An Assessment of the Potential of Plantain Peel Ash as a Potash Biocatalyst for Producing Reducing Sugar from *Phoenix dactylifera* Seed Pit

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ABSTRACT

Alkaline process is one of the most efficient pretreatments for hardwoods and agricultural residues; so, demand for green renewable alternatives to inorganic KOH (potassium hydroxide) for hydrolysis of cellulose, production of soap and lubricating grease, becomes imperative. This aim of this study is to assess the potential of plantain peel ash (PPA) as a bioalkali for lignocellulosic pretreatment of *Phoenix dactylifera* (Date palm) seed pit (DPSP) and compare this to synthetic KOH.

The combusted plantain peel ash had K_2O as the dominant oxide (54.2 wt %) using x-ray fluorescence (XRF) spectroscopy, the concentrations of KOH extracted from PPA were quantified titrimetrically using 0.1M HCl. The chemical hydrolysis of DPSP was carryout using Taguchi L9 orthogonal array design of experiment. The factors KOH/BioKOH concentrations (0.022–1.950%), Solid to liquid ratio (1:30-1:90 w/v), treatment times (15–60 min) at 121°C were optimized for the hydrolysis. The study indicated that the yield of reducing sugar from DPSP was 21.95 mg sugar/g substrate at 0.157 % BioKOH, 30 min reaction time and 1:30 w/v solid: liquid ratio; while 29.35 mg sugar/g substrate was generated using inorganic KOH at the same optimized conditions. There was no significant difference in carbohydrate conversion to reducing sugar using BioKOH compared to inorganic KOH. Therefore, the use of green renewable alkaline alternatives for hydrolysis of cellulosic waste to sugars, will increase the supply of sugar substitute for fuel and food.

(Keywords: date palm seed, lignocellulosic, bioalkali, plantain peel, reducing sugars)

INTRODUCTION

The world energy market, worth around 1.5 trillion dollars, is dominated by fossil fuels (Goldemberg, 2006) which are no longer regarded as sustainable, and which much lower availability. Therefore, the quest for sustainable and environmentally friendly energy sources has resulted in renewable feedstocks, such as biomass, that are rich sources of cellulose and hemicellulose.

Lignocelluloses are mainly made up of cellulose, hemicelluloses and lignin; these are bonded by covalent bonds, various intermolecular bridges, and van der Waals' forces thus forming a complex structure, and making them resistant to enzymatic hydrolysis and insoluble in water (O'sullivan, 1997). Therefore, an efficient, less energy intensive, and cost-effective pretreatment method is a necessity for producing fermentable sugars from these feedstocks, at economically viable cost. Pretreatment of biomass is a crucial step to overcome lignocelluloses recalcitrance in the conversion to ethanol.

Date pits have a hard seed coat that makes the seed components difficult to digest. It is necessary to process the seeds before feeding them to livestock (Zaid and Arias-Jiménez, 2002). Alkali treatments increase the digestibility of fibrous materials. Application of a 9.6% NaOH solution to ground date pits decreased neutral detergent fiber (NDF) content and increased *in vitro* digestion rates. This treatment was more effective on finely ground date pits (4 mm *vs.* 8 mm) (Al-Yousef, *et al*., 1986). Nasser *et al.* (2016) reported that the lignocellulosic components of date palm seed pit is: cellulose 32%, hemicellulose 30% and lignin 37%.

Partial degradation of cellulose and hemicellulose is possible during alkali pretreatment. A large number of reactions may take place at elevated temperature in the alkali conditions. The most important reactions are: dissolution of non-degraded polysaccharides; formation of alkali-stable end-groups referred to as peeling reactions of end groups (peeling-off); hydrolysis of glycosidic bonds and acetyl groups and decomposition of dissolved polysaccharides (Fengel and Wegener, 1984; PÉrez and Samain, 2010).

