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Bio-Ethanol Production from Cocoyam Peels using Enzymatic Hydrolysis

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Authors' contributions

This work was carried out in collaboration among all authors All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the great potential of cocoyam peels (non-edible waste material) as suitable substrate for bio-ethanol production using enzymatic hydrolysis process. The raw material was subjected to pretreatment prior to the hydrolysis and fermentation processes. Several experimental analyses to determine the suitability of this food waste as bioethanol feedstock -the proximate analyses, enzymatic hydrolysis, analysis of simple sugars, fermentation experiments, kinetics and optimization of the enzymatic hydrolysis and fermentation were done. Cellulase secreted from *Aspergillus niger* was used for the hydrolysis of the peels in a separate hydrolysis. Similarly, commercial saccharomyces cerevisiae was also used for the fermentation of the hydrolyzate. The kinetic studies revealed that Michaelis Menten model was suitable for the enzymatic hydrolysis and fermentation processes. The Seaman kinetic equation for the enzymatic hydrolysis was solved using Microsoft Excel Solver. The Box-Behnken of Response Surface Method (RSM) was employed to optimize the hydrolysis yield. From the numerical optimization solution, simple sugar

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yield from enzymatic hydrolysis was 57.5%. The result also showed that the highest ethanol yield of 7.15%v/v for 4 days with pH value of 7.3 was obtained.

Keywords: Bioethanol; fermentation; cocoyam peels and hydrolysis.

1. INTRODUCTION

Increasing demands for generation of clean energy globally has caused the development of and continuous research into innovative methods for renewable energy fuels from diverse sources [1-11]. This bioenergy derived from biomass seems to be the preferred option and also considered reliable substitutes to conventional fossil energy, in view of its intrinsic qualities [12-16]. Some of these bioenergy sources/qualities include biofuel being eco-friendly, sustainable, biodegradable, non-toxic and non-emission of greenhouse gases [17]. Consequently, generation of bioethanol resources from lignocellulosic biomass could be feasible via hydrolysis and fermentation process or technological advancement such as fast pyrolysis, after undergone suitable and adequate pretreatment for the resultant hydrolysis to take place since they are recalcitrant [1,18,19,8,20]. This by-product can also be manufactured by chemical process of reacting ethylene with steam used in cosmetic, thermometer, used as solvent, a preservative and most importantly, as a motor fuel [21].

Bioethanol can be produced via fermentation using two methods: Separate Hydrolysis and Fermentation (SHF) and Simultaneous Saccharification and Fermentation (SSF) with a batch and fed-batch methods [19]. Both employ starch to produce ethanol and comprise the same phases. In the first phase, enzymatic hydrolysis is applied employing $\propto$-amylase and glucoamylase enzymes for starch degradation thus reducing polysaccharides to monosaccharides and some disaccharides used in further fermentation. Enzymatic hydrolysis is quite a new approach when compared to concentrated-acid and dilute-acid hydrolysis. The significant advantages of enzymatic hydrolysis are high ethanol yield and safer operating conditions. Nevertheless, SHF lasts over 72hrs, while SSF only requires 36hrs. The time difference between both methods is attributed to the time required for hydrolysis. In SSF, enzymatic hydrolysis simultaneously occurs with fermentation (prior enzymatic pretreatment of starch), while in SHF, starch is completely hydrolyzed before fermentation. Ethanol produced using agricultural waste with separate hydrolysis and fermentation also have problems as the higher concentration of reducing sugars inhibits yeast growth [8,22,23]. In order to achieve high products yield from the hydrolysis and fermentation processes, it is important to optimize the variables that significantly affect the processes [24].

The FAOSTAT database indicates that Nigeria is the world's largest producer of cocoyam (3×10^6tonnes yr^{-1}) and has a specific waste index (SWI) of 0.3 [25,26]. This high quantity of the crop will invariably produce high volumes of waste, which also serves as raw material for bioethanol production. The peel characterizes as a skin and thin outer cortex of their tubes represents a major waste during processing constitutes about 10-13% of the tuber [27], having high carbohydrate content of 41.2-46.0%. Although many researchers have investigated on bioethanol production from various biomass materials, relatively little research attention have been devoted using the cocoyam waste produce. However, this study was carried out to evaluate the potential of cocoyam peels to produce ethanol.

2. MATERIALS AND METHODS

2.1 Sample Collection

The cocoyam (*Colocasia esculenta*) peels were collected from different homes at Emene in Enugu East L.G.A, Enugu State of Nigeria. The peels were gathered, washed thoroughly with distilled water and sun dried for two weeks. The sample was milled into powdered form using an electric grinder and sieved to a fine particle size of 250μm. It was further sun dried again for a week and then packaged in a well labeled air-tight container for analyses.

2.2 Proximate Analysis

2.2.1 The moisture content

The proximate composition of the cocoyam peels were determined sequentially following the method described by [28]. 5g of the sample was weighed into a glass Petri dish and placed in an oven at 100˚C for 1hr. The dish with the sample was allowed to cool in desiccators, after which

the weight was taken. The sample was repeatedly dried, cooled and weighed until constant weight was obtained. The moisture content was calculated following Equation 1.

$$\% \text{ Moisture} = \frac{W_1 - W_2}{W_0} \times 100 \quad (1)$$

Where W_0= Original weight of the sample

W_1 = Weight of sample plus the dish before drying
W_2 =Weight of the sample plus the dish after drying

2.2.2 The extractives content

The percentage extractives were determined from the dried residue of the moisture content determination. 1g of the dry powder was weighed, wrapped in a filter paper, and introduced into the thimble part of a Soxhlet extractor. The Soxhlet extraction was carried out using ethanol solvent for 4hours. The residue after the extraction was dried in an oven at 60˚C for 2hours and allowed to cool in desiccator before the final weight was obtained. The percentage extractives were calculated using Equation 2.

$$\% \text{ Extractives} = \frac{W_3 - (W_5 - W_4)}{W_3} \times 100 \quad (2)$$

Where:

W_3=Original weight of the dry sample
W_4=Weight of the empty filter paper
W_5=Weight of the residue plus the filter paper

