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Bioethanol Production Potential and Optimization from Cassava (*Manihot esculenta)* Peels Using *Saccharomyces cerevisiae*.

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Abstract

Biofuels are expected to reduce dependence on imported petroleum, reduce greenhouse gas emissions and revitalize the economy by improving agricultural products. There is an increasing demand for Bioethanol and Nigeria currently depends on the importation of ethanol to meet its local demand. This research work was aimed to evaluate the potentials of Cassava peel in bioethanol production using *Saccharomyces cerevisiae*. Hydrolysis of the substrate was carried out and the hydrolysate was detoxified with 0.8 g/L ammonium sulphate to remove inhibitory substance such as formic acid. The hydrolysate was further fermented using *S. cerevisiae* to convert glucose to ethanol. Highest amount of reducing sugars were obtained with 15g of Cassava peel having 5.51g/L. After the fermentation process the hydrolysate was subjected to double distillation and maximum ethanol was obtained at 30^0C temperature, pH 3.5, and 15g of the substrate after 96 hours of fermentation. Cassava Peels produced 23.05% ethanol with fermentation efficiency of 44.45%. There was no significant difference (P>0.05) between ethanol yield of Cassava peel using *S. cerevisiae* for hydrolysis and fermentation respectively. The use of cassava peel for bioethanol production as an alternative source of fuel provides a starting point for the improvements in cultivation and adsorption of cassava as well as improving food security.

Keywords: Bioethanol, Fermentation, Optimization, Glucose, Saccharification

1 Introduction

The quest by many countries for energy independence and the widespread awareness of the need to reduce green-house gas emissions has heightened the search for alternative energy sources (15). People's Livelihood diversification would require the understanding of society dynamics in terms of domestic energy consumption as well as investigating possible ways of producing energy from available resources (7). Biofuels are expected to reduce dependence on imported petroleum, reduce greenhouse gas emissions and revitalize the economy by increasing agricultural produce (12). Ethanol has been produced in batch fermentation with *Saccharomyces cerevisiae, Aspergillus niger, Mucor mucedo,* that cannot tolerate high concentration of ethanol (24, 32, 38).

Cassava peels waste contains 43.626% cellulose, hemicellulose 10.384% and lignin 7.646% (10). There are two main stages of bioethanol production, first is to hydrolyze, which breaks down cellulose into simple sugars with the help of acids or enzyme, second is by fermentation, which will convert sugar into alcohol through anaerobic respiration by microbes. Enzymes are used as catalysts for the break down reaction of lignocellulose into simple sugars. Enzymatic hydrolysis is relatively more efficient in producing sugar because it can minimize the

formation of toxic compounds as a result of acid hydrolysis process. However, the disadvantage of these methods is it takes much longer time than acid hydrolysis process and high cost of purified enzyme material.

The technological availability and awareness of Africans especially local farmers to the economic potential of utilizing agricultural products in bio-ethanol production poses a great problem. This research work will therefore examine the production of Bio-ethanol using *Saccharomyces cerevisiae* to produce ethanol from Cassava peels.

2 Materials and Methods

2.1 Source of Agricultural produce

Cassava (*Manihot spp)* was purchased in clean polythene bags from Monday Market Maiduguri Borno State, Nigeria, and identified by botanist, in the Department of Biological Science, University of Maiduguri.

2.2 Isolation of Isolates

Rotten Papaya was used for the isolation of *Saccharomyces cerevisiae*

2.3 Characterization and Identification of *Saccharomyces cerevisiae*

2.3.1 Carbohydrate (sugar) Fermentation Using API 20C AUX

An ampoule of API (API 20C AUX) suspension was opened and isolated colonies from yeast culture were scooped with initially sterilized inoculating loop aseptically and transferred into the ampoule, mixed properly until the suspension turbidity was equal to 0.5 McFarland standard.

