Production of Bioethanol from Hybrid Cassava Pulp and Peel using Microbial and Acid Hydrolysis

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Agricultural wastes are potential sources for the commercial production of biofuels because of their availability and low market price. In the present study, the viability of producing bioethanol from three varieties of cassava pulp and peel (CPP) was studied. Acid hydrolysis was performed by dispersing 20% w/v CPP in 100 mL of hydrochloric acid. Biological hydrolysis was performed by inoculating gelatinized CPP paste with *Aspergillus niger*. A set of un-gelatinized control samples was used to investigate the effect of heat pretreatment on the reducing sugar yield. The hydrolyzed samples were fermented with *Saccharomyces cerevisiae*, and the ethanol yield was determined. The reducing sugar yield was 110.7 g/L, 100.4 g/L, and 96.7 g/L from acid hydrolysis of three cassava varieties, while a yield of 98.9 g/L was obtained from cassava peel at 0.7 M and 50 min. The gelatinized pulp from the samples hydrolyzed with *A. niger* consistently produced more reducing sugar than the control samples. The highest ethanol yields were 54.8% and 33.1% obtained, respectively, from a heat-pretreated variety and cassava peel. Results from the conversion of cassava peel readily bring to light a more useful way of managing cassava wastes in the environment.

Keywords: Cassava pulp; Cassava peels; Hydrolysis; Fermentation; Ethanol; Glucose

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INTRODUCTION

The increasing global demand for energy has caused the development of and continuous research into innovative methods for generating renewable fuels from numerous sources, including waste. Biomass sources for bio-ethanol production have yielded interesting results and are being touted as a promising energy source of the future. Energy crops with high energy contents, such as maize, sugarcane, soybeans, Barbados nuts, sunflowers, and cassava, have been cultivated and used to produce biofuel (Adelekan 2010).

Ethanol is used worldwide in the production of alcoholic beverages, for clinical uses, the manufacture of pharmaceutical products, and as a solvent and fuel. The use of ethanol for energy is not particularly new. Ethanol was used as automotive fuel as far back as 1896 by Henry Ford, who designed his first car to run on ethanol (Goettemoeller and Goettemoeller 2007). Ethanol is an attractive substitute for premium motor spirits (PMS) because it is 98% pollution free, biodegradable, and renewable (Saha *et al.* 2014). Blends of 10% to 20% ethanol and PMS have also been used as transportation fuels (Purwadi 2006). Ethanol has been claimed to be the global energy source that could drive a

sustainable future for the planet (Amigun *et al.* 2008; Adelekan 2010; Nuwamanya *et al.* 2012). Ethanol has been produced and consumed by humans since prehistoric times because of its intoxicative effects. It can be produced through pretreatment, hydrolysis, and fermentation (Gnansounou and Dauriat 2005) of lignocellulosic material waste (Akaracharanya *et al.* 2011) and non-food biomass, which is termed second-generation bioethanol (Sanchez and Cardona 2008). Examples of this include cassava waste, rice husk, bagasse, corn stover, and municipal wastes, such as softwood newspaper, all of which are viable feedstocks for the production of second-generation bioethanol and other chemicals (Sanchez and Cardona 2008). First-generation ethanol is bioethanol obtained from crops containing sucrose or starch, such as sugar cane, sugar beets, corn, rice, millet, and cassava.

Hydrolysis of cassava peel and pulp is possible with acid and enzymes; the latter of which gives better yields because of a better selectivity for the desired products by the enzymes (Saha *et al.* 2014). Cassava is a major staple food in nearly all parts of Nigeria. In 2000, it was reported that Africa cultivates over 50% of the global cassava output, with Nigeria producing over 70% of this quantity (FAO and IFAD 2005). Cassava pulp is a solid product obtained by processing cassava roots. It is a fibrous substance that has a composition of approximately 60% starch (Li and Zhu 2011) and 20% cellulose fiber on a dry mass basis. This composition depends on the cassava variety and processing efficiency. Many studies have been performed on the utilization of cassava pulp as an animal feed and for the production of bioethanol (Suksombat *et al.* 2006; Khempaka *et al.* 2009). Cassava waste is usually large in volume; for example, an estimated 34 million MT to 40 million MT of cassava produces approximately 2.22 million MT of cassava leaves, 2.9 million MT of cassava peel, and 6.3 million MT of cassava pulp. Cassava waste can be processed and converted into value-added products, such as biomethane or biogas, bioethanol, biosurfactants, and biofertilizer (Khempaka *et al.* 2009), while some waste is used as feed for cattle and other ruminants (Osei *et al.* 1990; Ubalua 2007). However, a majority of the waste is left to rot. One disadvantage of using cassava waste is that it requires some form of drying (either with natural sunlight or electrical/charcoal-powered dryers) before incineration. If it is left to dry with natural air, it makes the environment unsightly and becomes a breeding ground for rodents, snakes, and other pests. This large volume of lignocellulosic material waste (Tewe 2004; Nörgård 2013) makes research attractive, as the raw material (cassava peel and pulp) is available all year-round in Nigeria.