2.2.3 The lignin

To determine the lignin content, the extractive free biomass was reweighed and added into a 250mL round bottom flask and 30mL of water, 2mL of acetic acid and 0.6g of sodium hypochlorite were added into the flask. The flask was refluxed for 4hrs. After which the residue was washed under vacuum filtration with water, ethanol, acetone and petroleum ether. The residue was collected with a filter paper of known weight and dried in the oven at 60˚C for 1hour, cooled and weighed again. The lignin content was calculated using the formula in Equation 3.

$$\% \text{ Lignin} = \frac{W_6 - (W_8 - W_7)}{W_3} \times 100 \quad (3)$$

Where,

W_6= Weight of the extractive free biomass
W_7= Initial weight of the filter paper
W_8= Final weight of the biomass and the filter paper

2.2.4 The hemicelluloses

The percentage hemicelluloses were determined from the lignin-free biomass. The residue after the lignin determination was weighed into a 250mL beaker. 100mL of 24% potassium hydroxide (KOH) was added into the beaker. The beaker with the contents was allowed to stand for 4hrs under room temperature. The biomass was thereafter filtered and washed with water, ethanol, acetone and petroleum ether. The final residue was filtered into a filter paper of known weight. The residue was dried in the oven at 60˚C for 2hrs, cooled in desiccators, and weighed again. The percentage hemicelluloses were calculated using Equation 4.

$$\% \text{ Hemicelluloses} = \frac{W_9 - (W_{11} - W_{10})}{W_3} \times 100 \quad (4)$$

Where,

W_9 = Weight of the original lignin-free biomass
W_{10}= Weight of the empty filter paper
W_{11} = Weight of the residue and the filter paper

2.2.4 The Cellulose and the ash

The cellulose and ash compositions were determined from the hemicelluloses-free biomass. The weight of the residue after the determination of hemicelluloses was reweighed into a porcelain crucible of known weight. The crucible was heated to ash in a muffle furnace at 600˚C for 4hours. The crucible with the ash was cooled in desiccators and weighed again. The Percentage cellulose and ash were determined using Equations 5 and 6, respectively.

$$\% \text{ Cellulose} = \frac{(W_{14} - W_{13}) \times 100}{W_3} \quad (5)$$

$$\% \text{ Ash} = \frac{(W_{13} - W_{12}) \times 100}{W_3} \quad (6)$$

Where,

W_{12}= Empty weight of the crucible
W_{13} = Final weight of the crucible plus the ash
W_{14} = Initial weight of the crucible plus the hemicelluloses-free residue

2.3 Pretreatment of the Biomass for Enzymatic Hydrolysis

Part of the ground biomass was pretreated to reduce the lignin and the hemicellulose contents before enzymatic hydrolysis. 100g of the ground tuber peels was soaked with 2M NaOH for 24hrs at room temperature. Thereafter, the residue was separated by filtration and washed repeatedly with distilled water until neutral pH. The residue was dried in oven at 60˚C for 24hrs, packed and sealed in another plastic container.

2.4 Cellulase Enzyme Production

2.4.1 Isolation of *aspergillus niger*

Aspergillus niger was used to produce the crude cellulase that hydrolyzed the cocoyam peels. The fungi was isolated and characterized at Microbiology Department of University of Nigeria Nsukka, Enugu State, Nigeria following the method of Omojashola and Jilani, [29]. To isolate the fungi, soil from groundnut husk dump site was crushed, sieved and diluted serially using sterile distilled water. Different dilutions of the soil was inoculated on the slant surface of Potato Dextrose Agar (PDA) medium in test tubes and incubated for 7days. Spores of *A. niger* were harvested by vortex and cellulase activities of the fungi detected by the disappearance of the red colour of Congo red solution around microbial colonies. Evaluation of the clear zones of each colony was estimated as radius (mm) of the clear zone minus the radius of the colony. *A. niger* colonies producing large clear zones were picked up [29].

2.4.2 Inoculums preparation

Reagent bottle were sterilized for the storage of crude enzymes inoculums. Inoculums for enzyme production were prepared by adding 10mL of citrate buffer (5.0 pH) to each test tube containing fully grown spores of *A. niger*. The test tubes were emptied into the sterilized bottles. The inoculums were estimated to have $2.8x10^6$ spores/mL [29] and thereafter stored in a refrigerator for future use.

2.4.3 Cellulase Enzyme production

The crude cellulase enzymes production was carried out in 250mL Erlenmeyer flasks with 50mL medium. The ingredients of culture medium include 30g/L alkaline pretreated biomass (dry biomass), 1g/L glucose, 6g/L ammonium sulfate, 2.0g/L KH_2PO_4, 0.3g/L $CaCl_2$, 0.3g/L $MgSO_4$, 0.005 g/L $FeSO_4$, 0.0016g/L $MnSO_4$, 0.0014g/L $ZnSO_4$ and 0.0037g/L $COCl_2$ [30]. The initial pH value was adjusted to 4.8 by adding 2.5mL citrate buffer solution (1mol/L) to the medium. The prepared medium was then autoclaved at 121°C for 30mins. The submerged fermentation started by inoculating the 50mL medium with 10ml of the fungi inoculums in a 250mL Erlenmeyer flask. The flask was incubated under shaker for 7days. The fermentation was terminated when the glucose level was zero. The medium was filtered and centrifuged to obtain the supernatant which is referred to as the crude enzyme. The quality of the crude enzymes was analyzed by incubating 3mL of the crude enzyme with 1mL of 10% cellulose solution for 1hour at 50°C. The simple sugar hydrolyzed was filtered and the concentration measured using the DNS method. The concentration of the simple sugar was applied to calculate for the quality of the enzyme.