An ampoule at API 20^{o}C medium was opened and approximately 100µlof the aliquot was transferred into the culture medium and was gently homogenized. The inoculation box (tray and lid) was prepared and 5ml of sterile distilled water was added into the honey combed wells while the strips 0 – 29 strips were placed in the box. The micro tube of the strips was filled with the yeast suspension obtained in the ampoule of API C medium with the inoculum level. The lid was placed onto the box and the preparation was incubated at 29^{o}C for 48hours. After 48 hours of incubation, the strips were read and the results were recorded. The isolates were confirmed using API web software (API 20C AUX).

2.3.2 Evaluation of *Saccharomyces cerevisiae* for physiological adaptation to produce ethanol

i. Flocculation

Flocculation test was carried out using the method of (43). The isolates were inoculated in 10ml yeast peptone medium in a tube and incubated at 28^{o}C for 48 hours. The tubes were agitated for visualization of flocculation. The amount of sedimentation formed in the test tube after 1 minute indicates flocculation.

ii. Ethanol Tolerance

Visual assessment of turbidity in the tubes was carried out according to the method described by (19). Five tubes containing 10%, 12%, 14% and 16% of ethanol and 0.5ml of yeast peptone were inoculated with a loopfull of yeast cells, respectively. These were incubated at 28^{o}C for 48 hours.

2.3.3 Inoculum preparation

Yeast colonies were cultured on Potato Dextrose Agar (PDA) medium for 72 hours, and cells were harvested using sterile water with 0.1% Tween 80 (4). Cells count was measured using Haemocytometer and adjusted to 2 x 10^{6}spores/ml.

Yeast Peptone Dextrose Broth (100ml) in 250ml flask capacity were inoculated with 48 hours old pure colonies of *Saccharomyces cerevisiae* from agar slant with the aid of an inoculating loop and incubated at 28 $\pm$ 2^{o}C on a

rotary shaker at 150 rpm for 48 hours. A 0.5 McFarland turbidity cells population was used to compare the size of the cell (50).

2.4 Mechanical pretreatment

Cassava peels were washed thoroughly with water and was allowed to air dry at room temperature before milling using ball mill and sieved through 2mm mesh size (21).

2.5 Proximate Composition of Substrates

Proximate Composition of Substrates was carried out using the procedure of the Association of Analytical Official Chemists (8).

2.6 Determination of Cellulose, Hemi cellulose and Lignin Content of Substrates

The cellulose, hemicelluloses and lignin content of substrates were determined using the method of Association of Official Analytical Chemists (8). Using Acid Detergent fibre (ADF), Neural Detergent Fibre (NDF), and Acid Detergent Lignin (ADL) reagents

2.7 Acidic pretreatment

Five grammes of substrate was placed in a conical flask containing 50ml of 5%, sulfuric acid and was autoclaved at 121^0C for 30mins.The autoclaved sample was filtered through No.1.Whatman filter paper; the residue was washed with distilled water and was oven dried at 60^0C.

2.8 Enzyme hydrolysis

Enzymatic hydrolysis was carried out according to the method described by (16). Five hundred milliliter capacity conical flasks were used for the enzyme hydrolysis of the substrate. The 5g, 10g and 15g, of each sample was put in the flask and 100ml of distilled water was added. The flasks were plugged with cotton wool and aluminum foil and then sterilized at 121^0C for 30min. Each flask was inoculated with 0.5ml suspension of 2 x 10^6 each of the standardized inocula and uninoculated flasks were used as control. The flasks were incubated at 37^0C for 5 days with frequent shaking to provide aeration and agitation. After the 5 days, the samples were filtered through what man filter paper.

2.9 Reducing Sugar Content

The sugar content of the hydrolysed substrate was determined using DNS method described by (33) with glucose as standard. It was determined by adding 3ml of 3, 5-Dinitrosalicylic acid (DNS) reagents to 3ml of the sample. The mixture was boiled for 10 minutes to develop the red-brown colour. One ml of 40% potassium tartarate solution was added to stabilize the color and cool to room temperature under running tap water. The absorbance was measured at 491nm using ultraviolet spectrophotometer. The reducing sugar content was determined by making reference to a standard curve of glucose concentrations.

2.10 Detoxification of Hydrolysate

This was carried out according to the method described by (30). The procedure removes the compound that inhibits/slows the fermentation process. A 0.8g/L of ammonium sulphate was weighed and added in each of the 100ml conical flasks containing the hydrolysates. The conical flasks were shake and inoculated with yeast isolates for fermentation to begin controls (non-inoculated medium) were also set.