Bio-alkali is the alkali derived from the ashes of burned biomaterials. When agricultural materials are burned in air, the carbohydrates, fats, proteins, and vitamins will volatilize. The resulting ashes contain oxides of the inherent minerals. Some of these are basic oxides of potassium and sodium, which when dissolved in water yield their corresponding hydroxides (alkali). Studies have shown that plantain peel ash can be used to produce soap (Yahaya, et al., 2012), and lubricating grease of good quality. Hence, it is anticipated that alkali produced from the ash of agricultural wastes can serve as potash biocatalyst in the hydrolysis of biomass.

The aim of this work is to determine the optimum conditions for the production of reducing sugar from date palm seed pit (DPSP) by BioKOH compared to the frequently used inorganic KOH hydrolysis at the optimized conditions.

EXPERIMENT

Extraction of Bioalkali (BioKOH)

Exactly 5.0 kg of plantain peels were collected from eateries in Kaduna, Nigeria, in June, 2017, and identified at Narict Bioresources Unit, Zaria, Nigeria. These were washed with deionized water and then dried in an oven at 60ºC, until they were dried enough to be broken and ground by an agate mortar and pestle. The dried peels were then burned inside a combustion pan with portable acetylene gas burner (Oxford, England) for 25 min at 110ºC. The ash obtained was then sieved through a 0.8 mm sieve and then assayed using XRF (X-supreme 8000 Oxford England). Exactly 0.06 kg of the ash obtained was boiled in 1 dm^3 deionized water for one hour at 100°C in a 2 dm^3 beaker using a Digital hotplate with stirrer (Stuart SD162). The slurry was kept for 12 hrs before draining the extract. After draining, the first extract/filtrate was kept in a separate sealed beaker and equal volume of cold deionized water as the volume of the first extract was poured into the slurry for further extraction. This was repeated the third time, and the extracts kept in different beakers. The specific gravity of the alkali and pH using a Hanna equipment (HI 9813-5, England) were measured (Onyegbado, et al., 2002; Onyekwere, 1996). The filtrate/extracts obtained were then quantified titrimetrically against 0.1M HCl for determination of the concentration of BioKOH (Babayemi, et al., 2010, Uyigue, et al., 2013).

Experimental Design

Response surface method (RSM) was used to determine the optimum conditions for the pretreatment of date palm seed pit (DPSP). Taguchi L9 design with three independent variables: reaction time, solid to liquid ratio, and concentration of inorganic KOH/BioKOH shown in Table 1, was applied to maximize the yield of reducing sugar from DPSP. The experimental design created by Minitab 16 software resulted in nine experimental trials

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Table 1: Summary of Experimental Design for Date Palm Seed Pit (DPSP) Hydrolysis.

Factors	Symbol	Range and levels		
Concentration (%w/v)	X_1	1.950	0.157	0.022
Solid:liquid ratio (w:v)	X_2	1:30	1:60	1:90
Reaction time (min)	X_3	15	30	60

Mechanical Treatment of Date Palm Seed Pit

Date palm seed pits (DPSP) were purchased from the local market in Zaria, Nigeria, in June, 2017, and was identified at Narict Bioresources Unit, Zaria, Nigeria. These were washed, air-dried and milled using Christy and Morris Laboratory mill (Chelmsford, England). This was then sieved through a diameter sieve of 0.8 mm and stored in sealed plastic bottle at room temperature in a desiccator.

Alkali/Bioalkali Pretreatment of Date Palm Seed Pit (DPSP)

One gram of the milled DPSP was separately soaked with 30, 60 and 90 cm^3 of 0.022, 0.157

and 1.950 % (w/v) bio-alkali that was extracted and quantified titrimetrically. Another set (for comparison) of one gram ground DPSP was soaked with inorganic KOH solution of equal concentrations, respectively, in a 250 cm^3 Erlenmeyer flask, and then autoclaved in a Dixons Surgical Instrument, Ltd, Switzerland at a temperature of 121°C for 15, 30, and 60 min in accordance with the Taguchi L9 experimental design shown in Table 6.