2.5 Enzymatic hydrolysis

Preliminary studies were conducted to determine the effects of process parameters on the yield of simple sugars and to determine the design space for optimization experiment. The enzymatic hydrolysis of the ground, pretreated cocoyam peels was performed in different 250mL Erlenmeyer flasks with a 20mL of the crude enzyme in 0.05 M citrate buffer solution (pH 5.0). 1g of the pretreated biomass was added in each flask and the flasks were incubated in an orbital shaker (140 rpm) at 30˚C [30] for a period. The pH of the mixture was adjusted using 1M NaOH and 1M HCl while analysis of simple sugars was carried out each day for about 5days. Mixtures were centrifuged and the simple sugar yield in the supernatant analyzed using the DNS method. Enzymatic hydrolysis of cellulose was considered to be represented by the formation of glucose, where the soluble sugar is assimilated:

$$(C_6H_{10}O_5)n + n(H_2O) \rightarrow nC_6H_{12}O_6 \quad (7a)$$

2.6 Analysis of Simple Sugars

Standard glucose calibration curve was prepared using the (Dinitrosalicylic acid) DNS method [31]. Standard glucose solutions of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2% were prepared by dissolving the equivalent amount of glucose powered in distilled water. The standard calibration curve was prepared by developing the standard solutions with DNS reagent and the absorbance recorded at 540nm in UV-spectrophotometer. The DNS

reagent was developed by dissolving 10g of DNS, 0.5g of sodium sulfite and 10g of NaOH with water in 1liter volumetric flask. To develop the standards with DNS, 3mL of each of the standard solutions was measured in test tubes. 3mL of the DNS reagent was then added to each of the tubes and the tubes were covered with aluminum foil. After which the color was developed by heating the tubes in boiling water for 10minutes. The tubes were allowed to cool and the absorbance recorded at 540nm. The absorbance was plotted against the glucose concentration and a standard equation was obtained. To test for the concentration of any sample, 3mL of the sample was heated with 3mL of DNS reagent and the absorbance of the color developed from the sample was used to calculate the simple sugar concentration in the sample.

2.7 Fermentation Experiment

The fermentation of enzymatic hydrolyzate was performed with commercial *saccharomyces cerevisea* obtained from the market. The effects of process parameters on the yield of ethanol were equally investigated before the optimization and kinetic studies. Fermentation was carried out in 100mL plastic bottles containing 20mL of the enzyme hydrolyzates. The pH of the hydrolyzates was adjusted using 1M NaOH and 1M HCl and the weight of the yeast varied between 0.1 to 0.3g. The containers were vigorously shaken for 30minutes for proper dissolution of the yeast cells into the hydrolyzates and then covered with cotton wools for escape of CO_2 as the fermentation bye product. The containers were allowed to stand for 1 to 3days according to the design runs, after which the mixture was filtered using vacuum filter. The fermentation filtrate was distilled using a simple distillation set up. The distillate was collected and the quality of ethanol was measured using a specific gravity/ethanol meter (DA-130N).

2.8 The Kinetics of the Enzymatic Hydrolysis and Fermentation Processes

The enzymatic hydrolysis and fermentation involved the use of enzymes, hence, the kinetics were studied using Michaelis Menten model. Equation 7 showed the linear form of the model and using numerical differentiation method, the rates of the reactions were determined. The linear plot of the inverse of the reaction velocity against the inverse of the substrate concentration provided a slope and intercept where the maximum reaction velocity and the kinetics constant (Michaelis Menten constant) were calculated according to Equation 7.

$$1/V = \frac{K_m}{V_m C_A} + \frac{1}{V_m} \quad (7)$$

$$\text{Where: the Velocity, } V = -\frac{dC_A}{dt}, \quad (8)$$

C_A = Substrate (glucose) concentration (g/dm^3)
V_m = Maximum velocity (g/dm^3.day)
K_m = Michaelis Menten constant (dm^3/g)

2.9 Optimization of Enzymatic Hydrolysis and Fermentation Processes

The optimization of the simple sugar yield in enzymatic hydrolysis and ethanol production in fermentation followed the same experimental design. The Box-Behnken design of Response Surface Methodology (RSM) of Design Expert (Version 6.06) was employed for both the enzymatic hydrolysis and fermentation processes. Hydrolysis and fermentation experiments followed the run order, whereas the yield of simple sugars and ethanol were the responses for the hydrolysis and fermentation reactions respectively. After the experiment, the statistical and optimization analyses were performed with Design Expert.

3. RESULTS AND DISCUSSION

3.1 The Proximate Characteristics of Cocoyam Peels

The proximate analysis values of the contents of the cocoyam peels are shown in Table 1. The moisture content was first determined then other contents were reported in dry basis. The peels have high percentage of cellulose in comparison to lignin and hemicelluloses; hence it is a good substrate for simple sugars production and ethanol subsequently.

3.2 The Quality of Cellulase Enzymes Produced from *Aspergillus niger*

The crude enzyme from *Aspergillus niger* was quantified by carrying out CMC assay. The result of the Carbonyl Methyl Cellulose (CMC) assay on the enzyme produced from submerged fermentation of the *Aspergillus* culture was 0.055IU/mL.

3.3 Enzyme Hydrolysis of Pretreated Cocoyam Peels

3.3.1 The effects of process parameters on enzymatic hydrolysis

The effects of time, pH and enzyme dosage on the yield of simple sugars in enzymatic hydrolysis of cocoyam peels are shown in Figs. 1 to 3 respectively. There was an increase in sugar yield with time until after 4days when the yield became constant. Maximum sugar yield was obtained on the 4th day hence other studies like the effects of pH and enzyme dosage were carried out for 4days. The kinetics studies were equally limited to 4days. This result of the preliminary study was equally used to choosing the design space for the optimization study. The result of the effects of pH showed a concave plot, indicating that the neutral pH region gave higher sugar yield. This result indicated that the design space for the optimization study should lie around the neutral regions.

The effects of enzyme dosage showed that the yield increased with enzyme dosage. At 15mL of enzyme per 20mL of the hydrolysis, the highest yield of simple sugars was observed, after which the yield became constant.

