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Impact of Acid, Alkali and Microbial Hydrolysis on Bioethanol Yield from Peels of Cassava, Yam and Potato Sourced From Food Vendors in Etsako West Local Government Area, Edo State, Nigeria

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ABSTRACT: This study evaluated the impact of acid, alkali, and microbial hydrolysis on ethanol yield from cassava, yam, and potato peels. Samples were sourced locally in Etsako West (LGA), Edo State, Nigeria using appropriate standard procedures. The RSM revealed that ethanol yield decreased with rising fermentation temperature but increased with substrate concentration and fermentation time. Optimal conditions were 14% substrate concentration, 34°C, and 55 hours of fermentation. Acid hydrolysates yielded the highest ethanol (0.22–0.27 mL/g), followed by alkali (0.21–0.25 mL/g) and microbial hydrolysates (0.14–0.20 mL/g). CPP acid hydrolysate achieved the highest fermentation efficiency (59.11%), followed by alkali (48.19%) and microbial (41.84%). ANOVA confirmed significant differences between hydrolysis methods, with acid and alkali methods yielding comparable results. While some ethanol quality parameters aligned with commercial standards, others varied significantly. Acid hydrolysis proved most effective, highlighting its potential for optimizing ethanol production from agricultural waste.

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Crude Oil, a mixture of hydrocarbons existing in liquid phase in natural underground reservoirs, has been the major source of world energy. However, following depletion of crude oil deposits, the world is in search of alternative energy sources (Pinaki *et al.*, 2015).Currently, bioethanol, an alcohol made by microbial fermentation of reducing sugars, is the most widely used liquid biofuel in the world (World Bioenergy Association, 2019). Agricultural wastes, which include residues from growing and processing of raw agricultural products, form a huge reservoir of biomass ready to be harnessed for bioethanol production. The estimates of agricultural waste generation places cassava as the highest, generating 33.7%, followed by yam 26.8% (Oyegoke *et al.*, 2023) Others include; maize (6.2%), sorghum (4.7%), oil palm (5.7%), rice paddy (3.7%). The average annual agro-waste generated from the largest cultivated crops in Nigeria was estimated to be 12.06 mega-tonnes (Oyegoke *et al.*, 2023).However, this potential reservoir of biomass is yet to be harnessed for bioethanol production. The challenge has been the development of a technology that is economical for the breakdown of lignocellulosic biomass to

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fermentable sugars (Mohan and Reddy, 2012). As a way forward, the use of microbial hydrolysis and fermentation in the bioconversion of agricultural wastes to ethanol is been researched vigorously. Therefore, the objective of this paper is to evaluate the impact of acid, alkali and microbial hydrolysis on bioethanol yield from peels of cassava, yam and potato sourced from food vendors in Etsako West Local Government Area, Edo State, Nigeria.

MATERIALS AND METHODS

Collection and Processing of Agricultural Wastes: Peels of cassava, yam and potato were collected from food vendors in Etsako West Local Government Area (6^o 58'N 6^o 18'E), Edo State, Nigeria. Samples were collected in clean plastic buckets with which they were transported to the laboratory for further processing. The peels were washed, oven-dried at 50^oC for 48 hours, ground using motorized blender (model: BL260500W) and sieved to have uniform particle size (0.2 mm). They were then packed in clean plastic containers and labeled as Cassava Peel powder (CPP), Yam Peel Powder (YPP) and Potato Peel Powder (PPP). The wastes were stored in a refrigerator prior to further treatment.

Isolation of Bacillus amyloliquefaciens and Zymomonas mobilis: Cow dung used for the isolation of *B. amyloliquefaciens* was collected from an abattoir in Auchi, Edo State. The cow dung sample was collected in sterile specimen bottles with which they were taken to the laboratory for further treatment. Freshly tapped palm wine was bought from a local palm wine tapper in Auchi, Edo State, Nigeria. It was collected in a clean 1 L plastic container with which it was transported to the laboratory and kept in the freezer prior to further use.

Preparation of Culture Media: Carboxymethylcellulose agar (CMC) used for the isolation of *B amyloliquefaciens* was prepared as described by Singh *et al*. (2013). Composition: 5 g of Carboxymethylcellulose agar, 0.5 g of K_2HPO_4, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of NH_4NO_3, 0.02 g of $FeCl_3 \cdot 6H_2O$, 0.01 g of $CaCl_2$ and 10 g of Agar in 500 mL of distilled water (Singh et al., 2013).

Malt Yeast Peptone Dextrose Broth used for the isolation of *Z mobilis* was prepared as described by Obire (2005). Composition: Malt extract 0.3 g, Yeast extract 0.3 g, Peptone 0.5 g, Glucose 2.0 g, distilled water 100 mL, ethanol 3% (v/v) pH 4.8.

Malt Yeast Peptone Dextrose Agar was prepared as described by Obire (2005). Composition: Malt extract 0.3 g, Yeast extract 0.3 g, Peptone 0.5 g, Glucose 2.0 g, Agar 2.0 g, distilled water 100 mL, ethanol 3% (v/v) pH 4.8. The media were autoclaved at 121^oC for 15 minutes and allowed to cool (lukewarm) before dispensing into Petri dishes.