2.11Fermentation of hydrolysate

A 100ml of each hydrolyzed sample of cassava peels were autoclaved at 121^0C for 15mins and 1ml of 0.5 McFarland turbidity equivalents to $1.5x10^8$cells of *Saccharomyces cerevisiae* was added. The flasks were incubated at 30^0C for 5days. The Bioethanol produced was double distilled in the distillation column. The distillates were collected for further analyses (47).

2.12 Estimation of Bioethanol Production

The percentage bioethanol yield was calculated based on the ethanol produced in grams from the amount of fermentable sugar (in grams) x 100. The fermentation efficiency was also calculated based on the following formula adopted from (17).

: Fermentation efficiency = Actual Yield/ Theoretical yield x 100

2.13 Optimization of Bioethanol Production

2.13.1Determination of the effect of varying amount of substrate on Bioethanol production

The substrate was varied from 5g, 10g, and 15g and hydrolyzed by enzymes to convert it to fermentable glucose which were further fermented to produce ethanol. The optimal amounts of substrate were determined by measuring the quantity of ethanol produced after fermentation.

2.13.2 Determination of the effect of varying time of fermentation

The effect of fermentation days was determined by taking samples from fermentation medium in hours 24hrs, 48hrs, 72hrs, 96hrs, and 120hrs to allow fermentation to yield more quantity of ethanol.

2.13.3 Determination of the effect of varying temperature

Suitable temperature for maximum production of ethanol by the yeast isolates was used for fermenting the hydrolysate at various temperatures namely 25, 30, 35, and 40°C in the incubator.

2.13.4 Determination of the effect of varying pH

Four sets of pH values were used for the selection of an optimum pH condition for ethanol production. Fifteen grams of the hydrolysate were used by adjusting the pH using HCl and NaOH to 3.5, 4.0, 4.5, 5.0 using digital pH meter. This was incubated at 30^oC for 96hours.

2.14 Confirmatory tests for ethanol

Confirmatory tests were carried out for both the produced ethanol and standard ethanol (19).

i. Flame Test

Small portion of the bioethanol was heated on a stainless-steel crucible slowly and then rapidly, the sample was observed for change in appearance, readily flammable, any odour produced, whether nonvolatile residue was left. The burning blue flame with characteristics smells of ethanol and no residue left indicated the presence of ethanol.

ii. Esterification

In a small test tube, 4 drops of ethanol and 4 drops of acetic acid were mixed; one drop of concentrated sulphuric acid was added. The mixture was warmed in a hot water bath for about 5 minutes, and 2.0ml of cold water was added. The production of a fruity smell after a while indicates the presence of ethanol.

iii. Determination of Boiling Point

A small volume (5ml) of the ethanol was put in a test tube sealed at one end and firmly attached to thermometer with a rubber band. The apparatus was clamped to a ring stand and the whole set up was immersed in a water bath. The ethanol boiled at 77.0^oC.

iv. Ethyl Acetate Test

One (1ml) of the ethanol was put in a test tube containing 0.5g of sodium acetate and a drop of concentrated H_2SO_4. The test tube and its content were gently heated for 1min. It was cooled and poured into 5ml of water in a boiling tube. The experiment was observed for fruity smell of acetate which indicates the presence of ethanol.

2.15 Statistical Analysis

The data generated were subjected to statistical analysis using One-Way Analysis of Variance (ANOVA) and Duncan Multiple range was used to compare the means. Significant difference was considered when $p<0.05$.

3 Results

3.1Isolation and Characterization of yeast Isolate

The yeast was isolated and identified as *Saccharomyces cerevisiae* using morphological and physiological characteristic as presented in Table 1. *Saccharomyces* species fermented 9 out of 20 sugars in API 20 CHL which included glucose, galactose, lactose, maltose, raffinose, Trehalose, Melezitose, Saccarose and methyl D-glucopyranoside. The isolates flocculates at 0.5-10ml in normal saline after 48hrs of incubation and was found to have high ethanol tolerance of up to 17%.