In the present study, the viability of producing bioethanol from three varieties of cassava pulp and peel was explored.

EXPERIMENTAL

Materials

Hydrochloric acid (HCl), (Sigma Aldrich, USA), sodium hydroxide (NaOH), (Sigma Aldrich, USA), di-ammonium phosphate pellets, dinitrosalicylic acid (DNS), Rochelle salt, and analytical glucose were all used in this experiment.

Three cassava cultivars with accession numbers IBA980505, IBA950289, and IBA010040 were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. They were cultivated and harvested from Covenant University Farm and respectively labeled as 5A, 4B, and 3A in the same order of the assigned accession numbers above. The cassava peels were sourced from a local market in Ota.

Equipment

The following equipment was used: a pH meter (AD1040, Adwa, Szeged, Hungary); magnetic stirrer; platform stirrer; hot plate; inoculation loop; roller mill; pails; platform shaker; magnetic stirrer/hot plate (JENWAY 1000, JENWAY, Essex, United Kingdom); weighing balance (OHAUS (Adventurer), Parsippany, USA); Ultraviolet Visible (UV/VIS) spectrophotometer (T90+, PG Instruments Limited, Leicesterhire, United Kingdom); centrifuge (SM-80-2, Surgifield Medical England, Essex, United Kingdom); and autoclave (SM-280-E, Surgifield Medical England, Essex, United Kingdom).

Sample Preparation

Dried pulp from three cassava varieties (3A, 4B, and 5A) were ground separately to fine powders with a roller mill and stored. The cassava peels were washed, spread out neatly, and sun-dried to a constant weight for one week. The dehydrated samples were homogenized to a powder with a roller mill and stored separately in air tight pails.

The basal salt medium was prepared by weighing and mixing the components (Table 1) in 1 L of distilled water.

Table 1. List of Basal Salt Ingredients

Compound	Quantity
KH_2PO_4 (99.5%)	2 g
$(NH_4)_2SO_4$ (99.0%)	1.4 g
$MgSO_4 \cdot 7H_2O$ (99.0%)	0.3 g
$CaCl_2$ (99.0%)	0.3 g
Urea	0.3 g
Tween 80	1 Ml
$FeSO_4 \cdot 7H_2O$ (99.0%)	5mg
$MnSO_4$ (99.0%)	1.6 mg
$ZnSO_4$ (99.0%)	1.4 mg
$CoCl_2$ (99.0%)	2 mg
Starch 1%	10 g
Medium was sterilized at 121 °C for 15 min	

Preparation of the A. niger spore suspension

Ten milliliters of sterile distilled water and three drops of tween 80 were added to a test tube and mixed gently. This mixture was then poured on a 5-d-old plate of *A. niger* isolate from sand and rotten tomatoes. A pair of sterile forceps was used to gently scrape the spores on the plate to ensure proper homogenization with the mixture. This mixture was then filtered using filter paper. The filtrate was centrifuged at 800 rpm for 10 min, and 10 mL of sterile distilled water was then added to the sediment.

Biological Hydrolysis by *A. niger*

Twenty grams of cassava pulp (varieties 3A, 4B, and 5A) and cassava peel were weighed and placed in distinct beakers. One hundred milliliters of distilled water were added to each of the samples and mixed thoroughly to obtain slurries. All of the samples were left to stand for 30 min to allow the constituents to swell properly. A set with a duplicate of each sample was created. The pH of the samples was measured using the pH meter. Each of the first set of samples was then autoclaved for 30 min at 120 °C for gelatinization, while the second set of samples was left as a control (un-gelatinized). One

milliliter of spore suspension containing 20×10^4 spores was inoculated in each of the sample mixtures. Fifty milliliters of basal salt medium were added to each of the samples. The samples were then placed on a platform shaker and allowed to mix at 130 rpm for 30 min. The sugar concentration of the samples was determined daily using the DNS method at 540 nm. The samples were then stored for fermentation.