3.3.2 The kinetics of the enzymatic hydrolysis of cocoyam peels

The kinetics of the enzymatic hydrolysis was studied using the Michaelis Menten model. The values of the kinetics parameters are shown in Table 2. The maximum velocity V_m and the kinetics constant K_m was obtained from the intercept and the slope of the graph of the inverse of velocity against the inverse of residual

Table 1. The proximate compositions of the cocoyam peels

Run	Moisture (%) on dry weight basis	Extractives (%)	Lignin (%)	Hemicelluloses (%)	Cellulose (%)	Ash (%)
1	7.33	15.70	5.50	25.80	43.00	10.00
2	7.53	16.40	5.70	23.90	43.00	11.00
3	7.37	16.30	5.60	24.50	41.60	12.00
AVR	7.41±0.11	16.13±0.38	5.60±0.10	24.73±0.97	42.53±0.81	11.00±1.00

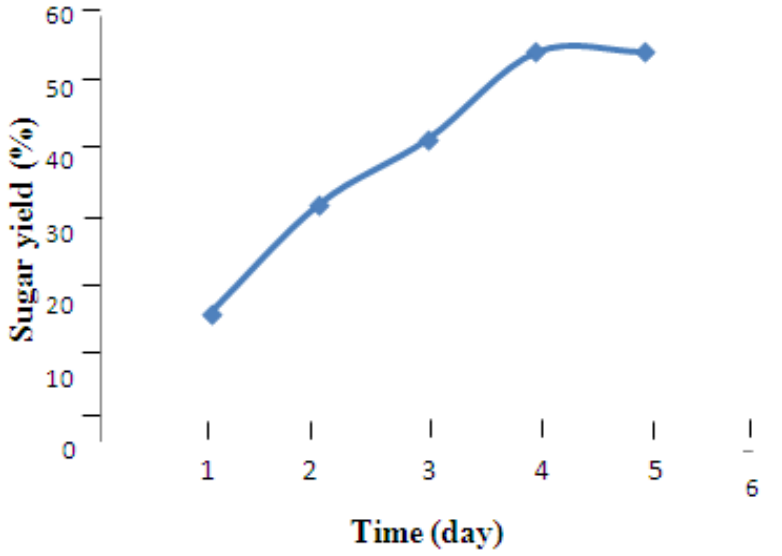

Fig. 1: The effects of time on enzymatic hydrolysis of cocoyam peels

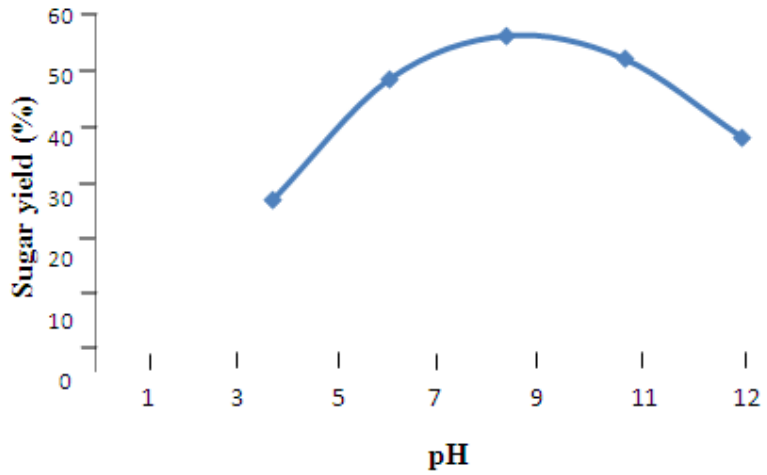

Fig. 2: The Effects of pH on enzymatic hydrolysis of cocoyam peels

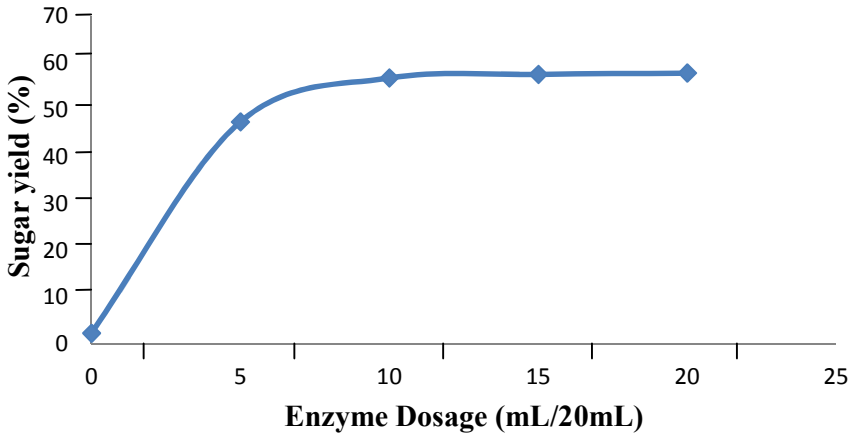

Fig. 3: The effects of enzyme dosage on enzymatic hydrolysis of cocoyam peels

cellulose concentration. The regression coefficient, R^2 was close to 1.0, which shows that kinetics data of the enzymatic hydrolysis of cocoyam peels fitted into Michaelis Menten model. The Michaelis Menten constant, K_m was high, and it measures the substrate concentration when the reaction velocity is half the maximum velocity (V_m). It can also be thought of as a measure of how well the substrate complexes with a given enzyme, otherwise known as its binding affinity. A low K_m indicates high affinity as the reaction approaches V_m more rapidly. A high K_m indicates that the V_{max} will only be reached if the substrate concentration is high enough to saturate the enzyme [32].

$$V = \frac{192.31C_A}{C_A + 613.87} \quad (9)$$

3.3.3 The optimization of the enzymatic hydrolysis of cocoyam peels

The experimental design matrix for the optimization of simple sugar yield is shown in Table 3. The responses were keyed back into the Design Expert for further analysis. A quadratic model was selected based on the fitness of the data and the statistical significances of the quadratic terms were analyzed using Analysis of variance (ANOVA). The mathematical model equation of the system was proposed based on the significant terms and the model was applied to optimize of the process parameters.

