Moroccan Journal of Chemistry
ISSN: 2351-812X
http://revues.imist.ma/?journal=morjchem&page=login
Omoniyi & al. / Mor. J. Chem. 7 N°1 (2019) 073-081

An assessment of the potential of plantain peel ash as a potash biocatalyst for producing reducing sugar from *Phoenix dactylifera* seed pit

Kehinde I. Omoniyi[a], Nsidibeabasi C. Nwokem[a], Yusuf O. Usman[b*] and Olanipekun O. Idowu[b]

[a]*Chemistry Department, Faculty of Physical Sciences, Ahmadu Bello University, Zaria, Nigeria.*
[b]*Basic Research Department, National Research Institute for Chemical Technology, Zaria, Nigeria.*

** Corresponding author:*
yusufusman1250@yahoo.com
Received 21 May 2018,
Revised 10 March 2018,
Accepted 10 March 2018

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 become imperative. The 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) compared to synthetic KOH. From the study, ashing 5.0 kg of plantain peels gave 9.4% ash. 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 carried out 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 time (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 29.35mg sugar/g substrate was generated using inorganic KOH at the same optimized conditions. There was no significant difference in the carbohydrate content of DPSP conversion to reducing sugar using BioKOH compared to inorganic KOH. Therefore, the use of renewable alkaline for hydrolysis of cellulosic waste to sugars, will increase the supply of sugar substitute for biofuel production, reduce cost of alkali and checkmate environmental hazard.

Keywords: Cellulose; date palm seed pit; hydrolysis; plantain peel ash, reducing sugar.

1.Introduction

The world energy market worth around 1.5 trillion dollars is dominated by fossil fuels [1], which are no longer regarded sustainable, with much lower availability. Therefore, the quest for sustainable and environmental friendly energy source has resulted to renewable feedstock, 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 it resistant to enzymatic hydrolysis and are insoluble in water [2]. Therefore, an efficient, less energy intensive and viable cost effective pretreatment method is a necessity for producing fermentable sugar. Pretreatment of biomass is a crucial step to overcome lignocellulose recalcitrant 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 [3]. Alkali treatments increase the digestibility of fibrous materials. Application of a 9.6% NaOH solution to ground date pits decreased neutral detergent fibre (NDF) content and increased *in vitro* digestion rates. This treatment was more effective on finely ground date pits (4 mm *vs.* 8 mm) [4]. Nasser *et al.* (2016) [5] reported the lignocellulosic components of date palm seed pit as: 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 [6,7]. Bio-alkali is the alkali derived from the ashes of burnt biomaterials. When agricultural materials are burnt 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. Studies have shown that plantain peel ash can be used to produce soap [8], 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. Therefore, there is need to explore renewable alkaline alternatives for hydrolysis of cellulosic wastes to sugars, for fuel and food use.

2. Materials and Methods

All Chemicals used are of analytical grade.

Extraction of Bioalkali (BioKOH)

Exactly 5.0 kg of plantain peels (*Musa parasidiaca*) were collected from eateries in Kaduna, Nigeria, in June, 2017, and identified at National Research Institute for Chemical Technology, NARICT Bioresources Unit, Zaria, Nigeria. These were washed with deionized water and then dried in an oven at 60^{o}C, until they were dried enough to be broken by hands; this was then ground by an agate mortar and pestle. The ground peel was then burnt inside a combustion pan with portable acetylene gas burner (Oxford, England) for 25 min and then placed in a muffle furnace (EF1 No F4191 Vecstar Ltd, UK) at 500^{o}C. 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 L deionised water for one hour at 100°C in a 2 L beaker, using a Digital hot plate stirrer (Stuart SD162). The slurry obtained was kept for 12 h 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 then measured [9.10].

