International Journal of Scientific & Engineering Research, Volume 15, Issue 2, February-2024
ISSN 2229-5518

Optimization of Glucose Yield from Acid, Alkali and Microbial Hydrolysis of Selected Agricultural Wastes.

*U J Ovueni, D V Adegunloye, A K Onifade, and O F Olukunle

Abstract— **In this study, agricultural waste materials such as cassava, yam, and potato peels, as well as maize, millet, and sorghum brans, were used. These materials were ground into fine powder using a disc mill and sieved to ensure a consistent particle size. The resulting powders were labeled as cassava peel powder (CPP), yam peel powder (YPP), potato peel powder (PPP), maize bran powder (MaBP), millet bran powder (MiBP), and sorghum bran powder (SBP). Chemical hydrolysis of the samples was carried out using Sulphuric acid and Sodium hydroxide, and the effect determined by evaluating the percentage contents of lignin, hemicellulose and cellulose as well as glucose yields. The effect of microbial hydrolysis, using *Bacillus amyloliquefaciens*, was also investigated. Acid and alkali hydrolysis was performed using five different concentrations ranging from 0.01 M to 0.25 M. Optimization of microbial hydrolysis was carried out by varying pH values (5 to 9), substrate concentration (0.02% to 0.1%), temperature (30°C to 50°C) and duration of saccharification (over a period of 48 hours). Results of the acid hydrolysis indicated an increase in the lignin and cellulose contents of the samples with increasing acid concentration. Lignin contents ranged from 8.50% (in SBP) to 45.16% (in M_aBP), cellulose ranged from 61.80% (in CPP) to 78.57% (in SBP). The hemicellulose contents, however, reduced during acid hydrolysis as acid concentration increased, from 21.88% (in PPP) to 10.71% (in SBP). Results of the alkali hydrolysis indicated a reduction in lignin, cellulose and hemicellulose contents of samples as concentration increased. For lignin values ranged from 16.08% (in PPP) to 9.10% (in SBP), cellulose values ranged from 36.89% (in SBP) to 20.27% (in M_iBP), while hemicellulose values ranged from 16.40% (in CPP) to 10.16% (in PPP). Glucose yields for both acid and alkali hydrolysis increased with increasing concentrations of hydrolytic agents. In the microbial hydrolysis, the gravimetric results show a gradual increase in percentage lignin and cellulose contents, while hemicellulose did not show defined trend. The glucose yields increased with rise in incubation temperature, with higher yields between 35°C and 40°C. Glucose yields were higher at pH 7 than at acid or alkaline pH. Increase in substrate concentration improved glucose yields, with highest yields recorded in 1% substrate concentration. Glucose yields also increased with incubation time. Highest yield of 0.237 mg/ml was observed with CPP with substrate concentration of 1%, a starting pH 7, incubated at 35°C for 48 hours.**

Keywords: Agricultural wastes, Acid, Alkali and Microbial Hydrolysis, Bioethanol, Glucose yield, Optimization.

1 INTRODUCTION

Bioethanol, which is produced through microbial fermentation, is the most commonly used liquid biofuel worldwide [1]. It is typically made from food crops such as corn, cassava, sugarcane, rice, and sweet potatoes. However, there are concerns about the sustainability of producing bioethanol from food crops, leading to interest in using lignocellulosic biomass for bioethanol production as an alternative [2].

Lignocellulosic biomass refers to the non-edible portion of food crops, which is made up of cellulose, lignin, and hemicellulose. This type of biomass is a rich source of hexose and pentose sugars, which can be used for the production of bioethanol [3]. The process of converting lignocellulosic biomass into ethanol involves several steps, including pretreatment, hydrolysis, fermentation, and ethanol recovery through distillation. The complex and resistant structure of lignocellulosic biomass requires the use of various pretreatment and hydrolytic processes in order to break down the carbohydrates into reducing sugars [4].

Various pretreatment and hydrolytic methods have been suggested, depending on the purpose of removing hemicellulose or lignin from the biomass. Dilute acid hydrolysis is promising as it is capable of high solubilization of hemicellulose. This process degrades most of the hydrogen bonds in hemicelluloses and partially degrades cellulose and lignin. In addition, acid hydrolysis breaks down hemicellulose to pentoses and hexoses, removes some of the lignin, and makes the cellulose structure more accessible, converting fractions to glucose [5]. Alkali hydrolysis using Sodium hydroxide at 121 °C has been shown to be effective in improving the digestibility of lignocellulosic biomass, by removing lignin, facilitate the digestibility of carbohydrates and enhancing reducing sugar yield [6]. Microbial saccharification of biomass has also been studied using fungi and bacteria. Several microorganisms have been proven to be capable of hydrolyzing biomass. Aerobic bacteria that require oxygen in the metabolism of the waste are much more effective in the degradation of waste rich in lignin content [7].