Isolation of Bacillus amyloliquefaciens: Bacillus amyloliquefaciens used for this work was isolated from cow dung. One gram (1 g) of cow dung was transferred into 10 mL of sterile distilled water in a test tube, and the tube suspended in a water bath maintained at 80^oC for 1 h. Thereafter serial dilution (10^{-1} to 10^{-6}) from the stock sample was carried out. Carboxymethylcellulose (CMC) agar prepared in a 500 mL conical flask was autoclaved at 121^oC for 15 minutes, cooled to lukewarm and dispensed onto sterile Petri dishes aseptically. From each dilution, 1 mL was plated on the solid CMC agar by spreading, and then incubated at 37^oC for 24 - 48 h.

Isolation of Zymomonas mobilis: Twenty milliliters (20 mL) of MYPD broth, previously autoclaved (121^oC for 15 minutes) and cooled and 3% (v/v) ethanol was added and dispensed into two screw-capped test tubes and inoculated with 5 mL of fresh palm wine. The culture was then incubated in an anaerobic jar at room temperature for a period of 48 hours, (Obire, 2005).

Malt Yeast Peptone Dextrose agar was prepared in a 250mL conical flask, and autoclaved at 121°C for 15mins. One milliliters of the overnight broth culture (MYPD broth) of the freshly tapped palm wine was put into a test tube containing 10 mL of sterile distilled water. From this stock culture, serial dilution was carried out by sequentially mixing 1 mL from it onto 9 mL of sterile distilled water in test tubes (aseptically) to give a suspension range of 10^{-1} to 10^{-6} (Adeleke *et al*, 2017). The dilutions were properly mixed to allow even distribution followed by inoculation of 1 mL from diluents10^{-2}, 10^{-4} and 10^{-6}onto sterile Petri-dishes aseptically, to which sterile molten Malt Yeast Peptone Dextrose agar was added. The plates were allowed to solidify and appropriately labeled and incubated anaerobically at 37^oC for 24 – 48 hours in an inverted position.

Discrete colonies observed were isolated, purified by streaking on freshly prepared media and incubated for 48 hours at 30^oC in an anaerobic jar. This procedure was repeated until a pure culture was observed. The identification of bacteria was based on morphological, biochemical and molecular characteristics. (Ona, *et al*., 2018).

Hydrolysis of Wastes: This was carried out using acid and alkali and *Bacillus amyloliquefaciens*.

Acid and Alkali Hydrolysis of Wastes: Dilute Sulfuric acid and Sodium Hydroxide were used for this process. A 50 g portion of the waste samples were placed in a 4 L metal container to which 2 L of 0.2M H_2SO_4 and NaOH were added respectively and the contents mixed thoroughly and sealed. The samples were then hydrolyzed at 121^{o}C for 1hour (Amenaghawon, 2017). Upon cooling the hydrolysates were neutralized with NaOH and H_2SO_4 respectively and concentrated to 500 mL by gentle boiling. The samples were cooled and stored in the refrigerator prior to fermentation.

Hydrolysis with Bacillus amyloliquefaciens: Fifty grams (50 g) of each of the wastes was weighed into 5 L beaker to which distilled water added to make a 1% substrate concentration. The mixture was autoclaved at 121^{o}C for 15 min. After cooling the substrate was inoculated with 20 mL of an overnight culture of *B. amyloliquefaciens* and incubated for 48 h at 35^{o}C, (Ezekiel and Aworh, 2018).The hydrolysates where filtered using sterile cheese cloth and concentrated to 500 mL by gentle heating.

Optimization of Fermentation of the Hydrolysates: The fermentation process was optimized using Response Surface Method as described by Oiwoh, *et al*. (2018). A three variable and five level Central Composite Design, consisting of 20 experimental runs was used for optimization of the reaction variables. The independent variables include: X_1; Substrate concentration ranging from 9.9 % to 30.09 %, X_2; Fermentation temperature ranging from 29.9^{o}C to 50.09^{o}C, and X_3; Fermentation time ranging from 35.5 hour to 131.4 hours (Table 1). The model was designed using the equation below:

$$Y = \int (x_1 , x_2 , x_3 , \ldots x_n) \quad (1)$$

Where Y is the response of the system; percentage ethanol yield, and x_n is the independent variables of action, the factors influencing the response, Y. The experimental design was aimed at optimizing the response variable Y so as to find a suitable approximation for the true correlation between the independent variable and the response surface. An empirical model was generated from the response using a second order polynomial equation (Oiwoh *et al*., 2018). The hydrolysates generated from the acid, alkali and microbial hydrolysis were fermented to ethanol using *Zymomonas mobilis*. A 1 L plastic gallon was used as the fermentation jar. A gas outlet was constructed by boring a hole on the cover of the gallon to which a 1 mm diameter rubber tubing (12 cm long) was inserted and glued to serve as a gas conduit hose. The other end serve as air lock. The hydrolysates were poured into the fermentation jars, and with the aid of paraffin wax, the jars were covered to prevent air entering. The fermentation was carried out based on the Response Surface Method Optimization result. Each fermentation jar was inoculated with a 10 mL of an overnight culture of *Z. mobilis* (Adegbehingbe, *et al*., 2021).

Table 1: Experimental ranges and level of independent variables

		Levels of Coded Variables				
		-α	Low	Medium	High	+α
Independent Variable	Symbol	-1.681	-1	0	1	1.6817
Conc.(%)	X_1	9.9	14	20	26	30.09
Temp. (^{o}C)	X_2	29.9	34	40	42	50.09
Time (H)	X_3	35.5	55	83.5	112	131.4

Distillation of fermented broth: The fermented liquids were passed through a sterile cheesecloth. The resulting broth was transferred into a distillation flask and cocked. The distillation equipment was assembled and placed on a heating mantle, which was adjusted to 78^{o}C. The distillation process continued until 100 mL of distillate was collected. The resulting distillate was then assessed for its percentage ethanol content, flash point, boiling point, viscosity, specific gravity, titratable acidity, and pH.