After the hydrolysis, the hydrolysate was separated using filter paper (Sharma, et al., 2013, Sukri, et al., 2014, Thanapimmetha, et al., 2011). Fourier transform infrared spectroscopy using FTIR 8400s Shimadzu, Japan, was carried out to determine the active groups in the hydrolysate. The total reducing sugars in the filtrate was assessed by the 3,5-dinitrosalicylic acid (DNS) method described by Miller (1959).

Statistical Analysis

Microsoft Excel 2016 at 95% confidence interval was used to evaluate the significant difference resulting from the contribution of each parameter to the production of reducing sugar.

RESULTS AND DISCUSSION

From the study, by ashing 5.0 kg of the plantain peels, 0.47 kg of ash was obtained which is 9.4% of the total plantain peel. There is close to report by Uyigue, et al. (2013) where 7.33 % ash content was obtained from 3.43 kg of empty-oil-palm-bunch. The pH values for the Bioalkali were 12.0, 11.2, and 10.2 for the first, second, and third extract, respectively; this confirms alkali production from plantain peel ash, and is in conformity to the work of Olabanji et al. (2012), who reported pH 12.88 for plantain peel ash solution. Based on this value, equal concentrations of corresponding inorganic alkali (KOH) was prepared having pH values of 13.0, 12.2, and 11.3, respectively. The variation in the pH values may be due to determinate errors in weighing measurements. The effect of increased pH on biomass lies on delignification and high yield of monosaccharide (Pedersen, et al., 2011).

The specific gravity values for Bioalkali for the 1st, 2nd and 3rd extract was 1.050, 1.010 and 0.900 mS/cm respectively. While that for the inorganic alkali (KOH) was 1.020, 1.000 and 0.900 mS/cm respectively. The actual concentration of potash (i.e., KOH) in the plantain peel ash extracts measured using 0.1M HCl gave mean volume of HCl used as 87.07, 7.03 and 0.97 cm^3 for the first, second, and third extract, respectively. This implies potash-alkali concentrations of 19.5 g/dm^3, 1.57g/dm^3 and 0.22 g/dm^3, respectively, for the three extracts (Tables 2 and 3).

Table 2: Physicochemical Parameters of Bioalkali

Parameters	1st Ext.	2ndExt.	3rd Ext.
pH	12.0	11.2	10.2
Spec. gravity	1.050	1.010	0.900
Cond. (mS/cm)	11.13	7.65	1.13
KOH (g/L)	19.50	1.57	0.216
KOH(%)	1.950	0.157	0.022

Table 3: Physicochemical Parameters of Inorganic Alkali.

Parameters	1st Sol.	2nd Sol.	3rd Sol.
pH	13.0	12.2	11.3
Spec. gravity	1.02	1.000	0.900
Cond. (mS/cm)	11.17	5.67	0.93
KOH (g/L)	19.50	1.57	0.216
KOH(%)	1.950	0.157	0.022