3.2 Proximate composition of Cassava Peels

Table 2 shows the proximate composition of Cassava Peel presented Protein content of 2.10%, Carbohydrate content of 54.69%, Moisture content of 20%, Fat content of 1.71%, Ash 2.30%, and Fibre 8.20%.

3.3 Cellulose, Hemicellulose and Lignin Content of Cassava Peels

Table 3 Shows Cellulose content of untreated Cassava peel to be 31.1% and pretreated 35.0%, Hemicellulose content of untreated Cassava peel 7.66% and pretreated 6.70%, and lignin content of untreated Cassava peel 31.4% and pretreated 27.0%.

3.4 Reducing Sugar Concentration after hydrolysis of Cassava peel

The results of reducing sugar hydrolysate of cassava peel were presented in Table 4. The highest concentration of reducing sugar was achieved at a substrate concentration of 15g. The reducing sugar production increased gradually with increase in substrate concentration. A maximum yield of 5.51g/L of reducing sugar was obtained and a minimum yield of 3.73g/L was obtained at substrate concentration of 5g after 5 days of hydrolysis.

3.5 Fermentation of products of Hydrolysate

The yield of ethanol production in gram of ethanol per gram of glucose in the hydrolysate of cassava peel is presented in Figure 1. The mass of Ethanol in the hydrolysate of cassava peel varied significantly between the substrate concentration and time. A 5g of cassava peel at 24hrs yields 0.148g and 15g yields 0.205g of ethanol after 96hrs of fermentation. The optimized conditions of Temperature and pH yields 0.227g at pH 3.5 which gives the highest ethanol yield and pH 5.0 yields the lowest with 0.205g in hydrolysate of cassava peels, temperature of 25^0C yields the highest ethanol of 0.223g and 40^0C yields 0.211g of ethanol.

Ethanol Yield from Fermentation of products of Hydrolysate substrate

The percentage yield of ethanol production in the hydrolysate of Cassava peel is presented in Table 5. Ethanol yield from Cassava peel varied significantly between the substrate concentration and the highest yield of 23.05% with fermentation efficiency of 44.45%.

3.6 Physicochemical Characteristics of Produced Ethanol

Table 5 shows the results of ethanol produced which was tested for other characteristics and compared with standard ethanol. Flame, Ethyl acetate, and Esterification tests shows positive, Boiling Point was 77.0^{o}C.

3.7 The influence of pH on bioethanol produced from the hydrolysate substrates (reducing sugar) is presented in Table 6. The optimum pH for ethanol production after fermentation with *S. cerevisiae* was found to be 3.5 at which the highest production of ethanol was recorded (23.88%); the minimum production of bioethanol was recorded at pH of 5.0.

The temperature for bioethanol from hydrolysate of substrate (reducing sugar) was optimized. Temperatures have profound effect on ethanol fermentation. The effect of different temperature on bioethanol production is presented in Table 7. The optimum temperature for maximal ethanol production was found to be 30^{o}C. Increase in temperature decrease the quantity of ethanol produced.

3.8 Optimum Condition for Bioethanol Production

Table 8 summarizes the optimum condition for high yield of ethanol. Temperature of 30^{o}C, pH 3.5 and substrate concentration of 15g were the condition that yielded the highest production of ethanol with 23.05% and fermentation efficiency of 44.45%.

Table 9 shows the differences between the percentage of ethanol obtained before and after detoxification with ammonium sulphate at optimum conditions.

Table 1: The Sugar Fermentation Pattern of *S. cerevisiae* (API 20 CHL)

S/N	Parameter	Observation
1.	Glucose	+
2.	Glycerol	-
3	Calcium 2-keto-gluconate	-
4	Arabinose	-
5	Xylose	-
6	Adonitol	-
7	Xylitol	-
8	Galactose	+
9	Inositol	-
10	Sorbitol	-
11	Methyl D-glucopyranoside	+
12	N-acetyl-glucosamine	-
13	Cellobiose	-
14	Lactose	+
15	Maltose	+
16	Saccharose	+

17	Trehalose	+
18	Melezitose	+
19	Raffinose	+
20	Fungus	*S. cerevisiae*
21	Flocculation	0.5-10ml (flocculates)
22	Ethanol tolerance	17%

Key: + = positive, - = negative

Table 2: Proximate Composition (%) of Cassava Peels

Parameters	Composition (%)
Moisture	20.0
Protein	2.10
Fat	1.71
Total Ash	2.30
Crude fibre	8.20
Carbohydrate	54.69

Table 3: Cellulose, Hemicellulose and Lignin Content of Untreated and Pretreated of Cassava Peel.