Efficiency of Fermentation: This was estimated using the theoretical ethanol yield and actual ethanol yield upon fermentation of hydrolysate using the expression below (Aminu *et al.*, 2018):

$$\text{FE (\%)} = \frac{Ethanol\ yield\ obtained}{Theoritical\ maximum\ yield\ from\ substrate} \text{ x } 100$$

Where FE = Fermentation Efficiency

Comparison of Ethanol Produced with Commercial Ethanol: This was done by comparing the chemical properties of ethanol produced from hydrolysates with that of commercially available (laboratory standard) ethanol.

RESULT AND DISCUSSION

Isolation of Bacillus amyloliquefaciens: Two discrete colonies were observed from the cow dung samples cultured in CMC agar. They were sub-cultured severally to obtain pure culture and designated cow dung isolate 1 and 2 (CDI1 and CDI2). They were then cultured in Potato dextrose broth to which 10% NaCl (w/v) was added and re-cultured onto CMC agar plates, also with 10% NaCl (w/v). Only CDI1 grew showing tolerance of NaCl up to 10%. Both organisms were Gram positive and the results of other biochemical test are shown in Table 2.

Table 2: Morphological and Biochemical characteristics of isolates from cow dung

Characteristics	CDI1	CDI2
Colour	creamy	White
Elevation	raised	Raised
Edge	entire	Entire
Surface	rough	Rough
Opacity	opaque	Opaque
Shape	Rod	Rod
Gram reaction	+	+
Endospore	+	+
Motility	+	+
VP	+	+
MR	-	-
Catalase	+	+
Oxidase	-	-
Indole	-	-
Urease	-	-
Growth on 10% NaCl	+	-
Mannitol	+	+
Glucose	+	+
Sucrose	+	+
Maltose	+	+
Fructose	+	+
Lactose	+	+
Arabinose	-	-
Possible Organism	*Bacillus amyloliquefaciens*	*Bacillus subtilis*

Isolation of Zymomonas mobilis From Palm Wine: Three discrete colonies were observed from the palm wine samples cultured on Malt Yeast Peptone Glucose (MYPG) agar. They were sub-cultured severally to obtain pure culture and designated Zymomonas isolate 1, 2 and 3 (ZI-1, ZI-2 and ZI-3). They were then cultured on nutrient agar plates and subjected to biochemical tests as shown in Table 3.

Table 3: Morphological and Biochemical characteristics of isolates from palm wine

Characteristics	ZI-1	ZI-2	ZI-3
Colour	creamy	creamy	Pale
Elevation	raised	raised	Convex
Edge	entire	entire	Undulating
Surface	moist	smooth	Rough
Opacity	translucent	opaque	Opaque
Shape	rod	rod	cocobacilli
Gram reaction	-	+	+
Endospore	-	-	-
Motility	+	-	-
VP	-	-	-
MR	-	-	-
Catalase	-	-	-
Oxidase	-	-	-
Indole	-	-	-
Urease	-	-	-
H_2S production	-	-	-
Mannitol	-	+	+
Glucose	+	+	+
Sucrose	+	+	+
Maltose	+	+	+
Fructose	+	+	+
Lactose	-	+	-
Possible Organism	*Zymomonas mobilis*	*Lactobacillus planterum*	*Leuconostoc* spp

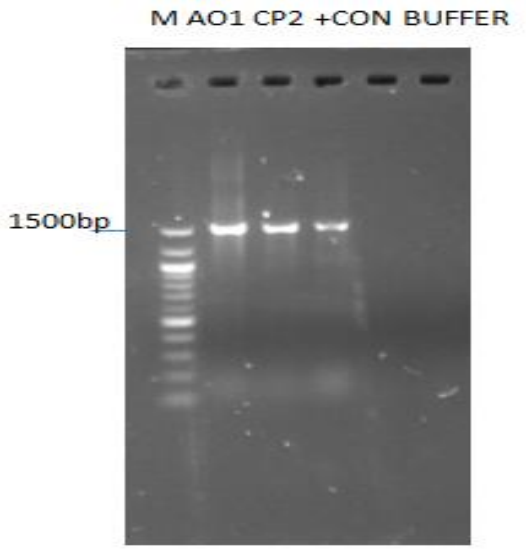

Plate 1: Agarose Gel showing the positive amplification of the 16s RRNA regions amplified from the selected Bacteria samples.

Table 4: NCBI blast showing the sequence identity of the isolates edited sequences

Sample ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
AO1	Bacillus amyloliquefaciens	2667	2391 4	99%	0	99.66 %	PQ15760 1
CP24	Zymomonas mobilis	2566	5132	99%	0	99.64 %	PQ15760 2