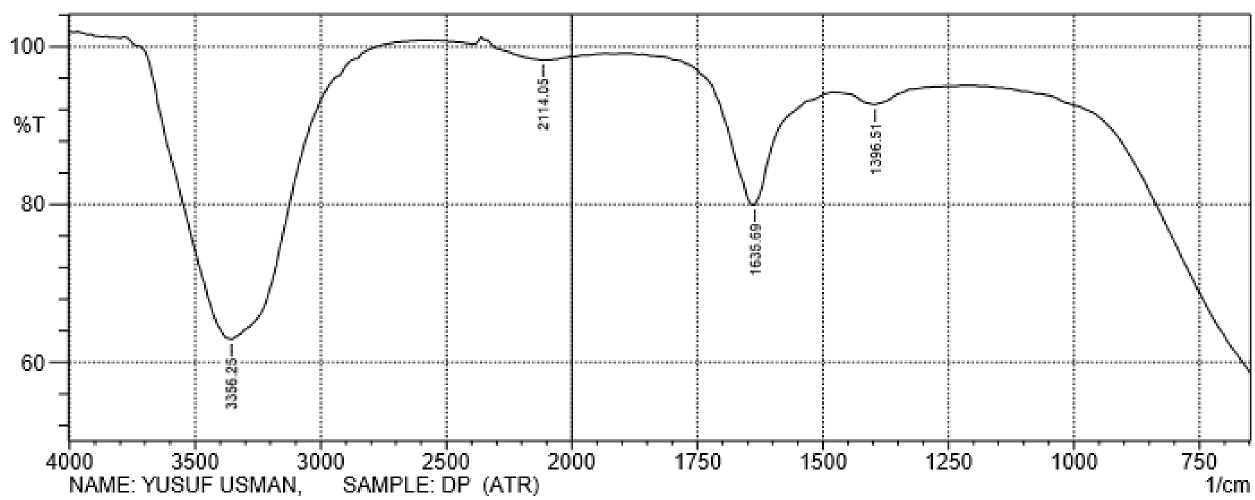
Low potash-alkali concentrations of these kind produce high yield of reducing sugar as asserted by Sharma, et al., 2013. This is more evident in Table 4, showing the oxides contained in the plantain peel ash. The major oxides present ranged from 0.0468 to 54.1881 wt%. The wt % for the oxides are in the ranking K (54.19) > Si (11.58) > P (10.49) > Ca (6.96) > Cl (5.08) > Mg (4.80) > S (3.30) > Al (2.77) > Fe (0.46) > Mn (0.17) > Zn (0.09) > Ti (0.07) > Sr (0.05) > Na (0.0) and Cr (0.0). The result of this study has slight disorder, and so confirms to the metal oxide arrangement in the work of Olabanji et al. (2012). Amongst other impurities, the oxide of potassium has the highest percentage of 54 wt % in the ash. So it serves as a viable source of potash biocatalyst for DPSP hydrolysis.

Table 4: X-Ray Flourescence Spectroscopy Analysis of Burnt Plantain Peel Ash.

Element	Conc (wt%)	Element	Conc (wt%)
Na_2O	0.0000	CaO	6.9613
MgO	4.8035	TiO_2	0.0726
Al_2O_3	2.7684	Cr_2O_3	0.0000
SiO_2	11.5773	Mn_2O_3	0.1689
P_2O_5	10.4901	Fe_2O_3	0.4582
SO_3	3.2959	ZnO	0.0874
Cl	5.0814	SrO	0.0468
K_2O	54.1881		

FTIR Spectra Analysis

As shown in Figure 1, It is evident from the spectra of DPSP hydrolysate using inorganic KOH and BioKOH, that reducing sugar was synthesized with bands at 3356, 1635, 1396 cm^{-1} and 3387, 1643, 1388, 1080 cm^{-1}, respectively, which indicates the presence of OH stretching vibration in OH functional groups, C=O stretching was also pronounced which serves as reducing sugar active group, CH_2 bending vibration in alkanes, C-O stretching representing pyranose ring skeletal vibration in ethers. (Table 5)



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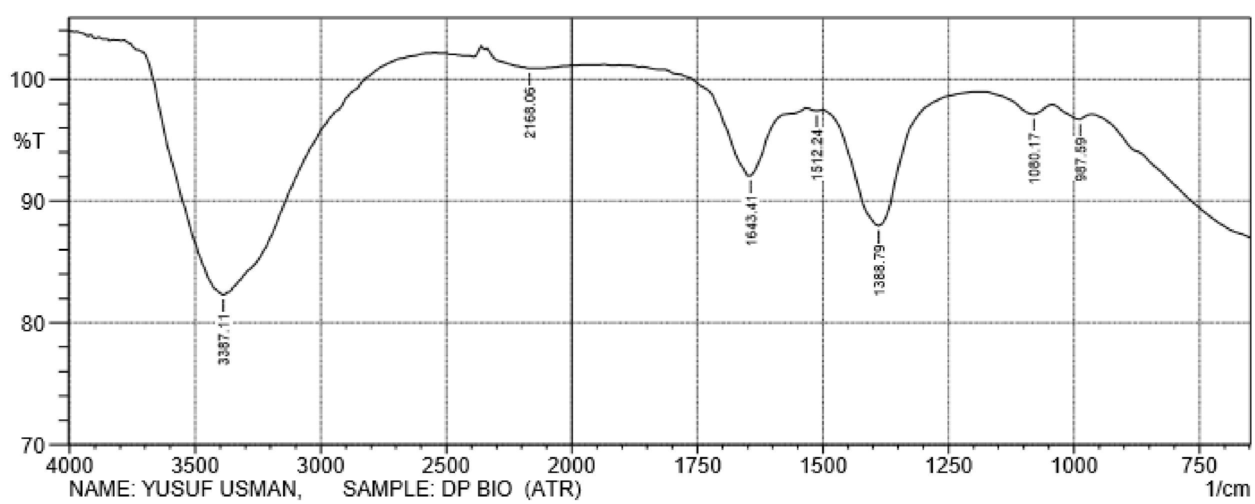