The average annual agro-waste generated from the largest cultivated crops in Nigeria was estimated to be 12.06 mega-tonnes [8]. The estimates of agricultural waste generation places cassava as the highest, generating 33.7%, followed by yam 26.8% [8] Others include; maize (6.2%), sorghum (4.7%), oil palm (5.7%), rice paddy (3.7%). 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 fermentable sugars [9]. This work was, therefore, designed to evaluate the impact of acid, alkali and microbial hydrolysis on the yields of glucose from selected agricultural wastes.

2 Collection of Samples

2.1 Agricultural Wastes

For a period of one month in February 2020, peels of cassava, yam, and potato, as well as brans of maize, millet, and sorghum were collected from farmers and food vendors in four locations - Ibienafe, Jattu, Iyerekwu, and Auchi - all located in the Etsako West Local Government Area (6° 58'N 6° 18'E) of Edo State, Nigeria. The materials were collected in clean plastic buckets and transported to the laboratory for further processing.

2.2 Processing of Agricultural Wastes

The peel and bran samples were oven-dried at 50°C for 48 hr, ground using motorized blender (model: BL260500W) and sieved (0.2 mm) to have uniform particle size. They were then packed in clean plastic containers labeled; Cassava Peel powder (CPP), Yam Peel Powder (YPP), Potato Peel Powder (PPP), Maize Bran Powder (MaBP), Millet Bran Powder (MiBP), and Sorghum Bran Powder (SBP). All the samples were stored in a refrigerator, prior to further treatment.

2.3 Cow dung sample for isolation of *Bacillus amyloliquefaciens*

Cow dung used for the isolation of *B. amyloliquefaciens* was collected from an abattoir also in Etsako West Local Government Area of Edo State, Nigeria. The cow dung was collected in a sterile specimen bottle with which it was transported to the laboratory for further treatment.

3 Materials and Method

3.1 Hydrolysis of Samples

3.2 Chemical Hydrolysis of Sample. Dilute Sulfuric acid and Sodium Hydroxide was used for this process.

3.2.1 Acid and Alkali Hydrolysis

Five different concentrations of Sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH) were used; 0.01M, 0.05 M, 0.1 M, 0.2 M, and 0.25 M. Ten grams (10 g) of the sample was put into a 250 ml conical flask to which 100 ml of each of H_2SO_4, and NaOH of the various concentrations, was added respectively [10]. The flasks and its contents were placed in an autoclave and the hydrolysis was carried out at 121°C for 1 hour "[11], [12]".

After hydrolysis the samples were allowed to cool and neutralized with NaOH (for acid hydrolysate) and H_2SO_4 (for alkali hydrolysate). The hydrolysates was centrifuged at 6,000 rpm, the supernatant was analyzed for reducing sugar content while the residue was dried and stored for gravimetric analysis of lignin, cellulose and hemicellulose contents [13]. For control, the same process was carried out using distilled water in place of H_2SO_4 and NaOH respectively.

3.3 Microbial Hydrolysis

This was carried out using the isolated *Bacillus amyloliquefaciens*. Fifty grams (50 g) of each of the waste samples was weighed into 500 ml conical flasks and made up to a final volume of 500 ml with distilled water, corked and sterilized at 121°C for 15 min. After cooling, the concentration of the medium was varied from 0.02% to 1.0% with sterile distilled water in 100 ml sterile conical flasks for hydrolysis [21].

The effects of temperature, pH, substrate concentration and duration of hydrolysis on the percentage yield of reducing sugars were investigated. Five substrate concentrations of the 'sample media' were prepared ranging from 0.02% to 1.0%. Hydrolysis was carried out using five different temperatures; 30°C, 35°C, 40°C, 45°C and 50°C. pH values ranging from 5 to 9 were used in this investigation over a period of 48 hours. The sample media for each set-up was inoculated with 1 ml of a 24 hours culture of *B. amyloliquefaciens* in Nutrient agar broth. Each set-up had an un-inoculated flask which served as control. A 10 ml aliquot of fermenting slurry of samples was drawn at interval, over the period of 48 hours for determination of glucose content (mg/ml) using DNS method [14].

3.4 Gravimetric Analysis of Hydrolyzed Samples

3.4.1 Determination of Lignin, Cellulose and Hemicellulose Contents

The amounts of lignin, cellulose and hemicellulose contents in the six agricultural wastes before and after hydrolysis were determined according to [15]. The experimental process involved the use of alcohol-benzene mixture (1:2), 72% H_2SO_4, concentrated HNO_3 (1.50 ml), 95% absolute ethanol, and 5% NaOH.