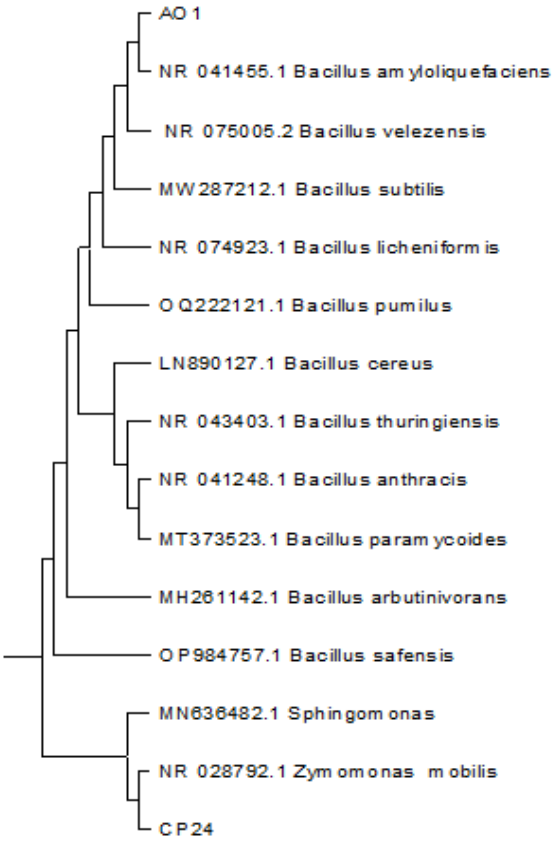

Plate 2: Phylogenic Tree of Isolates

Optimization of Fermentation: The optimization was done using a quadratic model determined based on the result of a twenty (20) run fermentation process carried out for each of the agricultural waste. The model for the analysis, in combination with the actual factors used was obtained as:

% Yield = 3.48774 + 0.210682 * A + -1.38757 * B + 0.634376 * C + 0.0875 * AB + 0.4625 * AC +- 0.4625 * BC + -0.783037 * A^2 + -0.111286 * B^2 + - 0.376451 * C^2 …… (2)

The most significant influence ($p<0.05$), as depicted from the Response Surface Method, was observed at 26% substrate concentration, 34^{o}C incubation temperature and a fermentation duration of 112 hours for cassava peel powder (CPP) and 14% substrate concentration, 34^{o}C incubation temperature and a fermentation duration of 55 hours for YPP and PPP. Figure 1 to 9 shows the three-dimensional surface plot of the three factors used to analyze the influence of each parameter and their pair-wise interaction on the percentage ethanol yield. Figure 1 shows the combined effect of substrate concentration and fermentation temperature on percentage ethanol yield from CPP. As the temperature increased, a corresponding increase in concentration resulted in a reduced ethanol yield.

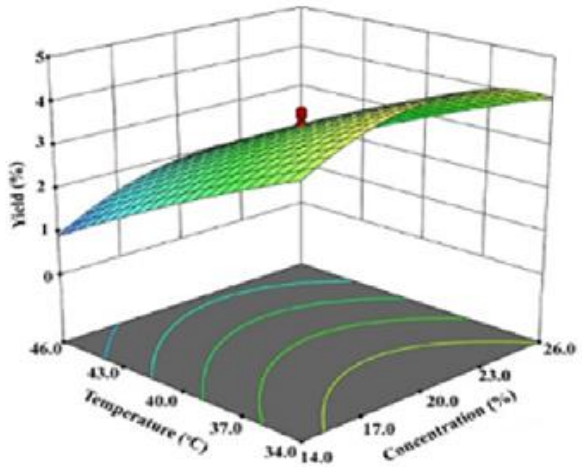

Fig. 1: Combined Effect of Temperature Substrate Conc. On Ethanol Yield from Cassava peels

In Figure 2, increase in fermentation time and a concomitant increase in substrate concentration resulted in increase in percentage ethanol yield. From the result of the optimization process, the variable factor 'temperature' has the greatest effect on the response (ethanol yield) compared to other factors. The variable factor 'time' showed the next high and significant influence on the percentage ethanol yield. The interaction of the substrate concentration with the variable factor 'time' appears significant on the response model.

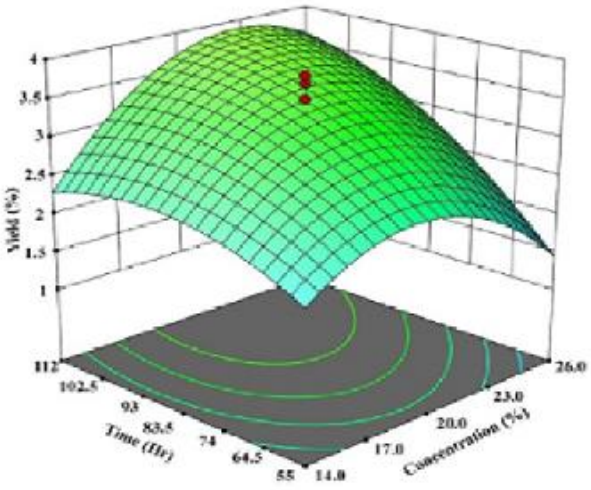

Fig. 2: Combined Effect of Time and Substrate Conc. On Ethanol Yield from Cassava peels

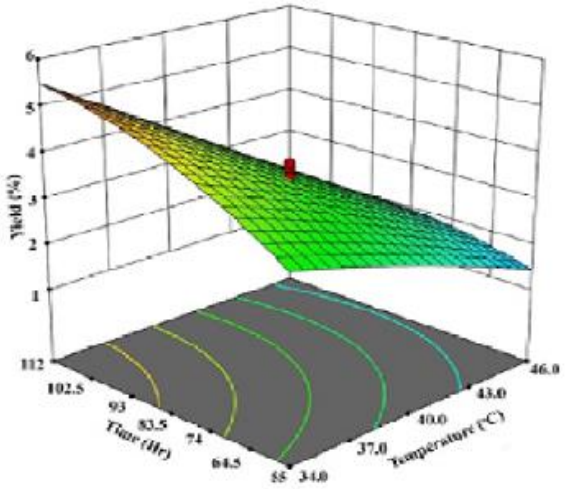

Fig. 3: Combined Effect of Time and Temperature on Ethanol Yield from Cassava peels