Potash-Alkali Contents of the Bio-Alkali Extracted from Plantain Peel Ash

The concentration of hydroxide ion in the filtrate/extract of the plantain peel ash was carried out titrimetrically, by measuring 25 mL of potash solution (filtrate) with a pipette into a 250 mL conical flask, three drops of phenolphthalein indicator was added, which turned the colour of the potash solution to pink. Then 0.1M HCl solution was used as the titrant against the potash solution (25 mL) at 2 to 3 mL drops at a time with the observance of a short drainage time between the acid drops, until a colourless solution was obtained at endpoint. This procedure was carried out in triplicate and the average titre value of HCl was recorded as V_1 (11.12) The actual concentration of the caustic potash present in the hydrolysate was measured based on the following equation:

$$KOH + HCl \rightarrow KCl + H_2O$$

The concentration of KOH was measured based on equation below,

$$C_{KOH} = (0.1 \times {}^{V_1}/_{25}) \times 56$$

Where: C_{KOH} = concentration of KOH, g/dm^3; and V_1 = average titre value of HCl for KOH neutralization, cm^3

Experimental Design

Response surface method (RSM) was used to determine the optimum conditions for the pretreatment of date palm seed pit (DPSP). Taguchi L9 orthogonal array 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. Microsoft Excel 2016 at 95% confidence level was used to evaluate the significant difference resulting from the contribution of each parameter to the production of reducing sugar.

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

		Range and levels		
Factors	Symbol			
Concentration (%v/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

The fruits of *Phoenix dactylifera* were purchased from the local market in Zaria, Kaduna in June 2017and identified at National Research Institute for Chemical Technology, Bioresources Unit, Zaria, Nigeria. The fleshy part was removed with knife. The date seeds were washed, dried in the sun for two days and milled using a size 8-inch Laboratory mill (40.101m, Christy and Morris Ltd, Chelmsford England). This was then sieved through a diameter sieve of 0.8 mm, and stored in sealed plastic bottle inserted into a dessicator at room temperature, prior to use.

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

One gramme of the milled DPSP was separately soaked with 30, 60 and 90 mL of 0.022, 0.157 and 1.950% (w/v) bio-alkali that was extracted and quantified titrimetrically. Another set of one gramme ground DPSP was soaked with inorganic KOH solution of equal concentrations respectively in a 250 mL Erlenmeyer flask, and then autoclaved (Dixons Surgical Instrument, Ltd, Switzerland) at a temperature of 121^{o}C for 15, 30 and 60 min. This reaction process was optimized using the Taguchi L9 orthogonal array design shown in Table 6. After the hydrolysis, the hydrolysate was separated using Whatman 125 mm filter paper [13,14,15]

Fourier Transform Infrared Spectral Characteristics of SPSC and DPSP

The infrared spectra of the hydrolysate of DPSP was measured with Attenuated Total Reflectance (ATR) Technology on a Fourier Transform Infrared Spectrophotometer (FTIR-8400S, Shimadzu, Japan) at NARICT, Zaria, Nigeria.
FTIR was carried out to determine/confirm the active groups in the hydrolysate.

Determination of Reducing Sugar by Dinitrosalicylic Acid Method (DNS Method)

A glucose standard curve was prepared by dissolving 100 mg of D-glucose in 100 mL of distilled water. From the working solution, 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL and 1.0 mL were collected and made up to 1.0 mL with distilled water in a volumetric flask, thus giving concentrations 0.2 mg/mL 0.4 mg/ mL 0.6 mg/mL, 0.8 mg/ mL and 1.0 mg/ mL respectively and 2.0 mL of DNS was added. The mixtures were then boiled for 5 minutes, cooled and made up to 10 mL with distilled water. After the homogenization of the reaction mixture, the absorbance was determined at a wavelength 540 nm with a HACH spectrophotometer (DR-2400 USA). The relationship between the glucose concentration and absorbance at 540 nm was plotted to determine the glucose standard curve. Figure 1.