3.4.2 Determination of percentage glucose content of hydrolyzed samples

DNS method was used to estimate the percentage glucose content for all samples hydrolyzed. Standard graph was plotted by using glucose solution (100µg/ml of working standard). Six (6) test tubes were used. 1 ml of the standard glucose solution was added to tube 1. Into tubes 2, 3, 4 5 and 6 were added 0.8 ml, 0.6 ml, 0.4 ml, 0.2 ml and 0.0 ml of the standard glucose solution respectively. The volume of the tubes was then made up to 1.0 ml with distilled water. To each of these tubes was added 2.0 ml of DNS reagent, shaken properly and placed in a water bath maintained at 90°C for 5 minutes. The tubes were cooled and 7 ml of distilled water added to stabilize the color. The absorbance was measured with a spectrophotometer at 540 nm, with solution in tube 6 taken as blank. The above procedure was repeated for 1.0 ml of extract and 1.0 ml of water for unknown estimation. The sugar contents of sample extracts were calculated by comparing their absorbance at 540 nm with the standard graph. The individual values were taken in duplicates [14]. The percentage saccharification was determined using the equation:

$$\%\text{Saccharification} = \frac{\text{Reducing sugars yield (mg/mL)} \times 0.9 \times 100\%}{\text{initial substrate concentration (mg/mL)}}$$

The factor 0.90 was used to convert polysaccharide to monosaccharide accounting for water uptake during hydrolysis [14].

3.5 Analysis of Data

The various treatments were carried out in duplicates and results expressed as mean ± standard deviation. The results were compared by one-way analysis of variance and Turkey HSD test. Significance was determined at $P \leq 0.05$ (95% confi-

dence level) for acceptance.

4 Results

4.1 Result from Gravimetric Analysis

Results of percentage lignin contents of samples hydrolyzed with sulfuric acid (Table 1) shows a general increase in lignin contents with increasing concentration of acid. Highest range of increase occurred in maize bran powder (MaBP), from 2.36% (in untreated sample) to 45.16% (in sample hydrolyzed with 0.25M H_2SO_4). Lowest increase was observed in sorghum bran powder, ranging from 2.70% to 8.50%, in untreated sample and sample hydrolyzed with 0.2M H_2SO_4 respectively.

Table 1: Percentage (%) lignin content of samples hydrolyzed using sulfuric acid

Sample	Untreated Sample	0.01 M	0.05 M	0.1 M	0.2 M	0.25 M
CPP	16.34±0.19b	20.28±0.13b	22.38±0.36b	23.29±0.13b	25.01±0.09b	24.76±0.04b
YPP	14.34±0.092ab	29.36±0.03c	30.99±0.05c	31.06±0.12bc	33.20±0.21c	32.22±0.18bc
PPP	11.59±0.11ab	26.99±0.12bc	27.58±0.13bc	28.15±0.15bc	30.14±0.26bc	29.28±0.15bc
MaBP	2.36±0.27a	15.31±0.05ab	20.28±0.25b	37.63±0.5c	34.38±0.22c	45.16±0.18d
MiBP	5.32±0.00a	9.93±0.35a	26.04±0.05bc	25.04±0.83b	29.45±0.28bc	24.49±0.26b
SBP	2.70±0.08a	5.47±0.26a	7.36±0.08a	8.07±0.04a	8.50±0.27a	7.70±0.24a

Results are means of duplicate treatments ± standard deviation.
Means followed by the same alphabet column wise indicate that there is no significant difference (p>0.05)
Legend: CPP: cassava peel powder, YPP: yam peel powder, PPP: potato peel powder, MaBP, MiBP & SBP: maize, millet & sorghum bran powders

Percentage lignin contents of samples hydrolyzed with sodium hydroxide (Table 2) show an increase in the lignin content as the concentration of NaOH increased. Highest increase was observed in CPP, with lignin content increasing from 16.34% (in untreated sample) to 46.70% (in sample hydrolyzed with 0.25M NaOH). However, in YPP, percentage lignin content decreased from 14.34% (in untreated sample) to 9.54% (in sample hydrolyzed with 0.25 M NaOH).

Table 2: Percentage lignin content of samples hydrolyzed using sodium hydroxide

Sample	Untreated Sample	0.01 M	0.05 M	0.1 M	0.2 M	0.25 M
CPP	16.34±0.19b	23.50±0.17b	32.83±0.27b	45.01±0.87b	46.70±0.01c	45.94±0.16c
YPP	14.34±0.09ab	10.47±0.03ba	9.97±0.00a	11.70±0.29a	9.98±0.33a	9.54±0.41a
PPP	11.59±0.11ab	21.43±0.22b	20.28±0.26ab	19.01±0.21ab	17.05±0.06b	16.06±0.65b
MaBP	2.36±0.27a	12.56±0.041a	9.70±0.25a	11.04±0.05a	10.15±0.07a	10.34±0.22a
MiBP	5.32±0.00a	11.08±0.19a	12.54±0.44	10.60±0.38a	10.21±0.03a	10.16±0.13a
SBP	2.70±0.08a	11.50±0.39a	9.16±0.05a	9.22±0.10a	9.10±0.27a	9.14±0.30a

Results are means of duplicate treatments ± standard deviation.
Means followed by the same alphabet column wise indicate that there is no significant difference (p>0.05)
Legend: CPP: cassava peel powder, YPP: yam peel powder, PPP: potato peel powder, MaBP, MiBP & SBP: maize, millet & sorghum bran powders.

