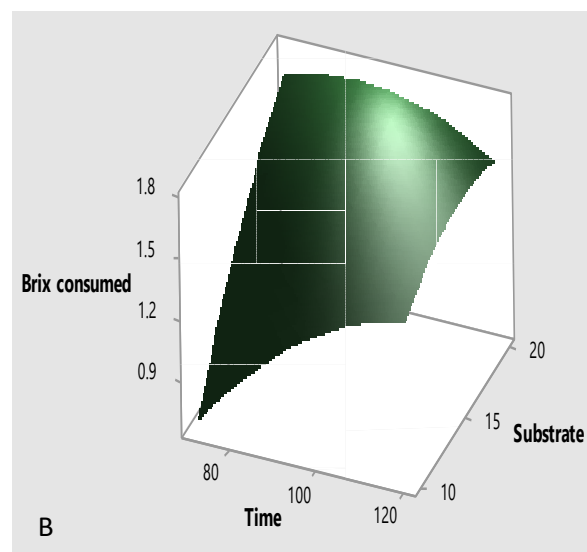
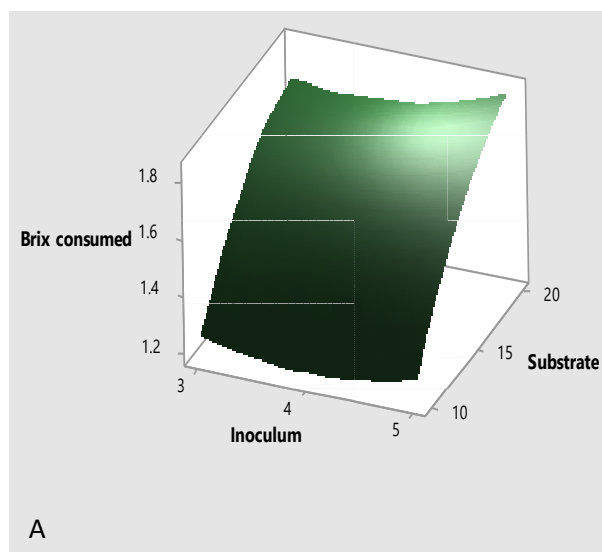
Component	Chemical composition (wt %)
Carbohydrate	55-70
Crude protein	6-8
Moisture content	6-8
Crude fat	3-6
Ash	8-10
Crude fibre	10-15

(Remi, 2023).

### Determination of brix and alcohol content

With flasks 11 set up at pH 8, 35°C, 72 hours of fermentation, inoculum size of 5, and substrate weight of 15g, the maximum yield of ethanol was 2.0g/l with a brix value of 1.1 °Bx. The lowest yield, however, was from flask 34 at pH 7, temperature 35°C, fermentation length 96 hours, inoculum size 6, and substrate weight 20g, yielding 1.1g/l with a brix value of 0.6 °Bx. The estimated production of ethanol under ideal circumstances is 1.3g/l. Compared to the second setup that ran under a different set of settings, this has a significantly higher alcohol concentration.

Response Surface plots which showed the interactions between the factors that affected the production of bioethanol from plantain peels using *Saccharomyces cerevisiae*, the main effects and optimization plots are shown in the figures below. Optimization plot shows that at pH 6, temperature of 40°C, fermentation time of 75 h, inoculum size of 5 and substrate weight of 20 g, maximum concentration of brix was 2.2 °Bx while the ethanol yield was 1.3 g/l.