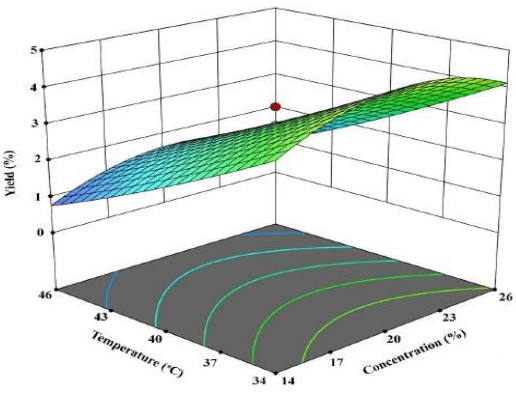

Fig. 4: Combined Effect of Temperature Substrate Conc. On Ethanol Yield from Yam peels

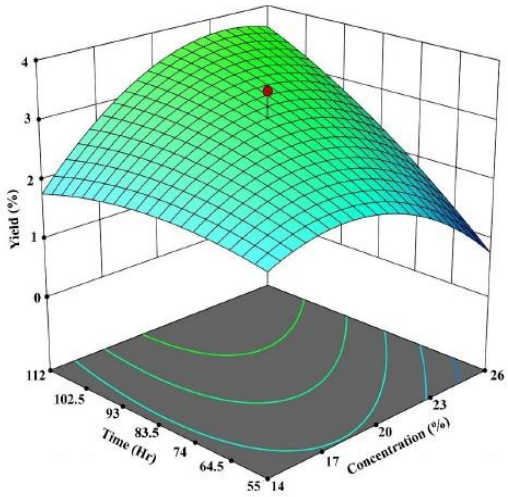

Fig. 5: Combined Effect of Time and Substrate Conc. On Ethanol Yield from Yam peels

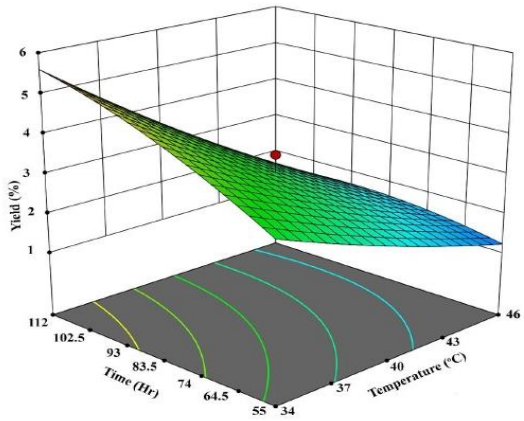

Fig. 6: Combined Effect of Time and Temperature on Ethanol Yield from Yam peels

Figures 4 - 6 shows the three-dimensional surface plot of the three factors used to analyze the influence of each parameter and their pair-wise interaction on the percentage ethanol yield. In 4 the combined effect of concentration and temperature on percentage ethanol yield from YPP is displayed. As the temperature increased, a corresponding increase in concentration resulted in a reduced ethanol yield. Figures 7 - 9 shows the three-dimensional surface plot of the three factors used to analyze the influence of each parameter and their pair-wise interaction on the percentage ethanol yield. Figure 7 shows the combined effect of concentration and temperature on percentage ethanol yield from PPP. As the temperature increased, a corresponding increase in concentration resulted in a reduced ethanol yield.

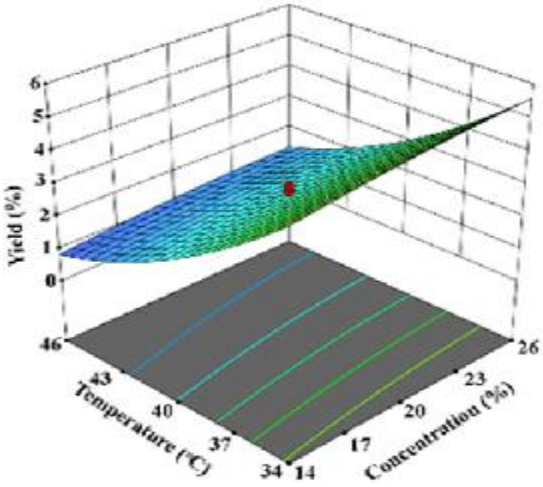

Fig. 7: Combined Effect of Temperature Substrate Conc. On Ethanol Yield from Potato peels

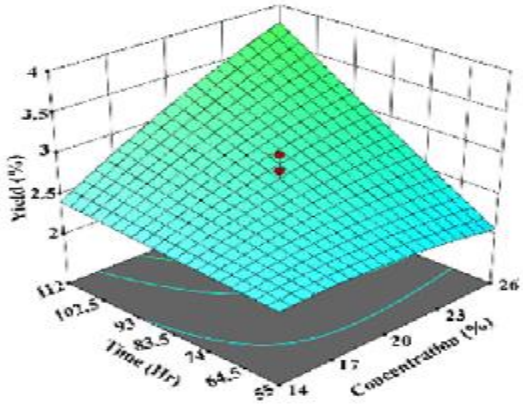

Fig. 8: Combined Effect of Time and Substrate Conc. On Ethanol Yield from Potato peels