Acid Hydrolysis

Twenty grams of cassava pulp (from varieties 3A, 4B, and 5A) and cassava peel were weighed and suspended in 100 mL of HCl at concentrations of 0.3 M, 0.5 M, and 0.7 M. The samples were kept on a hot plate at 100 °C for 20 min; this was also repeated for 30 min, 40 min, 50 min, 60 min, 75 min, and 90 min. After the hydrolysis process, 0.5 M sodium hydroxide was used to adjust the pH of the samples to 6.5 to prepare for fermentation of the samples. The samples were labelled and filtered to remove any solid non-hydrolysates. The glucose content of the hydrolysate was determined using the DNS method.

Fermentation and Distillation

One colony of yeast (*Saccharomyces cerevisiae*) isolated from palm wine was collected using a sterile inoculating loop and added to each of the hydrolyzed samples in a conical flask. Five grams of di-ammonium phosphate pellets were then added to each of the hydrolysates (di-ammonium phosphate provided the yeast with nutrients to facilitate rapid fermentation). The samples were cocked with cotton wool and left to stand for 4 d to ensure complete fermentation. The fermented broth was transferred into a round bottom flask and placed on a heating mantle fixed to a distillation column. Another flask was fixed to the other end of the distillation column to collect the distillate at 78 °C (boiling point of ethanol). This was done for each sample of the fermented broth. The distillate collected was measured using a measuring cylinder and expressed as the quantity of ethanol produced (g/L) by multiplying the volume of the distillate with the ethanol density (0.8033 g/cm) (Oyeleke and Jibrin 2009; Somda *et al.* 2011; Nörgård 2013).

Determination of the Glucose Concentration using the DNS Method

The DNS reagent was first prepared by dissolving 1 g of DNS acid in 20 mL of 2 M NaOH and 50 mL of distilled water. Thirty grams of Rochelle salt were added and distilled water was then added to reach a total volume of 100 mL. Rochelle salt was introduced to prevent the reagent from dissolving oxygen.

A sample of 0.2 mL of analytical glucose with a known concentration was placed in a test tube, and 1.8 mL of distilled water and 2 mL of DNS reagent were added. The solution was boiled for 5 min in a water bath. A color change from light brown to brick red was observed. The sample was cooled and diluted to 24 mL. This procedure was repeated for the four different glucose concentrations. The absorbance of the diluted samples at 540 nm was measured using the UV spectrophotometer. The data obtained was used to prepare a calibration curve, which was then used for determining the reducing sugar concentration for all of the hydrolysate samples.

Determination of the glucose concentration in the hydrolysate

A sample of 0.2 mL of the reducing sugar solution (hydrolysate) with an unknown concentration was placed in a test tube. The solution was made in the manner discussed previously. The absorbance of the diluted samples at 540 nm was then measured using the

UV spectrophotometer. Then, with the standardized calibration curve, the glucose concentration in the hydrolysate was determined.

RESULTS AND DISCUSSION

Glucose Production from Acid Hydrolysis of the Cassava Starch Substrates

The glucose yields produced from the acid and biological hydrolysis processes are presented below. Figure 1 shows that there was a gradual increase in the glucose production with time until a peak glucose yield of 97.5 g/L was attained at approximately 50 min for sample 3A. The peak glucose yields obtained were 96.43 g/L, 88.68 g/L, and 83.68 g/L at 60 min for samples 5A, 4B, and cassava peel, respectively.