Results of percentage cellulose contents of samples hydrolyzed with sulfuric acid are presented in Table 3. In most of the samples (CPP, MaBP, MiBP and SBP), there was a slight increase in percentage cellulose content with increasing acid concentration.

Percentage cellulose contents of samples hydrolyzed with sodium hydroxide (Table 4) indicate a general decrease with increasing concentration of NaOH. In CPP, cellulose content reduced from 43.03% in untreated sample to 32.88% in sample hydrolyzed with 0.25 M NaOH. Similarly in YPP and PPP, cellulose contents reduced from 45.11% and 54.34% (in sample hydrolyzed with 0.01M NaOH) to 34.12% and 33.18% (with 0.25 M NaOH) respectively.

Table 3: Percentage (%) cellulose content of samples hydrolyzed using sulfuric acid

Sample	Untreated Sample	0.01 M	0.05 M	0.1 M	0.2 M	0.25 M
CPP	43.03±0.04a	54.11±0.07ab	55.88±0.10b	58.71±0.37b	60.97±0.25b	61.80±0.15b
YPP	38.40±0.32a	45.82±0.03a	41.11±0.12a	38.30±0.36a	34.49±0.17a	32.44±0.25a
PPP	30.75±0.00a	57.00±0.03ab	54.50±0.26ab	48.73±0.16ab	42.27±0.00a	41.97±0.92a
MaBP	45.03±0.07ab	55.26±0.25ab	58.89±0.13b	61.36±0.11bc	62.90±0.05b	67.74±0.39c
MiBP	52.29±0.32bc	61.56±0.33b	65.54±1.39bc	68.16±0.75c	68.95±0.46bc	65.22±0.10bc
SBP	50.89±0.11c	66.70±0.29b	69.09±0.15c	71.72±0.39cd	78.57±0.36cd	77.21±1.43cd

Results are means of duplicate treatments ± standard deviation.
Means followed by the same alphabet column wise indicate that there is no significant difference (p>0.05)
Legend: CPP: cassava peel powder, YPP: yam peel powder, PPP: potato peel powder, MaBP, MiBP & SBP: maize, millet & sorghum bran powders

Table 4: Percentage (%) cellulose content of samples hydrolyzed using Sodium Hydroxide

Sample	Untreated Sample	0.01 M	0.05 M	0.1 M	0.2 M	0.25 M
CPP	43.03±0.04ab	53.60±0.16ab	43.70±0.36a	33.66±0.84a	34.10±0.11a	32.88±0.01ab
YPP	38.40±0.32a	45.11±0.05da	43.99±0.21a	41.09±0.43ab	38.91±0.31ab	34.12±0.66ab
PPP	30.75±0.00a	54.34±0.11ab	43.02±0.07a	40.88±0.31ab	38.82±0.61ab	33.18±0.21ab
MaBP	45.03±0.07ab	48.84±0.38a	56.33±0.01b	42.29±0.32ab	38.72±0.26ab	38.25±0.20b
MiBP	52.29±0.32b	51.23±0.002a	44.48±0.01a	36.93±0.09a	24.66±0.31a	20.27±0.05a
SBP	50.89±0.11b	61.78±0.02b	53.34±0.11ab	50.82±0.72b	48.24±0.07b	36.89±1.01b

Results are means of duplicate treatments ± standard deviation.
Means followed by the same alphabet column wise indicate that there is no significant difference (p>0.05)
Legend: CPP: cassava peel powder, YPP: yam peel powder, PPP: potato peel powder, MaBP, MiBP & SBP: maize, millet & sorghum bran powders

Percentage hemicellulose contents of samples hydrolyzed with sulfuric acid and sodium hydroxide (Table 5 and 6), showed a general decrease with increasing concentrations of acid and alkali. For acid hydrolysis, highest decrease was recorded in SBP, with hemicellulose content reducing from 25.95% (in untreated sample) to 10.71% (in sample hydrolyzed with 0.25 M H_2SO_4).

Table 5: Percentage (%) hemicellulose content of samples hydrolyzed using Sulfuric acid

Sample	Untreated Sample	0.01 M	0.05 M	0.1 M	0.2 M	0.25 M
CPP	40.62±0.24b	23.43±0.30ab	23.03±0.18ab	22.05±0.04b	21.26±0.15ab	20.60±0.38ab
YPP	37.58±0.23ab	25.49±0.012b	24.18±0.16b	23.78±0.33b	22.98±0.55b	21.39±0.04b
PPP	37.87±0.06ab	27.24±0.09b	25.00±0.22b	23.56±0.34b	22.98±0.22b	21.88±0.12b
MaBP	22.60±0.19a	19.41±0.30a	19.01±0.03a	17.01±0.16ab	15.32±0.11ab	14.44±0.22ab
MiBP	21.49±0.09a	18.49±0.01a	16.46±0.17a	14.70±0.41a	12.80±0.32a	12.16±0.05a
SBP	25.95±0.38a	16.51±0.09a	14.24±0.13a	13.90±0.09a	12.01±0.39a	10.71±0.39a