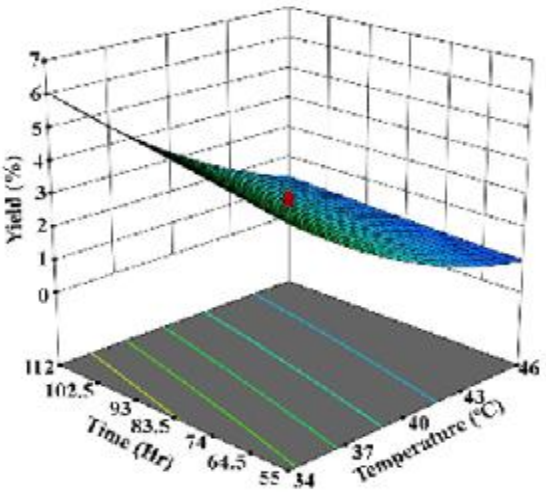

Fig. 9: Combined Effect of Time and temperature on Ethanol Yield from Potato peels

Fermentation of Hydrolysates: The ethanol yields from the fermentation of the hydrolysates are shown in Table 4.

Table 4: Ethanol yield (mL/g) of acid, alkali and *B amyloliquefaciens* Hydrolyzed Samples fermented with *Z mobilis*

	Ethanol yield (mL)		
Samples	Acid hydrolysis	Alkali hydrolysis	*B amyloliquefaciens*
CPP	0.23 ± 0.56^{a}	0.18 ± 0.56^{bc}	0.16 ± 0.35^{c}
YPP	0.23 ± 0.63^{a}	0.21 ± 0.23^{a}	0.19 ± 0.35^{bc}
PPP	0.26 ± 0.35^{a}	0.22 ± 0.98^{a}	0.16 ± 0.63^{b}

Results are mean of duplicate treatments ± standard deviation; Legend: CPP: cassava peel powder, YPP: Yam peel powder, PPP: Potato peel powder. Means within the column that share the same alphabet are not significantly different

Statistical analysis of the ethanol yield for the various processes showed the ANOVA result revealed the mean difference between Acid Hydrolysis and Alkali Hydrolysis is not statistically significant ($p>0.05$). However, the mean differences involving *B. amyloliquefaciens* are statistically significant compared to both Acid Hydrolysis and Alkali ($p < 0.05$).

Fermentation Efficiency: Estimates of the theoretical ethanol yield for the agricultural wastes are shown in Table 5, while the fermentation efficiency for the different wastes samples are presented in Figures 3 - 5.

Table 5: Theoretical ethanol yield for the agricultural wastes samples

Samples	Mass of sample Fermented (g)	% CHO content	Theoretical mass of CHO in samples (g)	Theoretical no. of moles of Glu	Theoretical no. of moles ethanol	Theoretical ethanol yield (mL)
CPP	50	67.84	33.92	0.1883	0.3767	21.99
YPP	50	78.02	39.01	0.2166	0.4332	25.29
PPP	50	75.47	37.735	0.2095	0.4190	24.47

Legend: CPP: cassava peel powder, YPP: Yam peel powder, PPP: Potato peel powder

In Figure 4, fifty grams (50 g) of the wastes (CPP, YPP, and PPP) were subjected to acid hydrolysis. The ethanol yield from this process varied among the wastes, ranging from 11.5 mL to 13.5 mL. The theoretical ethanol yield, which represents the maximum possible yield based on the input material, ranged from 21.99 mL to 25.29 mL. From the plot, cassava peel powder had the highest fermentation efficiency at 59.12%, followed by PPP and YPP respectively. In Figure 5, the result of the fermentation of the alkali hydrolysates are shown. The ethanol yield from the fermentation of the alkali hydrolysates ranged from 10.5 mL to 11.5 mL. The fermentation efficiencies for the different wastes were generally lower compared to the acid hydrolysates, with CPP having the highest efficiency at 47.75%, followed by PPP and YPP respectively.

The ethanol yield from the fermentation of wastes hydrolyzed with *B. amyloliquefaciens* are displayed in Figure 6. The ethanol yield from this process ranged from 7.2 mL to 9.8 mL. The fermentation efficiencies for *B. amyloliquefaciens* hydrolysates were generally lower compared to both acid and alkali hydrolysates. The highest was observed with CPP at 40.93% followed by YPP and PPP.

The properties of the ethanol produced compared with that of commercially available (laboratory standard) ethanol are shown in Tables 6 to 8. Comparing the quality of the ethanol produced from the acid hydrolysates with that of commercially available ethanol (Table 6), it is observed that ethanol produced from acid hydrolysates differs from the commercial ethanol in terms of ethanol content (58 – 68% as against 98%), flash point (51.4°C to 55.2°C as against 17°C), viscosity (1.23 to 1.25as against 1.20), and pH (5.2 to 6.8 as against 7.33 to 10.0).

The ethanol produced from the fermentation of microbial hydrolysates (Table 8) also differs significantly from the commercial ethanol in terms of ethanol content (36% to 48%), flash point (54.65°C to 57.8°C) boiling point (78.9°C to 82.1°C), and pH (4.2 to 4.7). The other parameters, such as viscosity, specific gravity, and titratable acidity, also show varying degrees of alignment and divergence with the commercial ethanol.