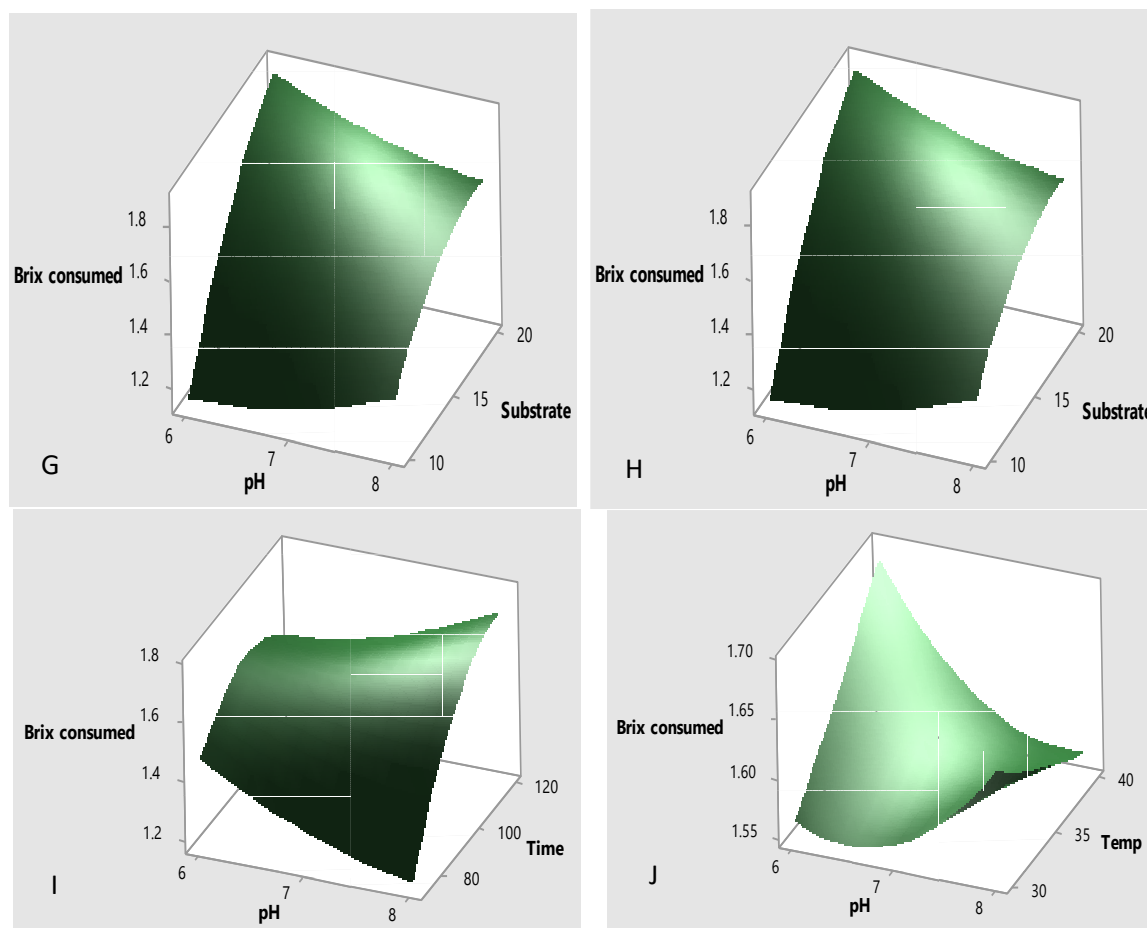
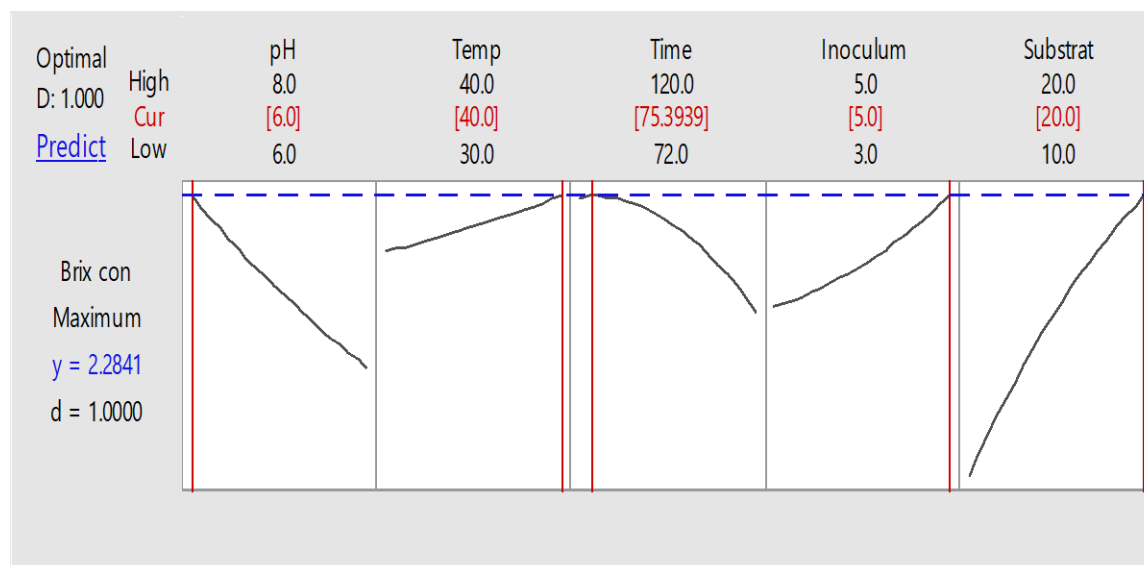


Fig. 1.1 Shows surface plots of brix converted in plantain peels with *Saccharomyces cerevisiae*

(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.

Fig 1.2 Optimization plot of plantain peels with *Saccharomyces cerevisiae*

The amount of brix utilized by the organism for all the flasks and the ethanol produced was estimated. This is shown in the table below.