Results are means of duplicate treatments ± standard deviation.
Means followed by the same alphabet column wise indicate that there is no significant difference (p>0.05)
Legend: CPP: cassava peel powder, YPP: yam peel powder, PPP: potato peel powder, MaBP, MiBP & SBP: maize, millet & sorghum bran powders

Table 6: Percentage (%) hemicellulose content of samples hydrolyzed using Sodium hydroxide

Sample	Untreated Sample	0.01 M	0.05 M	0.1 M	0.2 M	0.25 M
CPP	40.62±0.24b	25.32±0.09ab	21.32±0.03b	19.59±0.35b	16.95±0.15b	16.40±0.06b
YPP	37.58±0.23ab	30.53±0.24b	20.95±0.55b	17.28±0.18ab	14.65±0.17ab	12.21±0.06a
PPP	37.87±0.06ab	29.67±0.03b	13.74±1.71a	12.10±0.15a	12.56±0.55a	10.16±0.05a
MaBP	22.60±0.19a	19.30±0.02a	15.37±0.03a	13.84±0.25a	11.41±0.30a	10.49±0.25a
MiBP	21.49±0.09a	18.88±0.11a	16.41±0.29ab	15.92±0.19a	15.11±0.09ab	13.99±0.23ab
SBP	25.95±0.38a	20.28±0.07ab	17.04±0.23ab	14.60±0.05a	13.05±0.05a	12.55±0.34a

Results are means of duplicate treatments ± standard deviation.
Means followed by the same alphabet column wise indicate that there is no significant difference (p>0.05)
Legend: CPP: cassava peel powder, YPP: yam peel powder, PPP: potato peel powder, MaBP, MiBP & SBP: maize, millet & sorghum bran powders

The glucose yield (mg/ml) from the acid and alkali hydrolysis of samples is presented in Table's 7 and 8. All samples showed

increase in glucose yield with increasing acid and alkali concentrations. Highest glucose yield of 104.07 mg/ml, was observed in maize bran powder (M_aBP) hydrolyzed at 0.25 M H_2SO_4.

Table 7: Glucose content (mg/ml) of samples hydrolyzed using Sulfuric acid

Sample	Untreated Sample	0.01 M	0.05 M	0.1 M	0.2 M	0.25 M
CPP	5.41±0.05a	22.23±0.05ab	40.36±0.23b	61.63±0.24b	68.55±0.10ab	71.23±0.11ab
YPP	2.48±0.02a	10.37±0.39a	37.07±0.10ab	48.40±0.16a	60.45±0.27ab	64.27±0.15a
PPP	6.23±0.10a	24.28±0.16b	30.34±0.22a	47.55±0.33a	55.32±0.22a	60.19±0.03a
MaBP	6.93±0.18a	30.24±0.09c	42.78±0.31b	64.75±0.19b	80.55±0.09bc	104.07±0.03c
MiBP	5.39±0.27a	20.62±0.09ab	29.60±0.17a	52.62±0.07ab	72.46±0.25b	88.56±0.13b
SBP	6.09±0.12a	25.61±0.16b	41.42±0.11b	60.64±0.23b	74.44±0.12b	93.56±0.11bc

Results are means of duplicate treatments ± standard deviation.
Means followed by the same alphabet column wise indicate that there is no significant difference (p>0.05)
Legend: CPP: cassava peel powder, YPP: yam peel powder, PPP: potato peel powder, MaBP, MiBP & SBP: maize, millet & sorghum bran powders

Table 8: Glucose content (mg/ml) of samples hydrolyzed using Sodium Hydroxide

Sample	Untreated Sample	0.01 M	0.05 M	0.1 M	0.2 M	0.25 M
CPP	5.41±0.05a	23.50±0.17b	32.83±0.27b	45.02±0.87bc	46.70±0.01b	45.94±0.16b
YPP	2.48±0.02a	11.47±0.35a	12.92±0.00a	13.70±0.29a	17.98±0.33a	17.54±0.41a
PPP	6.23±0.10a	19.48±0.17b	24.34±0.07ab	39.36±0.09b	50.40±0.25bc	55.17±0.04bc
MaBP	6.93±0.18a	19.77±0.21b	25.43±0.20ab	36.27±0.03b	49.21±0.22b	54.45±0.03bc
MiBP	5.39±0.27a	17.28±0.16ab	33.48±0.07b	46.43±0.07bc	51.36±0.11bc	62.40±0.07bc
SBP	6.09±0.12a	19.24±0.13b	27.60±0.00ab	49.56±0.13c	58.44±0.33c	67.34±0.11c

Results are means of duplicate treatments ± standard deviation.
Means followed by the same alphabet column wise indicate that there is no significant difference (p>0.05)
Legend: CPP: cassava peel powder, YPP: yam peel powder, PPP: potato peel powder, MaBP, MiBP & SBP: maize, millet & sorghum bran powders

4.2 Optimization Microbial hydrolysis

Optimization of the microbial hydrolysis of sample using *Bacillus amyloliquefaciens* was carried out using different hydrolytic conditions such as substrate concentrations; 0.02% to 0.1%, pH ranging from 5 to 9, incubation temperature ranging from 30°C to 50°C, and duration of incubation from 6 to 48 hours. The gravimetric results of untreated samples and samples hydrolyzed using *B amyloliquefaciens* is presented in Table 9. There was a general increase in the percentage lignin and cellulose contents for all the samples. This increase may be attributed to increase in microbial population in the hydrolytic broth. The percentage hemicellulose content, however, did not show a marked increase or decrease.