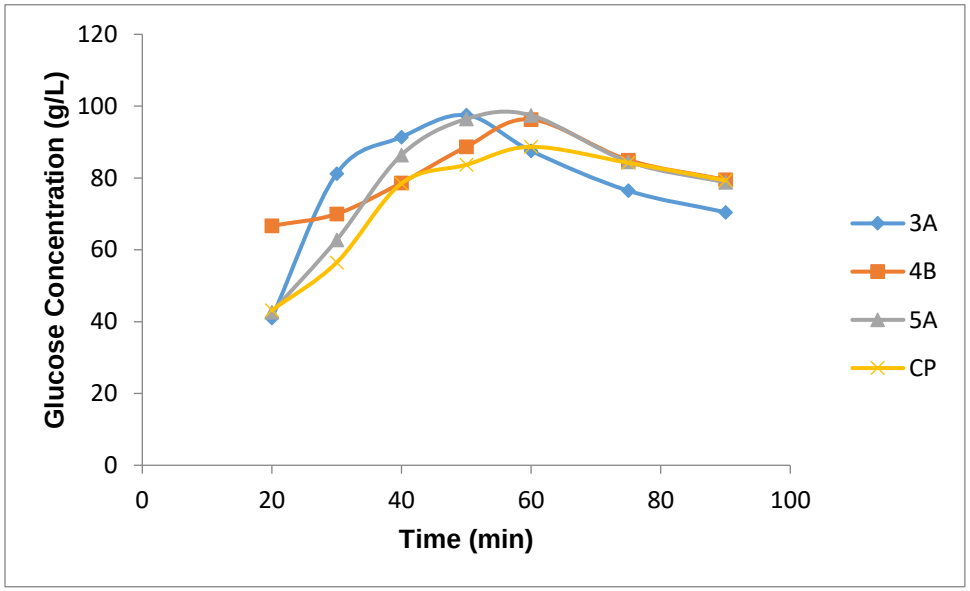

Fig. 1. Glucose concentration using 0.3 M HCl in the hydrolysis of the cassava pulp (3A, 4B, and 5A) and cassava peel (CP)

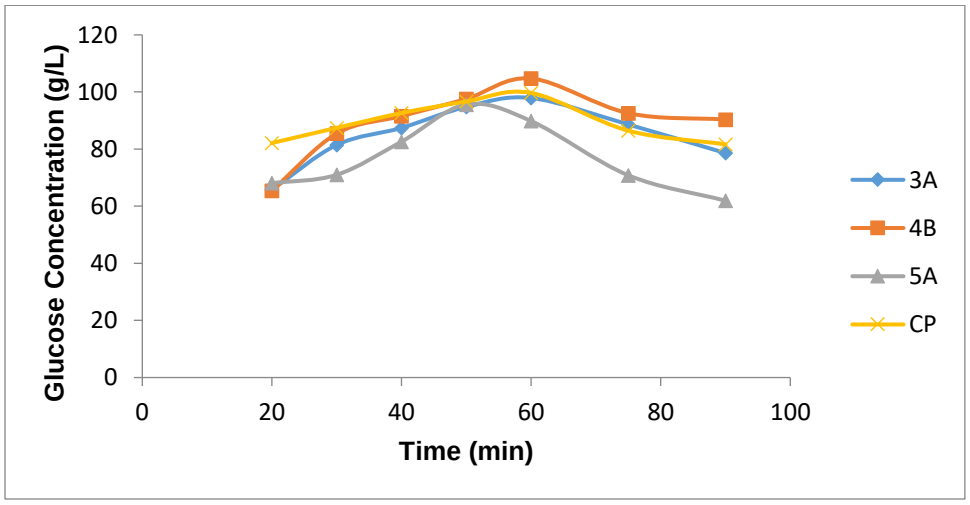

Fig. 2. Glucose concentration using 0.5 M HCl in the hydrolysis of the cassava pulp (3A, 4B, and 5A) and cassava peel (CP)

Figure 2 shows that variety 4B had the highest glucose yield of 104.7 g/L at approximately 60 min and 0.5 M HCl. This was followed closely by the other samples; cassava peel and 3A yielded 99.7 g/L and 97.9 g/L, respectively at 60 min, while 5A yielded 96.7 g/L at 50 min.