Composition	Untreated Cassava Peel (%)	Pretreated Cassava Peel (%)
Cellulose	31.1	35.0
Hemicellulose	7.66	6.70
Lignin	31.4	27.0

Table 4: Reducing Sugar Concentration (g/L) After hydrolysis of African Locust beans fruit pulp and Cassava Peels with *A. niger* after 5 days

Substrate (g)	Concentration(g/L)
5	3.73±0.01
10	4.51±0.02
15	5.51±0.01
Control	0.19 ±0.01

Values are presented as mean± SEM, n= 3

Table 5: Physicochemical Characteristics of Produced Ethanol

Parameters	Ethanol produced	Standard ethanol
Flame	+	+
Ethyl acetate	+	+
Esterification	+	+
Boiling point	77.0^{o}C	78.3^{o}C

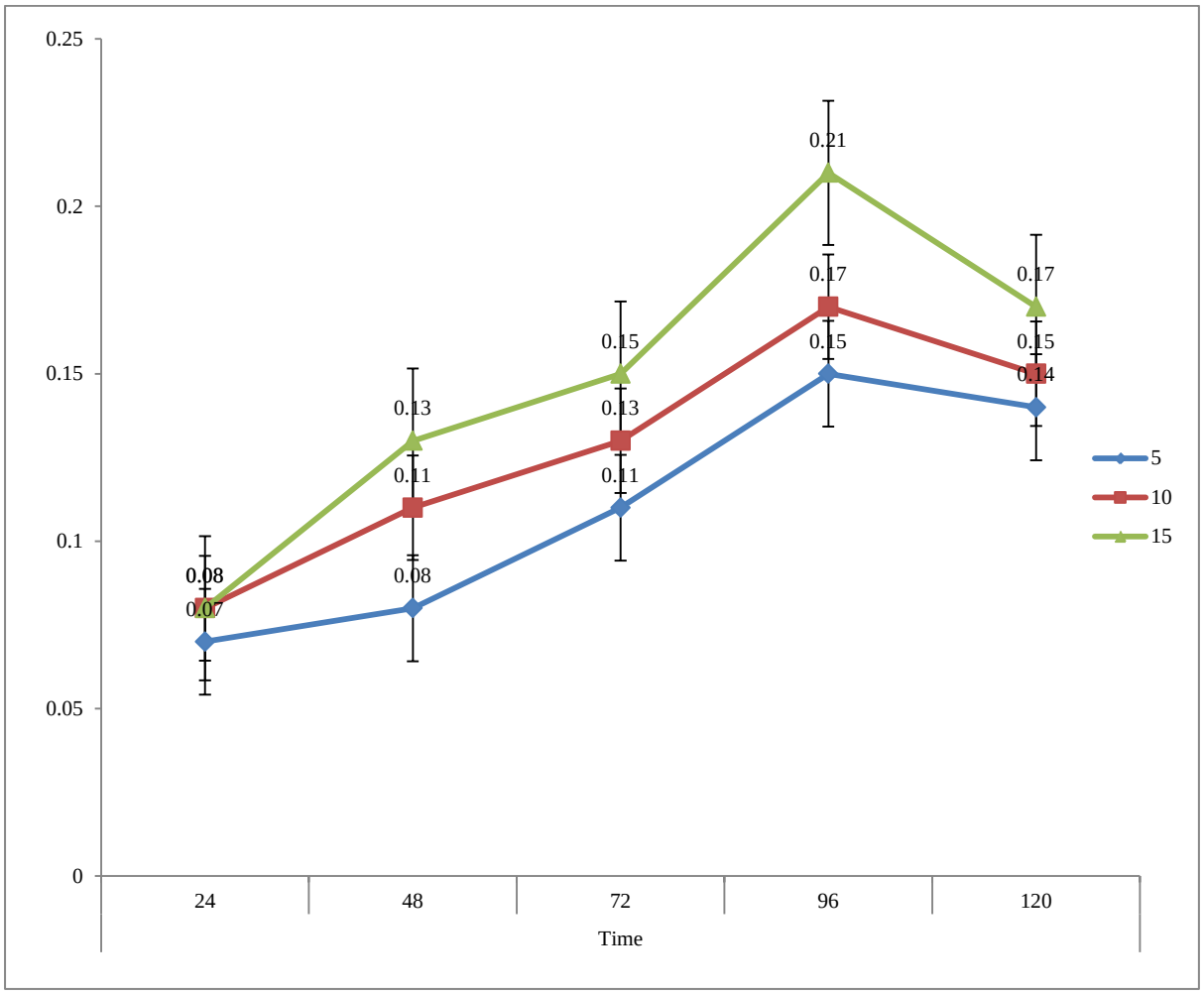

Figure 1: Ethanol Yield (g/g) Produced from Hydrolysate of Cassava peel at Temperature of 35°C and pH of 4.5.

Table 6: Effect of varying pH on Percentage (%) Bioethanol Production from hydrolysate of cassava peel at 96hrs, temperature of 35°C and 15g of the substrate.

pH Substrates (g)	3.5	4.0	4.5	5.0
Cassava peel	22.67±0.11 (43.75)	21.05±0.09(40.60)	20.51±0.00(39.55)	18.14±0.02(35.00)
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00 ± 0.00

Each value represents mean of three independent test ± standard error.
Value in bracket is fermentation efficiency

Table 7: Effect of varying Temperature on Percentage (%) Bioethanol Production from hydrolysate of Cassava peel at 96hrs, Temperature of 35°C and 15g of the substrate.

Temperature (°C)7 Substrates (g)	25	30	35	40
Cassava peel	21.8±0.00 (42.00)	23.05±0.04(44.45)	22.51±0.05(43.40)	22.32±0.06(43.05)
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Each value represents mean of three independent tests ± standard error.
Value in bracket is fermentation efficiency

Table 8: Optimum Conditions for Bioethanol Production

Substrate	Temperature (^{o}C)	pH	Substrate(g)	Ethanol (%)
Cassava peel	30	3.5	15	23.05(44.45)