Table 9: Percentage contents of lignin, cellulose and hemicellulose of samples hydrolyzed using B amyloliquefaciens

	Untreated			After Hydrolysis		
Samples	Lignin	Cellulose	Hemicellulose	Lignin	Cellulose	Hemicellulose
CPP	16.34±0.002b	43.03±0.003b	40.62±0.013b	21.39±0.002b	48.01±0.009ab	38.32±0.002b
YPP	14.34±0.013b	35.40±0.005a	37.58±0.009b	19.44±0.004b	40.11±0.001a	38.21±0.007b
PPP	11.59±0.008b	39.78±0.003a	37.87±0.011b	16.36±0.009ab	43.17±0.007a	36.27±0.001b
MaBP	2.36±0.007a	45.03±0.001b	22.60±0.006a	11.12±0.013a	49.83±0.004ab	23.41±0.003a
MiBP	5.32±0.005a	52.29±0.004b	21.49±0.001a	13.02±0.003ab	59.21±0.011b	19.39±0.011a
SBP	2.70±0.011a	59.89±0.007b	25.95±0.005ab	9.22±0.002a	62.28±0.001b	27.15±0.003ab

Results are means of duplicate treatments ± standard deviation.
Means followed by the same alphabet column wise indicate that there is no significant difference (p>0.05)
Legend: CPP: cassava peel powder, YPP: yam peel powder, PPP: potato peel powder, MaBP, MiBP & SBP: maize, millet & sorghum bran powders

Highest glucose yields were obtained after 48 hours of incubation at 1.0% substrate concentrations, and incubation temperatures of 35°C the results shown in Figures 1.

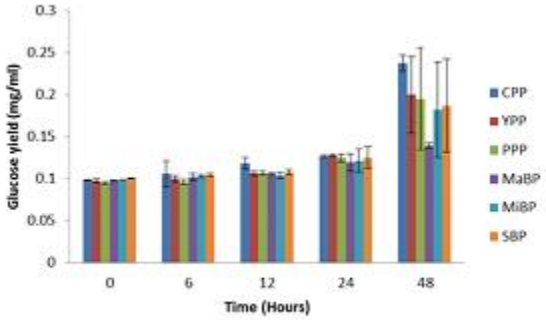

Figure 1: Glucose yield from microbial hydrolysis (Substrate conc. 1%, pH 7, 35°C)

Table 10: Percentage hydrolysis of samples hydrolyzed using acid, alkali and *B amyloliquefaciens*

Sample	Mass of Biomass	Theoretical Glu content (mg)	Glu Yield (mg) Acid Hydrolysis	Glu Yield (mg) Alkali Hydrolysis	Glu Yield (mg) Microbial Hydrolysis	Percentage Hydrolysis Acid (%)	Alkali (%)	Microbial (%)
CPP	1 g	425	71.23	46.70	23.70	15.05	9.86	5.01
YPP	1 g	256	64.27	17.98	20.00	22.42	6.27	6.97
PPP	1 g	623	60.19	55.17	19.50	8.69	7.97	2.81
MaBP	1 g	322	104.07	54.45	13.00	29.09	15.22	3.89
MiBP	1 g	550	88.56	62.40	18.20	14.49	10.21	2.97
SBP	1 g	304	93.56	67.34	16.70	27.09	19.93	5.54

Legend: CPP: cassava peel powder, YPP: yam peel powder, PPP: potato peel powder, MaBP, MiBP & SBP: maize, millet & sorghum bran powders

5 Discusion

Chemical and microbial hydrolysis of six waste samples; peels of cassava (CPP), yam (YPP), potato (PPP) and brans of maize (MaBP), millet (MiBP) and sorghum (SBP), were carried out. Sulfuric acid and sodium hydroxide, at varying temperatures, pH values and duration were used for the chemical hydrolysis, while *Bacillus amyloliquefaciens* was used for the microbial hydrolysis (saccharification).