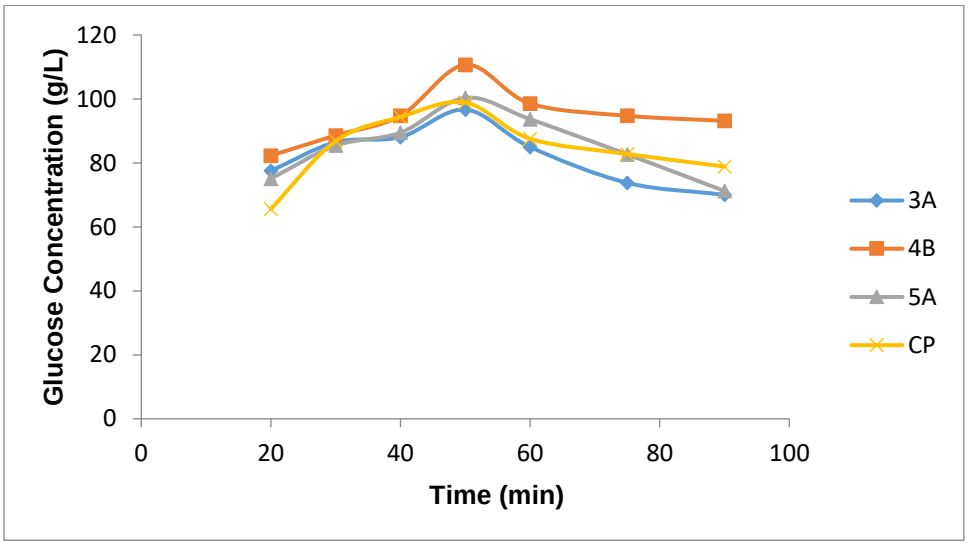

Fig. 3. Glucose concentration using 0.7 M HCl in the hydrolysis of the cassava pulp (3A, 4B, and 5A) and cassava peel (CP)

Figure 3 shows that sample 4B still exhibited the highest glucose yield of 110.7 g/L when 0.7 M HCl was used, but at 50 min instead. The other samples also each produced their maximum glucose concentrations at 50 min in the following order: 5A (100.4 g/L), cassava peel (98.9 g/L), and 3A (96.7 g/L). For all of the HCl concentrations used for hydrolysis, the glucose yield was observed to increase gradually with the time and concentration (Figs. 1 to 3) and reached a peak before beginning to decline. The results agreed with those of Ebabhi *et al.* (2013), who first observed that the reducing sugar yield usually increased directly as the acid concentration increased because of the higher availability of H^+ ions to break down the starch polymer. The overall highest reducing sugar yield at 0.7 M HCl (110.7 g/L) was obtained from cassava pulp variety 4B at 50 min and a temperature of 100 °C (Fig. 3). Also, cassava pulp variety 3A produced a maximum glucose yield of 96.7 g/L at 0.7 M HCl after a reaction time of 50 min, while a maximum yield of 100.4 g/L was obtained from cassava pulp variety 5A at the same acid concentration of 0.7 M and a reaction time of 60 min (Fig. 3). The highest reducing sugar yield from the cassava peel was 94.9 g/L and was obtained at 50 min of hydrolysis using 0.7 M HCl (Fig. 3).

Exposure of lignocellulosic materials to harsh conditions (high acid concentration and temperature) usually results in the breaking down of the recalcitrant hemicellulose-lignin matrix (Chamy *et al.* 1994). The glucose and reducing sugar yields obtained were the result of the combined hydrolysis of starchy and lignocellulosic materials that make up the cassava peel and pulp (Urbaneja *et al.* 1996; Agu *et al.* 1997; Zhu *et al.* 2002). It was observed that it took a longer reaction time for a high sugar yield to be obtained when a lower acid concentration was used. This was because at low acid concentrations, less H^+ ions were available for the breakdown of starch and lignocellulose; hence, the reaction progressed at a slower rate.

The gradual decline in the sugar concentration after the peak value, as was observed for all of the samples (Figs. 1 to 3), was attributed to dehydration reactions that occurred during acid hydrolysis, as well as the subsequent breakdown of the recovered reducing sugar to other compounds. The presence of excess H^+ ions resulted in the decomposition of the reducing sugars dissolved in the hydrolysate to undesirable compounds, such as furan aldehydes, carboxylic acids, acid salts, phenolics, and vanillin (Malester *et al.* 1988; Gopinatthan *et al.* 2015). These compounds generally inhibit the fermentation of the hydrolysate into ethanol (Nichols *et al.* 2008).

The results showed that variety 3A performed better under mild acid concentrations, unlike variety 4B, which yielded more glucose under higher acid concentrations. The preference of variety 5A could not be ascertained because it produced a relatively high glucose yield at the extreme concentrations of 0.3 M and 0.7 M HCl, while it produced the least glucose at 0.5 M HCl.

Glucose Production by Microbial Hydrolysis of Cassava Substrates

The set of gelatinized samples (3A1, 4B1, 5A1, and CP1) consistently produced more reducing sugar than the control samples (3A2, 4B2, 5A2, and CP2). This was because they provided better conditions for the growth of *A. niger* (Fig. 4). A factor responsible for this was that the gelatinized samples had a larger surface area that allowed the *A. niger* fungus to attach itself properly to the substrate and secrete the appropriate enzymes. However, *A. niger* molds could not effectively hydrolyze the un-gelatinized (control) samples because of the inhomogeneous nature of the starch suspension and the difficulty it has with absorbing water. This made it difficult for all the secreted amylolytic enzymes to be used up because the structure of the α-1, 4- and α-1, 6-glucosidic bonds of the starch granules in the control samples had not been properly disrupted or dispersed, as was the case with the gelatinized samples.