Value in bracket is fermentation efficiency

Table 9: Effect of Ammonium Sulphate as Detoxification Agent on Percentage (%) Bioethanol Production from hydrolysate of Cassava peels at optimum Temperature of 30^{0}C and pH 3.5 at 15g of the substrates.

Substrates	Detoxified	Non-Detoxified
Cassava peel	23.05±0.04(44.45)	16.10±0.09(31.50)

Each value represents mean of three independent tests ± standard error.

Value in bracket is fermentation efficiency.

4.0 Discussion

Microorganisms have long been considered as harmful entities contributing towards diseases and food spoilage but also playing their role for the welfare of human being. Presently, these microorganisms are being widely used in food industry for production of a large number of fermented food products and at the same time these are also very helpful for conversion of food and industrial wastes into value added useful products (23). *Saccharomyces cerevisiae* was isolated from rotten papaya. Successful isolation and identification of *Saccharomyces cerevisiae* from rotten papaya fruit using morphological characteristics and its ability to ferment glucose, sucrose and lactose is in line with the findings of (50) who reported the isolation of *Saccharomyces cerevisiae* from rotten papaya (31) Also reported the isolation of *Saccharomyces cerevisiae* from rotten guava. (35) isolated *Saccharomyces cerevisiae* from a variety of rot-ten fruits and barks of trees. *Saccharomyces cerevisiae* flocculates in normal saline after 48hrs of incubation and showed level of tolerance to ethanol of up to 17% which agrees with the findings of (27) who observed that the level of ethanol tolerance by *S. cerevisiae* was between 15% and 20%. It is also in line with the work of (26) who reported ethanol tolerance of up to 16%.