3.3.4 The analysis of variance table for optimization of enzymatic hydrolysis

The ANOVA table for the quadratic model of Box-Behnken design for the enzymatic hydrolysis of cocoyam peels is shown in Table 4. The table contains p-values for the model and the terms, from which the statistical significances were decided. The p-value for the model was less than 0.01 and the F-value was 44.32. This implies that there is less than 0.01% chance that F-value this large could occur due to noise. Therefore, the quadratic model was significant and could be applied to navigate through the design space. The p-values for the main factors effects, A(time), B(pH) and C(enzyme dosage) were less than 0.01 and this means that they were statistically significant. The factor squared effects of time (A^2), and pH (B^2) were significant at 0.01 level but that of the enzyme dosage (C^2) was significant at 0.06 level. The two-factor interactive effects (AB, AC and BC) were not significant as there p-values were more than 0.05. This means that the effects of one factor did not depend on the level of another factor. The lack of fit was not significant. This is another indication that the data fitted well into quadratic model because non-significant lack of fit is desired.

3.3.5 The model graphs and equations

The 3D plot of time and pH shown in Fig. 4 indicates that the optimum simple sugars yield at constant enzyme dosage could be located towards the end of time space and the middle of pH space. Fig. 5 shows that at constant pH, the optimum simple sugar yield could be located towards the end of both time and enzyme dosage spaces. Fig. 6 shows that at constant time, the optimum simple sugars yield was more influenced by the pH than the enzyme dosage and the optimum could be located towards the middle of pH space.

The mathematical model equation shown in Equation 10 contains only the significant terms as indicated by the ANOVA table.

$$Y = -6.5 + 9.4xA + 8.85xB + 1.68xC - 0.925\,A^2 - 0.689B^2 - 0.048C^2 \quad (10)$$

Where: A = Time, B = pH and C = Enzyme dosage

Table 2. The kinetics parameters of enzymatic hydrolysis

V_m(g/dm^{3}day)	K_m (dm^3/g)	R^2
192.308	613.865	0.991

Table 3. The response of box-behnken design for enzyme hydrolysis

STD	Run	Time (day)	pH	Enzyme Dosage (ml)	Simple sugar (%)
3	1	2	9	10	45
2	2	6	3	10	48
4	3	6	9	10	52
5	4	2	6	5	45
1	5	2	3	10	41
12	6	4	9	15	54
14	7	4	6	10	57
7	8	2	6	15	53
10	9	4	9	5	47
8	10	6	6	15	57
9	11	4	3	5	44
11	12	4	3	15	51
6	13	6	6	5	51
16	14	4	6	10	58
15	15	4	6	10	55
13	16	4	6	10	56
17	17	4	6	10	56

Table 4. The ANOVA table for enzymatic hydrolysis

Source	Sum of Squares	DF	Mean Square	F Value	Prob> F	
A	72	1	72	65.45455	< 0.0001	
B	24.5	1	24.5	22.27273	0.0022	
C	98	1	98	89.09091	< 0.0001	
A2	57.64211	1	57.64210526	52.40191	0.0002	
B2	161.8526	1	161.8526316	147.1388	< 0.0001	
C2	6.063158	1	6.063157895	5.511962	0.0513	
AB	0	1	0	0	1.0000	
AC	1	1	1	0.909091	0.3721	
BC	0	1	0	0	1.0000	
Residual	7.7	7	1.1			
Lack of Fit	2.5	3	0.833333333	0.641026	0.6276	not significant
Pure Error	5.2	4	1.3			
Cor Total	446.4706	16				

3.3.6 The numerical optimum solution

The numerical optimum solution is shown in Table 5. The optimum solution was verified by conducting another hydrolysis experiment with 1g of pretreated cocoyam peels using enzyme dosage of 14ml/20mL at a pH of 7.0 and for a period of 5days. The yield of simple sugar was observed to be 57.5%, which is in close agreement with the theoretical value, hence the optimum solution was verified.

3.4 The Ethanol Fermentation Results

3.4.1 The effects of process parameters on ethanol yield

The effects of time, pH and Enzyme dosage on ethanol yield at constant temperature are shown in Figs. 7-9, respectively. The ethanol obtained from enzyme hydrolyzate was quite higher in concentration. Ethanol yields increased with time and stayed constant after 4days of fermentation. The yields of ethanol showed peak around pH 7, after which the yields dropped at higher pH. The yields equally showed peaks at around dosage of 4mL/20mL mixtures and after which the yields declined.

3.4.2 Kinetics of ethanol fermentation

The kinetics study of the fermentation process followed the same Michaelis Menten model, which was applied with enzyme hydrolysis. The calculations for the initial sugar concentration before fermentation while the calculations for the reaction velocities followed the same pattern. The kinetics parameters are shown in Table 6. The regression coefficients (R^2) were close to 1.0 but the data with enzyme hydrolyzate fitted perfectly well to the Michaelis Menten model. The kinetics constant for the fermentation of enzyme hydrolyzates was lower which signifies that the enzyme substrate affinity of the enzyme hydrolyzate was higher.

3.4.3 Optimization of fermentation process

The Box-Behnken design matrix with responses for fermentation of enzyme hydrolyzates is shown in Table 7. The analyses of the responses were carried out with Design Expert and the software suggested quadratic models for the fermentation of enzyme hydrolyzate. The quadratic models were validated by the Analysis of Variance (ANOVA) as shown in Tables 8.

3.4.4 The ANOVA of the quadratic models of the ethanol fermentations

The ANOVA Tables show that the quadratic model and the main factor effects, which are time (A), pH (B) and yeast dosage (C) were significant at 0.05 levels because their p-values were less than the significant level. The squared effects of time (A^2) and pH (B^2) were significant while that of yeast dosage (C^2) was not significant for enzyme hydrolyzate. None of the two-factor interactive effects (AB, AC and BC) was significant at 0.05 level for the fermentation of enzyme hydrolyzate. The lack of fit p-values was higher than 0.05, meaning that the lack of fit was not significant. Non significant lack of fit was desired.