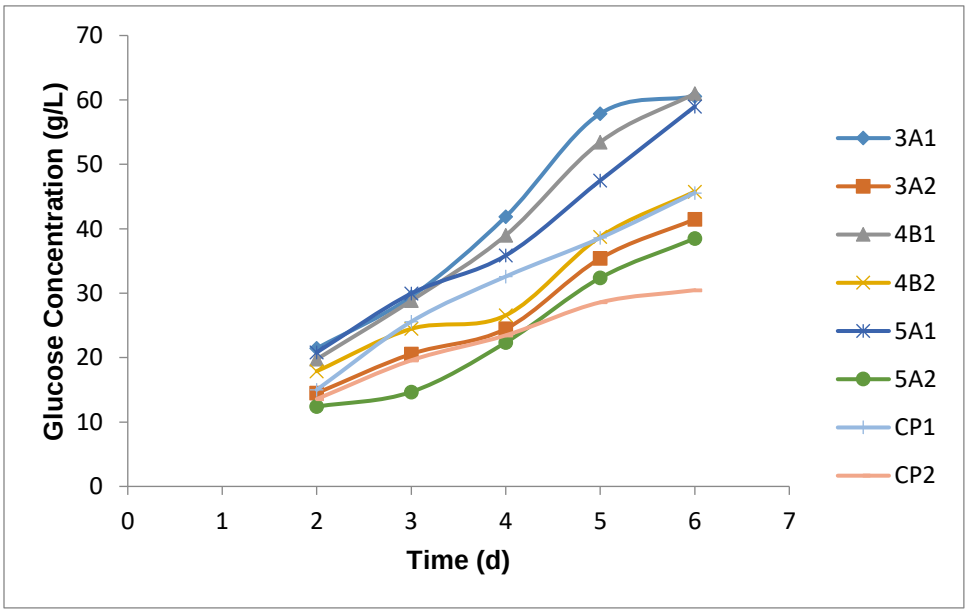

Fig. 4. Glucose yield from biological hydrolysis using *A. niger*; gelatinized samples: 3A1, 4B1, 5A1, and CP1; and control samples: 3A2, 4B2, 5A2, and CP2

Relatively, of the four samples the highest reducing sugar yield of 61.0 g/L was obtained from the heat-pretreated cassava variety 4B after 6 days of hydrolysis while the reducing sugar yields obtained from the other samples – heat pretreated and gelatinized cassava peel, cassava pulp varieties 3A and 5A were, respectively, 45.6 g/L, 60.6 g/L, and 59.0 g/L. The reducing sugar yield obtained from the cassava peel was similar to the results obtained by other researchers (Woiciechowski *et al.* 1999; Yoonan and Kongkiattikajorn 2004), who performed cassava peel hydrolysis using *A. niger*. In addition to α-amylase and glucoamylase secretion, *A. niger* also produced cellulases (Yoonan and Kongkiattikajorn 2004). Cellulase enzymes (cellobiohydrolases, β-glucosidases, and endoglucanases) worked synergistically to catalyze the degradation of the lignocellulose (Hoshino *et al.* 1997; Hanif *et al.* 2004). The impressive reducing sugar yield recovered from the cassava peel was attributed to the combined hydrolysis of both the residual starch (starch from the cassava flesh removed with the peel during peeling of the cassava) and lignocellulose present in the sample from the enzymes produced by *A. niger*.

Comparison of the Recovery of Ethanol from the Acid and Microbial Hydrolysis of Cassava Substrates

Tables 2 through 6 show the percentage of ethanol recovered from 20 g of various varieties of cassava substrates (3A, 4B, 5A, and cassava peel) and using two different production techniques (acid and microbial hydrolysis). When acid hydrolysis was performed, four cassava substrates and three different acid concentrations (0.3 M, 0.5 M, and 0.7 M) were used with various reaction times. Tables 2 through 6 show that the cassava substrate, acid concentration, reaction time (hydrolysis), and production method were key parameters in determining the percentage of ethanol recovered.