Table 1.3 Brix consumed by *Saccharomyces cerevisiae* and the estimated ethanol produced from plantain peels

Flasks	Brix (°Bx)	Potential Alcohol (mls)
Flask 1	1.8	1.0
Flask 2	1.5	0.8
Flask 3	2	1.1
Flask 4	1.5	0.8
Flask 5	1.6	0.9
Flask 6	1.9	1.1
Flask 7	1.3	0.7
Flask 8	1.5	0.8
Flask 9	1.3	0.7
Flask 10	1.3	0.7
Flask 11	2	1.1
Flask 12	1.9	1.1
Flask 13	1.6	0.9
Flask 14	1.2	0.7
Flask 15	1.6	0.9
Flask 16	1.7	1.0
Flask 17	1.5	0.8
Flask 18	1.8	1.0
Flask 19	1.6	0.9
Flask 20	2	1.1

Flask 21	1.4	0.8
Flask 22	1.2	0.7
Flask 23	1.8	1.0
Flask 24	1.6	0.9
Flask 25	1.3	0.7
Flask 26	1.8	1.0
Flask 27	1.7	1.0
Flask 28	1.9	1.1
Flask 29	0.5	0.2
Flask 30	1.2	0.7
Flask 31	1.5	0.8
Flask 32	1.3	0.7
Flask 33	0.8	0.4
Flask 34	1.1	0.6
Flask 35	1.8	1.0
Flask 36	1.7	1.0
Flask 37	1.3	0.7
Flask 38	1.4	0.8
Flask 39	1.1	0.6
Flask 40	1.5	0.8
Flask 41	1.2	0.7
Flask 42	1.7	1.0
Flask 43	1.8	1.0
Flask 44	1.3	0.7
Flask 45	1.7	1.0
Flask 46	1.7	1.0
Optimized	2.2	1.3
Flask		

## Discussion

The outcome of this study shows that bioethanol yield of 1.3 g/l was obtained by employing *Saccharomyces cerevisiae* to digest unripe plantain peels. under optimized conditions of pH 6, temperature 40°C, inoculum size 5%, substrate weight 20 g, and fermentation time 75 h. This represents a significant improvement over the highest yield of 1.1 g/l from the conventional method of bioethanol production. This improvement can be attributed to precise control of physicochemical parameters which collectively influence the efficiency of saccharification and fermentation. The improved yield corroborates the findings of Khan et al. (2017), who reported that pre-treatment and parameter optimization significantly enhance ethanol productivity from starchy and cellulosic biomass.

Results of plantain peels with *Saccharomyces cerevisiae* shows that highest ethanol yield was seen with the inoculum size increased to MacFalands standard 5. According to Irfan (2010), an increase in inoculum size leads to increased biomass concentration and results in increased bioethanol concentration.

The optimum temperature for alcohol production was at seen at 40°C. This conforms to the work of Manikandan et al. (2006) who achieved maximum concentration of ethanol yield from cassava at 40°C. High temperature supports



the hydrolysis of starch into simple sugars which leads to constant availability of glucose concentration throughout the experiment.

Higher yield of ethanol was seen at fermentation time of 72 h. Shorter fermentation time causes inefficient fermentation due to inadequate growth of microorganisms (Zabed et al., 2014). The longer the fermentation time, the more the toxic inhibitory materials produced. As a result of high concentration of ethanol in the fermented broth, the system may become unfavourable for the organism (Nagodawithana et al., 1974). Nadir (2009) reported that the highest ethanol concentration of 40.11g/l was gotten after 64 h and this dropped to 37.24g/l after 72 h of fermentation with sweet sorghum.

The result also showed that 20 g of the substrate gave the highest yield of brix conversion. The lowest yield was found with substrate weight of 10 g. This agrees with the work of Laopaiboon et al. (2007) who stated that high ethanol productivity and yield in batch fermentation can be obtained by using higher initial sugar concentration. High substrate loading for industrial fermentation is feasible and hence always desired (Nagodawithana et al., 1974). From the result, highest yield of ethanol was obtained at pH of 6. Ganigue et al. (2016) 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.

The maximum ethanol yield was 1.1 g/l with a brix value of 2 °Bx but with the optimal conditions of pH 6, temperature of 40°C, inoculum size of MacFalds standard 5, substrate weight of 20 g and fermentation time of 75 h., maximum ethanol yield of 1.3 g/l was seen with a brix value of 2.2 °Bx.

### Conclusion

The use of unripe plantain peels as a lignocellulosic biomass aligns with global efforts toward utilizing agro-waste for sustainable bioethanol production. The present study establishes unripe plantain peels as a viable feedstock for bioethanol production, with yield enhancement possible through careful optimization of fermentation parameters.

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