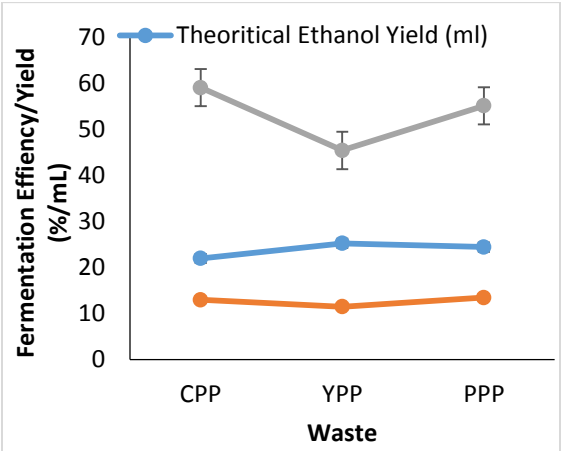

Fig 4: Fermentation efficiency of acid hydrolysate
key: CPP-cassava peel powder, YPP: Yam peel powder, PPP: Potatoe peel powder

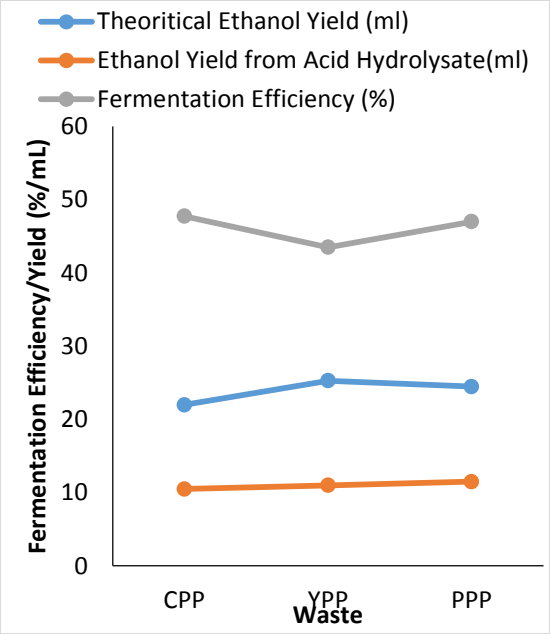

Fig 5: Fermentation efficiency of alkali hydrolysate
key: CPP-cassava peel powder, YPP: Yam peel powder, PPP: Potatoe peel powder

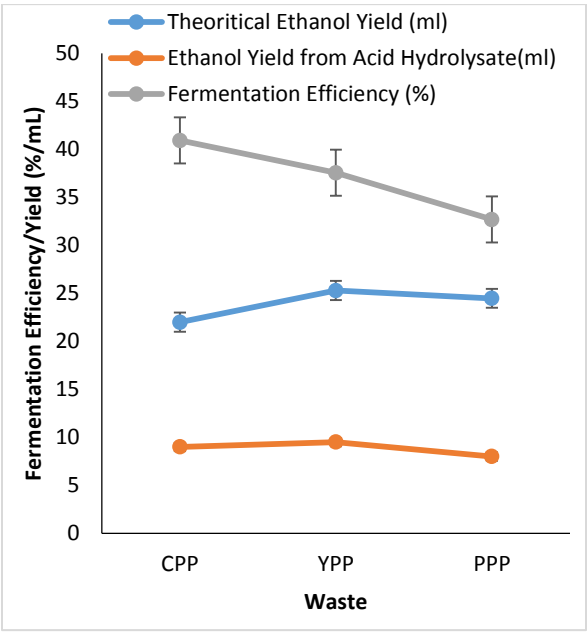

Fig 6: Fermentation efficiency of *B amyloliquefaciens* hydrolysate
key: CPP-cassava peel powder, YPP: Yam peel powder, PPP: Potatoe peel powder

Table 6: Properties of ethanol produced from acid hydrolysates

Parameters	CPP	YPP	PPP	commercial ethanol
% Ethanol	65.9	57.8	67.7	98
Flash Point (°C)	53.8	55.2	52.45	17
Boiling Point (°C)	78.9	79.5	78.9	78.37
Viscosity	1.24	1.23	1.23	1.2
Specific gravity	0.89	0.89	0.87	0.75-0.85
Titra. Acidity	12.2	13.75	12.1	16.0-18.0
pH	5.9	5.2	5.8	7.33-10.0

Table 7: Properties of ethanol produced from alkali hydrolysates

Parameters	CPP	YPP	PPP	commercial ethanol
% Ethanol	53.9	54.8	56.0	98
Flash Point (°C)	55.8	56.3	57.8	17
Boiling Point (°C)	79.7	79.4	78.7	78.37
Viscosity	1.24	1.25	1.24	1.2
Specific gravity	0.89	0.91	0.91	0.75-0.85
Titra.Acidity	32.6	22.75	28.5	16.0-18.0
pH	3.7	4.5	4.4	7.33-10.0

Table 8: Properties of ethanol produced from *B **amyloliquefaciens*** hydrolysates

Parameters	CPP	YPP	PPP	commercial ethanol
% Ethanol	46.05	47.95	41.85	98
Flash Point (°C)	56.1	54.65	56.95	17
Boiling Point (°C)	80.7	79.9	81.5	78.37
Viscosity	1.25	1.24	1.24	1.2
Specific gravity	0.893	0.913	0.884	0.75-0.85
Titra. Acidity	14.7	22.1	21.8	16.0-18.0
pH	4.4	4.2	4.6	7.33-10.0

Peels of cassava, yam, and potato, were utilized as feedstock for bio-conversion to ethanol, involving hydrolysis and fermentation. Acid, alkali and *Bacillus amyloliquefaciens* were used for the hydrolysis of the substrates. The acid and alkali hydrolysis were carried out following the method described by Olanbiwoninu and Odunfa (2012) and Amenaghawon (2017). Microbial hydrolysis was carried out using *Bacillus amyloliquefaciens*.