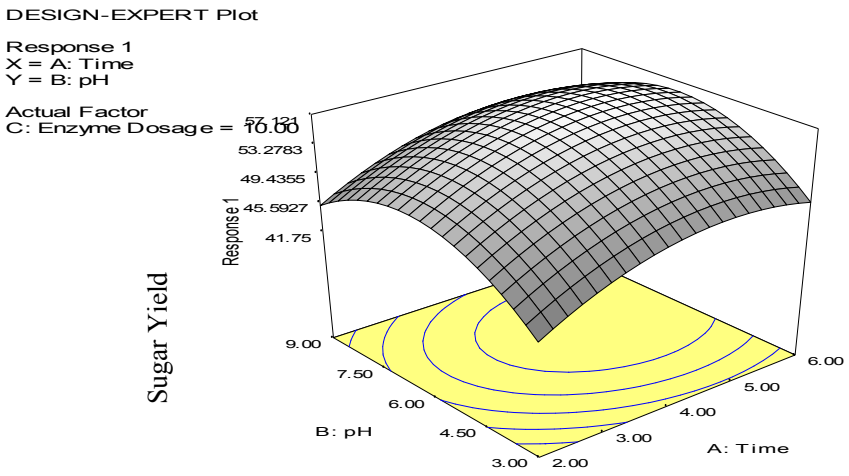

Fig. 4: The 3D plot of time and ph against simple sugar yield

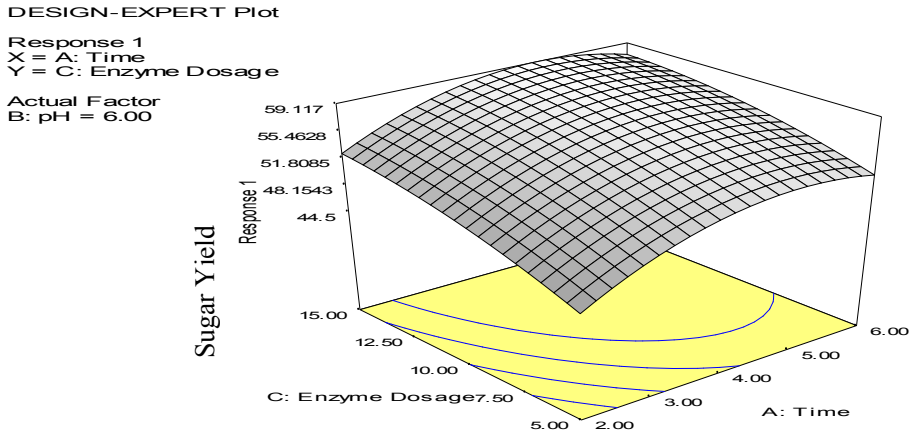

Fig. 5: The 3D plot of time and enzyme dosage against simple sugar yield

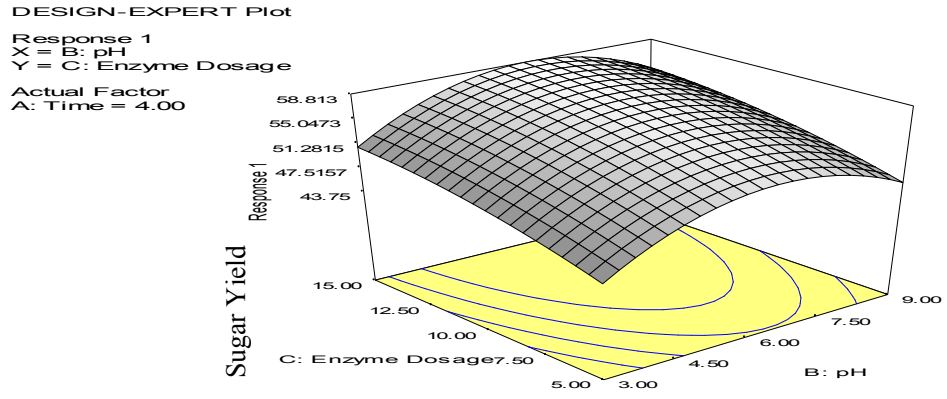

Fig. 6: The 3D plot of ph and enzyme dosage against simple sugars yield

Table 5. The numerical optimum solution for enzymatic hydrolysis

Time(day)	pH	Enzyme Dosage (ml/20mL)	Sugar Yield (%)	Actual Yield (%)
5.0	7.02	14.27	58.73	57.5