Table 2. Ethanol Recovered from Cassava Variety 4B

HCL Conc. (M)	Hydrolysis Time (min)	Volume of Ethanol Recovered (mL)	Weight of Ethanol Obtained (g)	Ethanol Recovered from 20 g of Cassava (%)
0.3 M	20	3.3	2.60	13.02
	30	7.3	5.76	28.80
	40	6.6	5.21	26.04
	50	10.3	8.13	40.63
	60	12.8	10.10	50.50
	75	5.7	4.50	22.49
	90	6.4	5.05	25.25
0.5 M	20	4.3	3.39	16.96
	30	5.4	4.26	21.30
	40	5.0	3.95	19.73
	50	8.8	6.94	34.72
	60	13.4	10.57	52.86
	75	9.3	7.34	36.69
	90	6.4	5.05	25.25
0.7 M	20	5.7	4.50	22.49
	30	8.4	6.63	33.14
	40	7.5	5.92	29.59
	50	13.9	10.97	54.84
	60	10.0	7.89	39.45
	75	9.3	7.34	36.69
	90	6.5	5.13	25.64

Table 3. Ethanol Recovered from Cassava Variety 5A

HCL Conc. (M)	Hydrolysis Time (min)	Volume of Ethanol Recovered (mL)	Weight of Ethanol Obtained (g)	Ethanol Recovered from 20 g of Cassava (%)
0.3 M	20	2.3	1.82	9.07
	30	4.6	3.63	18.15
	40	4.8	3.79	18.94
	50	6.5	5.13	25.64
	60	5.6	4.42	22.09
	75	3.7	2.92	14.60
	90	4.6	3.63	18.15
0.5 M	20	3.2	2.53	12.62
	30	4.8	3.79	18.94
	40	4.5	3.55	17.75
	50	6.4	5.05	25.25
	60	7.3	5.76	28.80
	75	5.3	4.18	20.91
	90	4.6	3.63	18.15
0.7 M	20	4.1	3.24	16.17
	30	6.3	4.97	24.85
	40	5.3	4.18	20.91
	50	8.1	6.39	31.95
	60	8.3	6.55	32.74
	75	3.5	2.76	13.81
	90	5.2	4.10	20.51

Table 4. Ethanol Recovered from Cassava Variety 3A

HCL Conc. (M)	Hydrolysis Time (min)	Volume of Ethanol Recovered (mL)	Weight of Ethanol Obtained (g)	Ethanol Recovered from 20 g of Cassava (%)
0.3 M	20	1.3	1.03	5.13
	30	3.6	2.84	14.20
	40	4.8	3.79	18.94
	50	7.9	6.23	31.17
	60	8.7	6.86	34.32
	75	4.7	3.71	18.54
	90	5.2	4.10	20.51
0.5 M	20	3.4	2.68	13.41
	30	4.6	3.63	18.15
	40	7.8	6.15	30.77
	50	6.7	5.29	26.43
	60	3.9	3.08	15.39
	75	5.3	4.18	20.91
	90	3.7	2.92	14.60
0.7 M	20	4.3	3.39	16.96
	30	7.4	5.84	29.19
	40	9.4	7.42	37.08
	50	3.4	2.68	13.41
	60	5.3	4.18	20.91
	75	2.0	1.58	7.89
	90	5.3	4.18	20.91

Table 5. Ethanol Recovered from the Cassava Peel

HCL Conc. (M)	Hydrolysis Time (min)	Volume of Ethanol Recovered (mL)	Weight of Ethanol Obtained (g)	Ethanol Recovered from 20 g of Cassava (%)
0.3 M	20	2.3	1.81	9.07
	30	3.4	2.68	13.41
	40	4.6	3.63	18.15
	50	4.5	3.55	17.75
	60	3.8	3.00	14.99
	75	4.2	3.31	16.57
	90	3.2	2.52	12.62
0.5 M	20	1.3	1.03	5.13
	30	2.5	1.97	9.86
	40	4.6	3.63	18.15
	50	3.6	2.84	14.20
	60	5.8	4.58	22.88
	75	3.6	2.84	14.20
	90	3.2	2.52	12.62
0.7 M	20	2.3	1.81	9.074
	30	2.8	2.21	11.05
	40	4.3	3.39	16.96
	50	6.7	5.29	26.43
	60	8.4	6.63	33.14
	75	3.6	2.84	14.20
	90	3.5	2.76	13.81