Optimization of fermentation was carried out using Response Surface Methodology (RSM). The independent variables selected were substrate concentration, temperature and duration (time) of fermentation. The response being monitored was percentage ethanol yield. Generally, the singular effect of temperature and time were significant ($P>0.05$). While the combined effect of substrate concentration and time as well as temperature and time were also significant. The most significant influence ($p<0.05$) was observed at a substrate concentration, temperature and time of 14%, 34°C and 55 hours, respectively. This was adopted for this study.

The result of the ethanol yield from the hydrolysates (Table 4) ranged from 0.16 mL to 0.26 mL, with highest ethanol yield recorded in CPP hydrolyzed using H_2SO_4. The lowest yield (0.16 mL) was recorded for CPP and PPP hydrolyzed by *B amyloliquefaciens*. The low yield of ethanol observed in the microbial hydrolysate may be associated with hydrolytic process. If the saccharification (microbial hydrolysis) yield low amounts of reducing sugars, this will ultimately affect the ethanol output which is depended on the conversion of available reducing sugars to ethanol. Following statistical analysis, there

was no significant difference in the ethanol yield from acid hydrolysate and alkali hydrolysates, however, there was significant difference in the ethanol yield from the chemically hydrolyzed samples and that hydrolyzed by *B amyloliquefaciens* ($P>0.05$).

The result of the fermentation efficiency (Figure 4 - 6) is a reflection of the ethanol yield. The lowest fermentation efficiency of 36% was recorded for hydrolysates of *B amyloliquefaciens* (Figure 6). The most efficient fermentation process was observed with acid hydrolyzed substrates (68%). The observed lower ethanol yield with alkali hydrolysates compared to acid hydrolysate may be attributed to the fast reaction rate of NaOH. Kumar and Wyman (2009) reported that alkaline hydrolysis causes swelling, leading to a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates resulting in fast generation of glucose, which if not harnessed may be degraded.

The result of the properties of ethanol produced from the various hydrolysates compared with that of standard ethanol showed favorable comparisons in some parameters. The pH of ethanol from acid hydrolysates ranged from 5.2 to 6.8, indicating acidity, as against 7.33 to 10.00 for standard ethanol indicating neutral to alkalinity. Titratable acidity ranged from 12.1 to 18.5 which compared favorably with that of standard ethanol: 16.0 to 18.0. Specific gravity of the ethanol produced (acid hydrolysates) ranged from 0.85 to 0.92 with that of standard ethanol ranging from 0.75 to 0.85. The viscosity (1.23 – 1.25) was also consistent with that of standard ethanol (1.20). The boiling point (78.9 – 79.8) was also consistent with that of standard ethanol; 78.37. Flash point (51.4 – 55.2), however, skewed largely from that of standard ethanol (17.0). The percentage ethanol content (55 % - 68.2 %) also varied widely from that of standard ethanol (98 %) indicating high water content. This can be improved upon by double distillation. The properties of ethanol from alkali hydrolysates were similar to that of acid hydrolysates. Specific gravity and viscosity ranged from 0.89 -0.92 and 1.23 – 1.25 respectively. Boiling point ranged from 78.7 -79.0. The pH, however, was more acidic than that of acid hydrolysates. This may be due to acid used in neutralizing the hydrolysate after alkali hydrolysis. The percentage ethanol content (53.9 % – 67.7 %) also indication high water content. The properties of ethanol from microbial hydrolysates indicate low ethanol content, ranging from 41.87% to 47.95%, compared to that of acid and alkali. Specific gravity and viscosity ranged from 0.884 - 0.913 and 1.24 – 1.25 respectively. Boiling point ranged from 79.9 -81.5. The pH ranged from 4.2 to 4.6, while flash point ranged from 54.65 to 56.95. The low ethanol content may be due to slower rate of reaction with regards to biochemical processes within living organism. The acidic pH may be attributed to the optimal pH for ethanol production for *Z. mobilis*. In a report by Zhang and Feng (2010), in which the optimal conditions for ethanol fermentation using the sweet potato was studied, it was observed that optimum ethanol production occurred at pH 4, substrate concentration of 20%, and inoculum size of 7.5% fermented for 24 hours.

Conclusion: The investigation has disclosed a comparison between acid, alkali, and microbial hydrolysis on the breakdown of lignocellulosic biomass into fermentable sugars for bioethanol production. According to the findings, acid hydrolysis demonstrated greater efficiency, followed by alkali and microbial hydrolysis, respectively. Statistical analysis reveals a significant differences among the three hydrolysis methods. Furthermore, the results indicated no significant difference between acid and alkali hydrolysis. However, both methods significantly differed from microbial hydrolysis. The result of the ethanol yield from *B. amyloliquefaciens* hydrolysis cannot be universally applied to all microbial hydrolysis processes due to variations in the hydrolytic efficiencies and substrate preferences among different saccharolytic microorganisms. To provide a more comprehensive comparison of acid, alkali, and microbial hydrolysis processes, utilizing *B. amyloliquefaciens* in degrading other waste samples, along with employing other cellulolytic bacteria, will provide a more general comparison of acid, alkali and microbial hydrolytic processes.

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Data Availability: Data are available upon request from the first author.

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