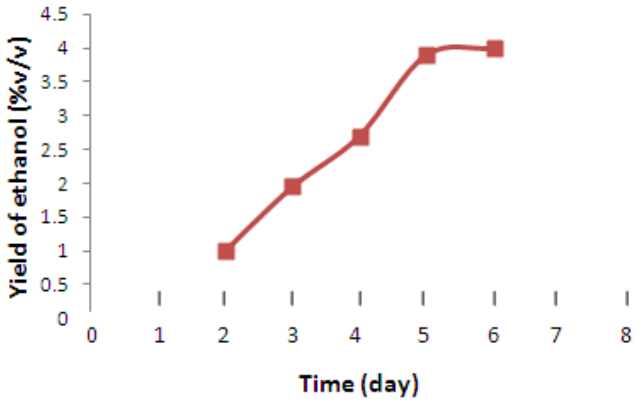

Fig. 7: The effects of time on ethanol yield

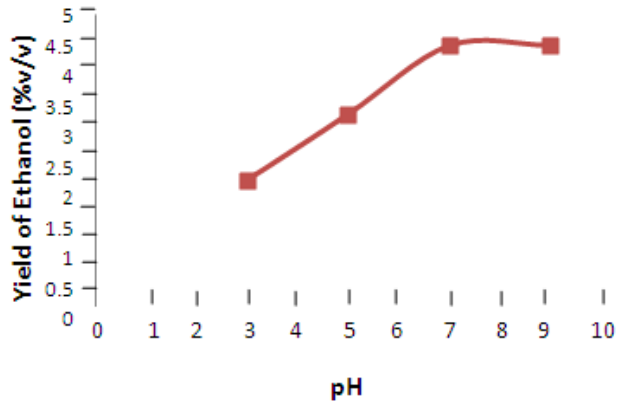

Fig. 8: The effects of pH on ethanol yield

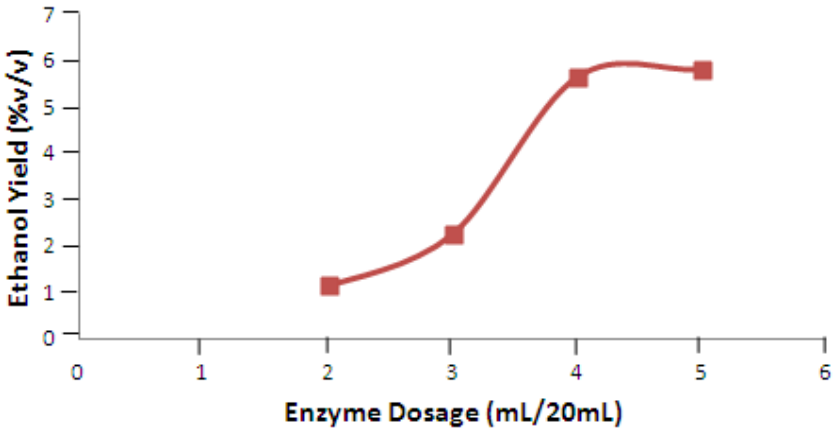

Fig. 9: The effects of enzyme dosage on ethanol yield

Table 6. The kinetics parameters for the fermentation reactions

Substrate	V_m	K_m	R^2
Enzyme Hydrolyzate	21.786	43.443	0.956

Table 7. Design matrix with responses of ethanol yields from enzyme hydrolyzate

Std	Run	Time (day)	pH	Yeast Dosage (g/20mL)	Enzyme Hydrolyzate Ethanol (%v/v)
1	15	2	3	1.5	1.5
2	16	6	3	1.5	3.6
3	17	2	9	1.5	2.8
4	1	6	9	1.5	4.8
5	13	2	6	1	4.7
6	9	6	6	1	5.1
7	8	2	6	2	5.7
8	10	6	6	2	6.2
9	5	4	3	1	3.6
10	6	4	9	1	4.5
11	12	4	3	2	4.2
12	4	4	9	2	5.9
13	14	4	6	1.5	6.1
14	7	4	6	1.5	6.3
15	2	4	6	1.5	6.1
16	11	4	6	1.5	6.6
17	3	4	6	1.5	5.9

Table 8. The ANOVA table of quadratic model for fermentation of enzyme hydrolyzate

Source	Sum of Squares	DF	Mean Square	F-Value	Prob> F
A	3.125	1	3.125	14	0.0072
B	3.25125	1	3.25125	14.5656	0.0066
C	2.10125	1	2.10125	9.4136	0.0181
A^2	4.865789	1	4.865789	21.79873684	0.0023
B^2	16.01053	1	16.01053	71.72715789	< 0.0001
C^2	0.378947	1	0.378947	1.697684211	0.2338
AB	0.0025	1	0.0025	0.0112	0.9187
AC	0.0025	1	0.0025	0.0112	0.9187
BC	0.16	1	0.16	0.7168	0.4252
Residual	1.5625	7	0.223214		
Lack of Fit	1.2825	3	0.4275	6.107142857	0.0565
Pure Error	0.28	4	0.07		
Cor Total	32.14471	16			

3.4.5 The quadratic model equations of the fermentation process

The mathematical model equations for ethanol yield as a function of time, pH and yeast dosage are shown in Equation 11 for fermentation of enzyme hydrolyzate of cocoyam peels. The model equations in actual terms do not contain the non-significant terms as indicated through the ANOVA table.

$$\text{Ethanol Yield}(\%) = -6.01 + 2.45 \times \text{Time} + 2.63 \times \text{pH} - 3.48 \times \text{EnzymeDosage} - 0.27 \times \text{Time}^2 - 0.22 \times \text{pH}^2 + 1.20 \times \text{EnzymeDosage}^2 \quad (11)$$

3.4.6 The 3D graph of time and pH against the ethanol yield

Time and pH have strong influence on the yield of ethanol from enzyme hydrolyzate. The 3D plot shown in Fig. 10 indicate that the optimum yield of ethanol at constant temperature and yeast dosage could be located at the middle of pH and towards the end of time in the design space. The high influence of time and pH on the yield of ethanol agrees with the ANOVA report where the p-values of both time and pH were less than 0.01, showing that both time and pH had

significant effects of ethanol yield even at 0.01 level.

3.4.7 The 3D graph of time and yeast dosage against ethanol yield

The 3D Graphs of time and enzyme dosage against ethanol yields for enzyme hydrolyzate are shown in Fig. 11. It can be seen that time variations had more influence on ethanol yield than yeast dosage variations. The optimum ethanol yield at constant temperature and pH could be located towards the end of time space, as the effect of enzyme dosage was not much significant. The less significance effect of yeast dosage variation can be seen from the p-values in the ANOVA table, where the p-values were slightly more than 0.05.

3.4.8 The 3D graph of pH and yeast dosage against ethanol yield

The 3D graph of pH and enzyme dosage against ethanol yield for enzyme hydrolyzate is shown in Fig. 12. The effects of pH had more impact on the optimum ethanol yield as can be observed from the graphs. The graphs equally show that the optimum ethanol yield at constant time and temperature could be located at the middle of pH space.

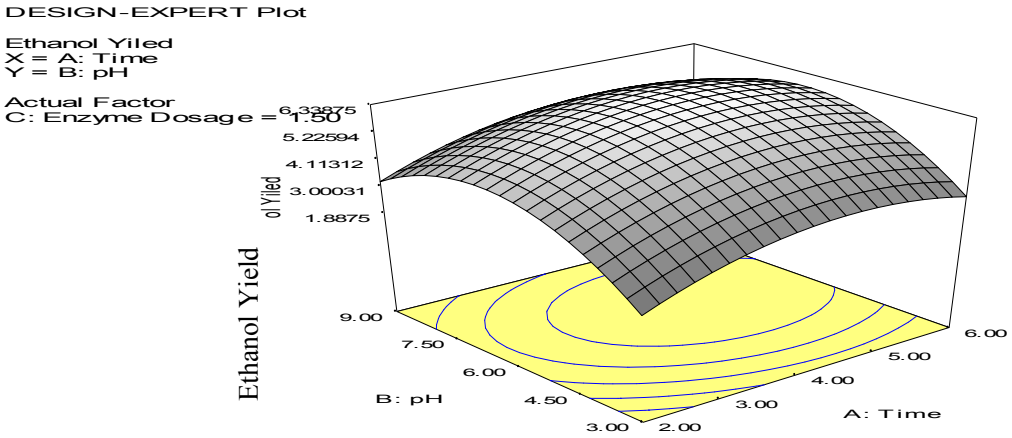

Fig. 10: The 3D graph of time, pH and ethanol yield for fermentation of the enzyme hydrolyzate