Figure. 1: FTIR Spectrogram of DPSP Hydrolysate for (A) Inorganic KOH and (B) BioKOH Hydrolysis.

Table 5: Infrared Spectra Analysis of DPSP Hydrolysate.

Sample Region (cm^{-1})			Assigned to
DPSP (BioKOH)	DPSP (Inorganic KOH)		
1080	------	m-s	C-O (C-6 skeletal vibrations/ Pyranose ring skeletal vibration
1388	1396	m	CH_3C-H (Bending vibration in Alkane)
1643	1635	m-s	C=O (Stretching)
3387	3356	s-broad	O-H (stretching vibration)

Key: M- medium, S – strong

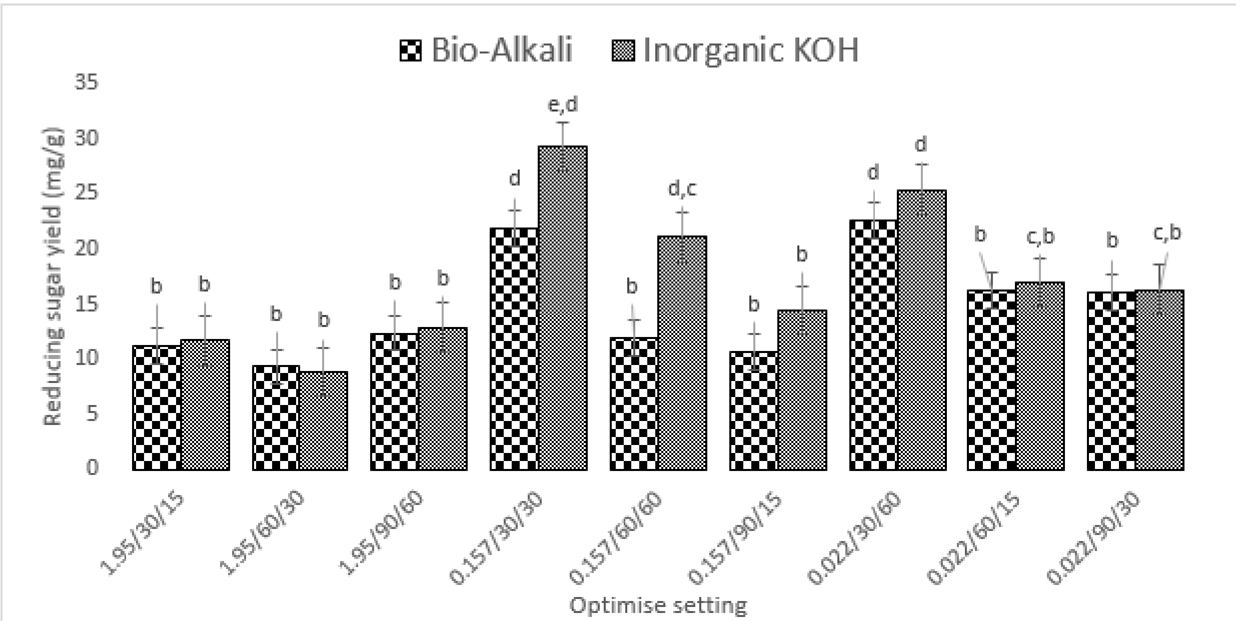

Figure 2: Reducing Sugar Yield of DPSP Pretreated with BioKOH and Inorganic KOH.