The proximate composition of untreated cassava peels reveals a carbohydrate content of 54.69%. This agrees with the work of (44) who presented carbohydrate content of up to 50%. This is also in consistent with the work of (41) that reported Percentage Carbohydrate of 35.83% from Cassava peels.

Fiber extracts analysis of Untreated Cassava Peels presented a Lignin content of (31.4%), Hemicellulose (7.6%) and Cellulose (31.1%). Pretreated Cassava Peels constitutes a lignin content of (27.0%), Hemicellulose (6.7%), and Cellulose (35.0%). The percentage of fiber extracts is higher in untreated Cassava Peels than the treated one. This is in line with the work of (14) that presented a lignin content of untreated rice husk of (20%), Hemicellulose (35%) and Cellulose (38%) in the substrates and Lignin (15%), Hemicellulose (32%) and Cellulose (41%) from the treated cassava peels. This is similar to the work of (32) Reported that typical lignocellulosic biomass contains (15-25% w/w), hemicellulose (23-32%), and cellulose (38-50%). Diluted sulphuric acid pretreatment of the substrates was found to cause swelling leading to an increase in internal surface area and disruption of the lignin structure which makes cellulose available for enzyme action (46) reported a two- and five-fold higher activity from acid pretreated groundnut shell compared to alkali pretreated. Pretreatment of any lignocellulosic biomass is an important tool for practical cellulose conversion processes, which is required to alter the structure of cellulosic biomass to make it accessible to the enzymes that convert the carbohydrate polymers into fermentable sugar (28). Hydrolysis of pretreated cassava peels was carried out for depolymerization of cell wall carbohydrate fraction into fermentable sugar.

The hydrolysis of 15g of cassava peels yielded maximum reducing sugars of 5.51g/L after 120hrs of incubation. The reducing sugar concentration in the hydrolysate increased as the hydrolysis time increases with increase in substrate concentration. This corresponds with the work of (1) who reported maximum glucose concentration of 5.51g/L from cassava peels. (5) Also reported that maximum glucose was obtained after 120hrs of incubation. This is also in line with the work of (15 and 26), which yielded a maximum glucose concentration from the highest substrates concentration after 120hrs of incubation.). Various yield of sugar from cellulosic materials has been reported (20, 39 and 50). These results suggest that the reduced sugars are the intermediate in ethanol production. The results were compared with those obtained from non-inoculated (controls) medium incubated in the respective media.

In detoxification, yeast strains mostly *Saccharomyces* species are capable of utilizing a wide range of nitrogen sources for growth. However, not all nitrogen sources equally influence growth (40). Ammonium compounds have been implicated as a good nitrogen source, reason been that yeast cells easily convert the molecule into glutamate and glutamine.

Therefore, media supplementation with nitrogen source is necessary for yeast cell growth during fermentation, this in turn impart the cell tolerance to stresses such as ethanol and formic acid (48). This result showed an increase in ethanol production after ammonium sulphate was added. The percentages of ethanol produced from Cassava peels after ammonium sulphate was added increases from 16.10% to 23.05%. This collaborates with the work of (48) where the addition of nitrogen sources improved ethanol production during fermentation stress.

Fermentation of the product of the substrate (Cassava peels) hydrolysates by *Saccharomyces cerevisiae* yielded ethanol in respect of the sugar concentrations obtained. Hydrolysate of the substrate with high sugar concentration yielded the highest concentration of ethanol. This indicates that the yield of ethanol is directly proportional to the concentration of sugar in the fermenting fluid. The concentration of ethanol increases with increase in fermentation time. The maximum ethanol production was obtained after 4 days of fermentation, where the ethanol production increases gradually and decreased after the fourth day. The reduced ethanol production after the fourth day may be due to the toxic effect on growth of yeast. Theoretically, the maximum conversion efficiency of glucose to ethanol is 51% on weight basis (9). Since growth commences during the aerobic phase, some amount of sugar gets used up before the anaerobic stage which is characterized by ethanol production (18). This work is similar to the one reported by (42) which shows the maximum ethanol concentration was obtained after 96hrs in submerged shake-flask fermentation of mahula flowers. It is also similar to the work of (49) that reported maximum ethanol yield after 6 days of fermentation, (15) also reported the maximum ethanol concentration was obtained after 6days of fermentation. (26) Produces a maximum ethanol concentration at 72hrs of incubation.