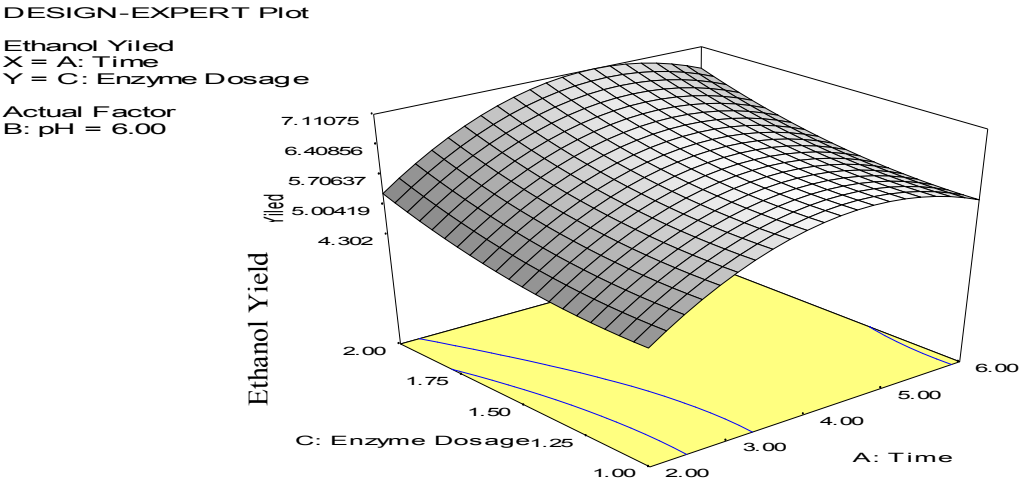

Fig. 11: The 3D graph of time, yeast dosage and ethanol yield for fermentation of the enzyme hydrolyzate

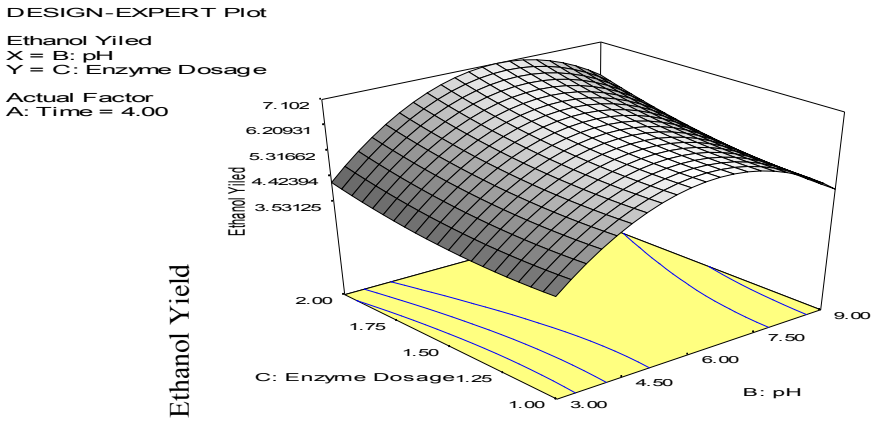

Fig. 12: The 3D graph of pH, yeast dosage and ethanol yield for fermentation of the enzyme hydrolyzate

Table 9. The optimum numerical solution for fermentation of enzyme hydrolyzate of cocoyam peels

Substrate	Time (days)	pH	Yeast Dosage (g/20mL)	Ethanol (%v/v)	Actual Ethanol (%v/v)
Enzyme Hydrolyzate	4	7.3	2	7.04	7.15

Table 10. The refractive indices of ethanol produced

	Ethanol (%v/v)	Refractive Index
Ethanol from Enzyme Hydrolyzates	7.15	1.3376
Standard Ethanol	7.15	1.3373

3.4.9 The numerical optimum solutions of ethanol yields for the fermentation of enzyme hydrolyzate of cocoyam peels

The numerical optimum solutions for the yield of ethanol as a function of time, pH and yeast dosage in fermentation of enzyme hydrolyzate of cocoyam peels are shown in Table 9. The theoretical yield of ethanol from enzyme hydrolyzate of cocoyam peels was verified by conducting fermentation of the enzyme hydrolyzate for 4days, at pH of 7.3 and yeast dosage of 2.0g/20mL. The ethanol yield recorded was 7.15%(v/v) and this was comparable to the theoretical yield of 7.04%(v/v). Therefore, the optimum ethanol yield was 7.15%(v/v) for enzyme hydrolyzate.

3.4.10 The characterization of ethanol produced

The refractive indices of the ethanol produced from the enzyme hydrolyzate of cocoyam peels were recorded as shown in Table 10. The values were compared with the standard refractive indices of ethanol of the same concentration with the ones produced from cocoyam peels [33]. The refractive indices of the ethanol produced from cocoyam peels were close to the refractive indices of standard ethanol, which confirms that ethanol was produced from cocoyam peels.

4. CONCLUSION

The result of this study confirmed that ethanol produced from cocoyam peels are potential alternatives to conventional ethanol. Despite the ability to use peels for ethanol production, the yield can be influenced by several factors especially temperature, pH, time and substrate concentration hence needs optimization of the process parameters. The maximum ethanol yield 7.15%v/v obtained from the fermentation process using co-culture of *Aspergillus niger* and *saccharomyces cerevisiae* was carried out for four days. This method of starch fermentation to ethanol will significantly improve the economy by reducing the cost of production of bioethanol.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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