Table 6. Ethanol Recovered from the *A. niger* Hydrolyzed Samples

Substrate	Ethanol Recovered from Heat-pretreated Samples (mL)	Weight of Ethanol Obtained (g)	Ethanol Recovered from 20 g of Cassava (Heat Pretreated) (%)	Ethanol Recovered from the Control (mL)	Weight of Ethanol Obtained (g)	Ethanol Recovered from 20 g of Cassava (Control) (%)
4B	14.5	11.44	57.20	7.8	6.15	30.77
5A	9.8	7.73	38.66	3.2	2.53	12.62
3A	10.8	8.52	42.61	4.3	3.39	16.96
Cassava Peel	5.2	4.10	20.51	1.3	1.03	5.13

Acid Hydrolysis of the Cassava Substrates

Tables 2 through 5 show that the acid concentration had a notable effect on hydrolysis and hence on the percentage of ethanol recovered from 20 g of each cassava substrate used. It was generally observed that, irrespective of the cassava substrate used, the same performance trend was observed for the three acid concentrations. When the acid concentration was higher, the percentage of ethanol recovered was higher. The 0.7-M acid concentration consistently produced the highest percentage of ethanol. This was closely followed by the 0.5-M acid concentration, except for variety 3A, where the percentage of ethanol recovered using 0.3 M HCl was higher than that obtained using 0.5 M HCl. For all of the other varieties, 0.3 M HCl produced the lowest ethanol percentage.

Ethanol Recovered from the Various Cassava Substrates

The different cassava samples used to produce ethanol performed well and obtained yields of more than 30%. Cassava sample 4B had the highest ethanol yields of 54.8%, 52.8%, and 50.5% using 0.7 M, 0.5 M, and 0.3 M HCl, respectively. This was closely followed by sample 3A with ethanol yield of 37.3% then 33.1, and 32.7% ethanol yields from cassava peel and sample 5A using 0.7 M HCl respectively.

Enzyme Hydrolysis of the Cassava Samples using *A. niger*

Aspergillus niger was inoculated into two substrate types (gelatinized and un-gelatinized) of the cassava samples. The results are shown in Table 6. The heat-pretreated samples (gelatinized) performed better than the control samples (un-gelatinized). Again, the heat-pretreated cassava type 4B produced the highest percentage (57.2%) of ethanol, followed by type 3A (42.6%) and 5A (38.7%). The cassava peel had the lowest ethanol production (20.5%). The same trend was observed with the control samples, except that the ethanol recovered from them was generally lower than that from the heat-pretreated samples. The highest percentage of ethanol was recovered from the un-gelatinized type 4B at 30.7%, and the lowest was 5.1% from the un-gelatinized cassava peel.

Comparison of the Ethanol Yield from the microbial and Acid Hydrolysis of the Cassava Samples

While the saccharification time for acid hydrolysis of the cassava samples was 20 min to 90 min, that of enzyme saccharification was 2 d to 6 d. This implied that acid hydrolysis will yield more ethanol by a factor of 94 at a minimum. Within 6 d, the heat-pretreated cassava sample type 4B that was hydrolyzed by *A. niger* produced 57.2% ethanol, which was similar to the 54.8% yield from the same cassava type 4B that was acid hydrolyzed for only 90 min. With the exception of the cassava peel, the other cassava varieties that underwent heat treatment and microbial hydrolysis followed the same trend that was observed for the acid hydrolysis treatment. Following microbial treatment, the cassava type 3A, cassava type 5A, and cassava peel samples produced 42.6%, 38.7%, and 20.5% ethanol, respectively, in 6 d, while the acid treatment of these cassava samples produced 37.1%, 32.7%, and 33.1%, respectively, in 90 min. The results further revealed the consistency of cassava type 4B in producing bioethanol, as high yields (more than 50%) were obtained irrespective of the processing method used.

CONCLUSIONS

1. This study confirmed that acid saccharification of the cassava peel and pulp is more efficient than enzyme saccharification using *A. niger*. While acid hydrolysis took 90 min to achieve the maximum glucose yield, it took the fungus 6 d to achieve the same result.
2. Cassava pulp variety 4B consistently produced the highest yield of reducing sugar, and hence the highest yield of ethanol, irrespective of the method used (acid or microbial hydrolysis).
3. The results from the acid and microbial hydrolysis processes were encouraging and revealed the potential for cassava pulp variety 4B to be used as a lignocellulosic material for ethanol production.

4. Furthermore, the heat-pretreated (or gelatinized) cassava samples yielded more ethanol compared with that from the control (or un-gelatinized) counterparts, irrespective of the hydrolysis method used. However, the samples hydrolyzed by *A. niger* produced more ethanol compared to the acid hydrolyzed samples.

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