Temperature and pH are the most important factors to consider for bioethanol production by *S. cerevisiae*. The optimum pH for ethanol production was found to be 3.5 at which the highest production of ethanol was obtained followed by pH 4.0. The ethanol concentration decreases with increased in pH. The decrease in ethanol concentration could be due to lesser enzyme activity at pH above the optimum. This is in agreement with the report of (36) that studied the effect of pH on ethanol production from carob pod by *S. cerevisiae* and found that the maximum ethanol yield was obtained at pH of 3.5. This result agrees with the findings of (51, 15 and 22), which show the optimum pH as 3.5 for bioethanol production. Also, recent studies on co-culture fermentation

of tapioca flour as substrate symbiotic strains of starch digesting *A. niger* and non-starch digesting and sugar fermenting *S. cerevisiae* in a batch fermentation showed the optimum values of pH and temperature for ethanol were found to be 3.5 and 30^{o}C respectively. (25 and 3) found that maximum ethanol concentration from solid potato waste was obtained at pH 3.5.

The findings of this research indicate that maximum ethanol yield of 23.05% in Cassava peels was produced at temperature of 30^{o}C followed by temperature of 35^{o}C. The ethanol yield decreased with increase in temperature from 30 to 40^{o}C. However, temperatures beyond 30^{o}C showed a fall in ethanol production which is in line with the findings of (45) who also reported 30°C as the optimum temperature for maximum ethanol production using starch employing co-culture of amylolytic yeast and *S. cerevisiae.* (13) Also reported maximum ethanol productivity from beet molasses by *S. cerevisiae* Y-7 after 72 hours of incubation at 30°C. Thus, optimum temperature for fermentation of Cassava peels was found to be 30°C. At temperatures higher than optimum, less ethanol production was observed. Decline in ethanol yield at increased temperature might be due the inactivation of enzymes involved in ethanol production pathways (39). These observations are consistent with the findings of (37, 15 and 26). The optimum condition achieved for ethanol production from Cassava peels was pH of 3.5, temperature of 30^{o}C and fermentation period of four days (96 hours) and 15g substrate concentration for *S. cerevisiae.* This is in line with the work of (15) who reported ethanol yield of 6.99% from rice husk at optimal parameters of pH 3.5, fermentation period of 120 hours, temperature 30^{o}C and 15g substrate concentration using *S. cerevisiae.* (26) Obtained maximum ethanol concentration at the optimum condition of temperature 30^{o}C, pH 4.5 using *S. cerevisiae* as the microorganism and different varieties of Mango kernels as the substrate. The variation in condition for optimization of ethanol produced by different researchers using different substrate give an indication that the optimization condition for optimum ethanol production depend on the substrate used. This was confirmed by (6) who showed the fermentation parameters for different strain of *S. cerevisiae* were significantly different when grown on banana pulp. (11) Reported that fermentation pattern for strains of *S. cerevisiae* on different substrates (Mango, Banana) were different with different strains producing maximum ethanol concentration on different substrates.

The percentage of bioethanol produced after optimization was 23.05% with fermentation efficiency of around 44.45%. This corresponds to the work of (2) who reported 96hrs to be the optimum time of fermentation while producing bioethanol from cassava peels using *S. cerevisiae.* (45) Reported 30^{0}C as optimum temperature for maximum ethanol production using co-cultures of amylolytic yeast and *cerevisiae.*

5.0 Conclusion

The use of cassava peel for bioethanol production as an alternative source of fuel provides a starting point for the improvements in cultivation and adsorption of cassava as well as improving food security. These studies show that cassava peel produces 23.05% with fermentation efficiency of 44.40% under optimum conditions of temperature 30^{0}C, pH 3.5, 96hrs and 15g of the substrates. It is recommended that more research should be carried using same substrates with different organisms to determine the organisms that produce higher concentration of ethanol.

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