*Mean values of experimental runs between BioKOH and inorganic KOH pretreatment, sharing the same letters are non-significantly different at 95% confidence interval.

Alkali and Bioalkali Pretreatment of Date Palm Seed Pit (DPSP)

From the study, the yield of reducing sugar was high at pretreatment conditions 0.157 % (w/v) inorganic KOH/BioKOH, 1:30 cm^3 (w/v) solid: liquid ratio, and 30 mins reaction time. There was no significant difference between the use of Bio-KOH and inorganic KOH for hydrolysis of DPSP.

The nine experiments were designed as shown in Table 6. From the study, the yield of reducing sugar ranges between 9.28 and 22.56 mg/g and 8.83 and 29.35 mg sugar/ g substrate for Bioalkali and inorganic alkali, respectively. By varying concentration of the alkalis, solid: liquid ratio and reaction time, the maximum reducing sugar yield at the optimum conditions was 21.95 and 29.35 mg sugar/g substrate using BioKOH and inorganic KOH respectively as observed at the experimental run number 4. This clearly shows that there was a decrease in the reducing sugar yield at increased pretreatment conditions for the depolymerization of carbohydrate (cellulose and hemicellulose) (Figure 2). This is so, because lesser energy was used at a reaction time of 30 min, low liquid volume and appreciably low inorganic KOH and Bioalkali concentrations.

CONCLUSIONS

The estimation of reducing sugar by the pretreatment of lignocellulosic biomass (cellulose and hemicellulose) using inorganic KOH and BioKOH was achieved through a statistical Taguchi L9 orthogonal array Design.

Table 6: Design Matrix of Taguchi L9 Array and Experimental Results Obtained for Bio alkali and Inorganic KOH Hydrolysis of Date Palm Seed Pit.

Runs	Conc (%)	Factor Settings		Reducing Sugar	Yield (mg/g)
		Solid:liquid Ratio (w/v)	Reaction Time (min)	BioKOH	Inorg. KOH
1	1.950	30	15	11.25 ±0.61	11.68 ±1.29
2	1.950	60	30	9.28 ±3.82	8.83 ±0.38
3	1.950	90	60	12.36 ±2.46	12.90 ±2.44
4	0.157	30	30	21.95 ±0.82	29.35 ±0.17
5	0.157	60	60	11.95 ±0.35	21.09 ±3.86
6	0.157	90	15	10.59 ±3.93	14.46 ±3.06
7	0.022	30	60	22.56 ±0.83	25.41 ±1.02
8	0.022	60	15	16.34 ±2.15	17.02 ±0.83
9	0.022	90	30	16.09 ±5.08	16.36 ±1.84

*Applying IBM SPSS statistics 20, each value for Red. Sugar yield using BioKOH & InorganicKOH is an average of 3 trials and the standard deviations given.

The result indicated that the optimal conditions for the production of reducing sugar from pretreated date palm seed pit by inorganic KOH and BioKOH hydrolysis was 0.157 % (v/v) of inorganic KOH and BioKOH, solid to liquid ratio of 1:30 (w/v) and reaction time of 30 min; which gave maximum reducing sugar retention of 21.95 and 29.33 mg sugar/g substrate for BioKOH and inorganic KOH respectively.

Also, the study indicated that there was no statistically significant difference between the hydrolysis of DPSP using BioKOH compared to inorganic KOH. Therefore, the study provides scientific data relevant for ascertaining the stoichiometric parameters for pretreatment of DPSP using alkali obtained from plantain peel ash prior to production of reducing sugar. Therefore, plantain ash can serve as Bioalkaline, and can be scaled up for other industrial benefits.

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