The result of the gravimetric analysis of the samples (hydrolyzed and untreated) for percentage contents of lignin, cellulose and hemicellulose are presented in Tables 1 to 7. The percentage lignin contents of the samples hydrolyzed with sulfuric acid increased with increasing acid concentration. This may be attributed to acid breaking the matrix structure of the fibre contents of the sample into cellulose, hemicellulose and lignin. The process involves decrystallization of the fibre content and the subsequent hydrolysis to release fermentable sugars. This result is consistent with that of other wokers who reported increase in lignin content with increasing acid concentration and time of hydrolysis of sugarcane bagasse [16]. The authors attributed this increase in lignin content to the summation of lignocellulosic derivatives of lignin; pseudo-lignin with lignin itself. The cellulose contents of the samples hydrolyzed with sulfuric acid also showed slight increase with increasing acid concentration. In CPP, percentage cellulose content increased from 46.75% (as seen in the untreated sample) to 61.80% (in 0.25M H_2SO_4). This trend was also observed in M_aBP, M_iBP and SBP, where cellulose contents increased from 42.28%, 54.31% and 63.84% (as observed in the untreated sample) to 67.74%, 65.22% and 77.21% (at 0.25M, H_2SO_4) respectively. This result is also consistent with the findings of other researchers who reported a slight increase in cellulose contents of lignocellulosic biomass during acid and alkali pretreatments [17].

Hemicellulose contents, generally reduced for all the samples hydrolyzed with sulfuric acid as concentrations increased. This may be attributed to the amorphous nature of hemicellulose and the ease with which it is depolymerized [18].

Alkali hydrolysis of the wastes samples resulted in a reduction in lignin, cellulose and hemicellulose contents as NaOH concentration increased. A number of reasons may be postulated for this occurrence; depolymerization of hemicellulose, breakdown of the lignin-cellulose complex and the depolymerization of cellulose to dimeric and monomeric carbohydrates. Previous workers noted that alkaline hydrolysis has the highest reaction rates followed by acid hydrolysis [19]. Sodium hydroxide treatment of lignocellulosic biomass causes swelling, leading to an increase in in the surface area, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates and disruption of the lignin structure [6].

The observed increase in glucose yield with increasing acid concentrations is consistent with previous work where an increase in reducing sugar yield up to 0.6 M H_2SO_4 was recorded [20]. Glucose yields also increased with increasing alkali concentrations. This may be associated with increased solubility of lignin-cellulose complex, as well as increased available polymers for hydrolysis. The observed lower glucose yield in samples hydrolyzed with sodium hydroxide compared with that of Sulphuric acid, may be due to its fast reaction rate and the possible decomposition of glucose to acid. It has been documented that despite the high rate of alkaline hydrolysis, obtaining high yield of sugar is difficult because mono and dimeric carbohydrates such as glucose and fructose are severely attacked by alkalis to form organic acid [19].

Glucose yield in the microbial hydrolysis increased with rise in incubation temperature, with higher yields between 35°C and 40°C. This may not be unconnected to the optimum temperature growth range for *B amyloliquefaciens*. The decline in glucose yield in incubation temperatures above 40°C, indicate a decrease in growth based on adverse conditions of temperature.

Higher glucose yields at pH 7 than at acid or alkaline pH, indicate *B amyloliquefaciens* prefer neutral pH to acidic or alkaline environment. Improved glucose yields with increase in substrate concentration can be related to increased substrate availability for microbial saccharification. Higher glucose yields with increased incubation time may be due to higher reaction time for microbial hydrolysis. The highest yield of 0.237 mg/ml was observed with CPP with substrate concentration 0.1%, a starting pH 7, incubated at 35°C for 48 hr.

Comparing the percentage hydrolysis of the various samples (Table 10), acid hydrolysis portends to be more efficient, followed by alkali and *B amyloliquefaciens*, respectively. Acid hydrolysis of cotton fibre has been reported to be five times higher than that of *Trichoderma reesei* [20]. They opined that the acid hydrolysis was more efficient due to surface sulfation. They however posited, that based on the energy needs and the environmental hazards of chemical hydrolysis, microbial hydrolysis was still potentially more promising.

6 Conclusion

An overview of the percentage hydrolysis of the various samples depicts acid hydrolysis to be most efficient, followed by alkali and *B amyloliquefaciens*, respectively. The cellulose contents of the samples hydrolyzed with sulfuric acid increased with a corresponding increase in acid concentration. Hemicellulose contents, generally reduced for all the samples hydrolyzed with sulfuric acid as concentrations increased. Alkali hydrolysis of the waste samples resulted in a reduction in lignin, cellulose and hemicellulose contents as sodium hydroxide concentration increased. There was a general increase in the percentage lignin and cellulose contents for all the samples hydrolyzed using *B amyloliquefaciens*, as microbial population in the hydrolytic broth increased. From the study it can be deduced that acid hydrolysis of peels of cassava, yam, potato, as well as brans of maize, millet and sorghum gave a better glucose yield, followed by sodium hydroxide and *B amyloliquefaciens* respectively.

7 References

[1] B. A. Edirin and A. O. Nosa, "A comprehensive re view of biomass resources and biofuel Production potential in Nigeria." *Research Journal in Engineering and Applied Sciences* 1(3) 149-155, 2012.

[2] J. Y.Yoo and S. M. Lee, "Development of bioethanol production process from lignocellulosic biomass using a thermophilic and ethanol-tolerant yeast." *Journal of Microbiology and Biotech nology*, 30(8), 1131–1139, 2020.

[3] Y. Yerima, M. U. Liman, L. G. Hassan, S. Umar, and I. M. Inuwa, "Bioethanol production from agricultural wastes: A review." *Journal of Renewable Materials*, 8(10), 1267-1278, 2020.

[4] M. E. Himmel, S.Y. Ding, and D. K. Johnson, "Bio mass recalcitrance: engineering plants and enzymes for biofuels production," *Science*, vol. 315, no. 5813, pp. 804–807, 2007.

[5] M. Balat, H. Balat, and O. Cahide, "Progress in bio ethanol processing." *Progress Energy Combust Science* 34, 1-23, 2007.

[6] J. S. Tumuluru and A. Kumar " Comparative study of bioethanol production from different lignocrllulosic biomass feedstocks." *Journal of renewable energy,* 147, 416-429. 2020.

[7] A. M. Mshandete, L. Björnsson, A. K. Kivaisi, and B. Mattiasson, "Effect of aerobic pre-treatment on pro-

duction of hydrolases and volatile fatty acids during anaerobic digestion of solid sisal leaf decortications residues." *African Journal of Biochemical Research* 2(5):111-119, 2008.

[8] Oyegoke T., Oyegoke A.F., Fasanya O.O., and Ibrahim A.G. (2023) State of Agro-wastes Management in Ni geria: Status, Implications, and Way Forward In: Waste Recovery and Management An Approach Toward Sustainable Development Goals, pp 241 – 272, CRS Press Taylor and Francis Group, London

[9] P.R. Mohan and O.V.S. Reddy, "Production and Optimization of Ethanol from Pretreated Sugar cane Bagasse using *Saccharomyces bayanus* in Simulta neous Saccharification and Fermentation." *Microbiology Journal*, 2(2): 52–63, 2012

[10] A. A. Olanbiwoninu, and S. A. Odunfa, "Enhancing the Production of Reducing Sugars from Cassava Peels by Pretreatment Methods." *International Journal of Science and Technology*. 2(9) 650 – 652, 2012.

[11] N.A. Amenaghawon, E.A. Okieimen, and O. Salokun, "Statistically Designed Separate Hydrolysis And Fermentation Process for Optimised Ethanol Pro duction from Pumpkin Pod." *FUTA Journal of Re search in Sciences*, 13 (1):1-9, 2017.

[12] Z. Anwar, M. Gulfraz, M.J. Asad, M. Imaran, Z. Akram, S. Mohmood, A. Rehman, P. Anwar, and A. Sadiq. "Bioethanol productions from rice polish by optimization of dilute acid pretreatment and enzy matic hydrolysis." *African Journal of Biotechnology,* 11: 992-998, 2011.

[13] T.T. Jiang, Y. Liang, X. Zhou, Z.W. Shi, and Z.J. Xin, "Optimization of a pretreatment and hydrolysis process for the efficient recovery of recycled sugars and unknown compounds from agricultural sweet sorghum bagasse stem pith solid waste." *Peer Journal* 6: e6186, 2019.

[14] Venkatachalapathy G, Krishnappa R.K.and Sirangala T.G.(2014). Estimation of sugar and bioethanol from different decaying fruits extract. *Advances in Ap plied Science Research*, 2014; 5(1): 106-110

[15] X. Bai, C. Shi, D. Wang, H. Cao, and Y. Liu, "Determi nation of lignin and cellulose content in biomass based on gravimetric method." *Bioresources*, 15(4), 8314-8327, 2020.

[16] D. M. Carvalho and J.L. Colodette, "Comparative study of acid hydrolysis of lignin polysaccharides in biomass." *BioResources* 12 (4): 6907 – 6923, 2017

[17] A. A. Adeolu, and L. David, "Experimental determi nation of the effects of pretreatment on selected Nigerian lignocellulosic biomass in bioethanol pro duction." *Scientific Reports* 11:557, 16pp, 2021. http://creat iveco mmons .org/licen ses/by/4.0/

[18] M. Balat and H. Balat, "Progress in bioethanol pro cessing." *Prog Energy Combust Sci* 4:551-573, 2008.

[19] S.C. Rabelo, R.M. Filho, and A.C. Costa, "A compare son between lime and alkaline hydrogen peroxide pretreatments of sugarcane bagasse for ethanol pro duction". *Appl Biochem Biotechnol,* 144: 87-100.

[20] I.M. Onoh, G.O. Mbah, and F.E. Iloamu zor,"Optimization of Acid Hydrolysis of Sweet Potato Peels to Produce Fermentable Sugar." *Journal of Engineering* 08 (6): 20 – 26, 2018.

[21] S. Prasad, J. Prateck, H.B. Rudrapatra, and V. Nada nathanga, "Preparation and characterization of cellulose nanowhiskers from cotton fibre by con trolled microbial hydrolysis." *Carbohydrate Polymers* 83(1); 122 – 129, 2011.