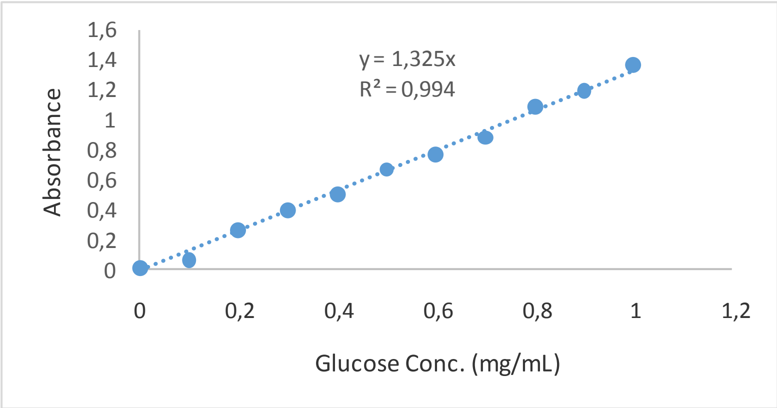

Fig.1. Calibration Curve of glucose for determination of Cellulose

The concentration of reducing sugar present in the hydrolysed sample of DPSP was determined by adding 2 mL of DNS acid to 0.5 mL of each of the hydrolysed samples and boiled for 5 min in a Water bath Shaker (Stuart SBS40, UK), then the solution was made up to 10 cm^3 with deionised water. The absorbance of each of the sample mixture was determined at 540 nm using a HACH portable spectrophotometer (DR-2400, USA). Thus, the concentration values was extrapolated from the glucose standard curve [16].

Statistical Analysis

Student s' t-test was used to compare the significant difference between the use of Bio-KOH and inorganic KOH for the hydrolysis of DPSP, with significant difference considered at $P < 0.05$.

3.Results and Discussion

Bioalkali Extract and Quantification

From the study, by ashing 5.0 kg of the plantain peels, 0.47 kg of ash was obtained, which implies a percentage yield of 9.4%l. There is close to report by Uyigue, et al. (2013) [12] 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 with the work of Olabanji, et al. (2012) [17], who reported pH 12.88 for plantain peel ash solution. Based on this values, 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 [18]. **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

The specific gravity values for Bioalkali in the 1st, 2nd and 3rd extract was 1.050, 1.010 and 0.900 mS/cm respectively. While that for the inorganic 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 (Table 2 and 3). 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 54.1881wt%. 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). It was observed that with slight dissimilarity, the order of the metal oxide is in agreement with the work of Olabanji et al. (2012) [17]. 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		

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 min reaction time. There was no significant difference between the use of Bio-KOH and inorganic KOH for hydrolysis of Date palm seed pit (DPSP).

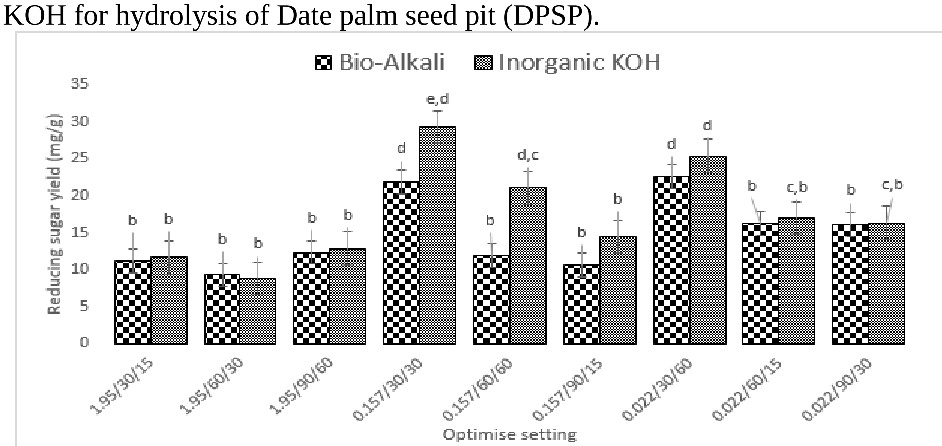

Figure 3: Reducing Sugar Yield of DPSP Pretreated with BioKOH and Inorganic KOH

*Mean values of experimental runs between BioKOH and inorganicKOH pretreatment, sharing the same letters are non-significantly different at 95% confidence interval. 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 3). It was observed that at lower concentrations of the Bioalkali and inorganic KOH, the yield of reducing sugar was high and this is similar to the findings of Sharma *et al.* (2013) [13] where switch grass was treated with 0.5% KOH at 21°C after 12 h.

FTIR Spectra

As shown in Fig. 2, it is evident from the spectra of DPSP hydrolysate using inorganic KOH and BioKOH, that reducing sugar was synthesized with broad bands assignment at 3356, 1635, 1396 cm^{-1} and 3387, 1643, 1388, 1080 cm^{-1} respectively, which indicates the presence of H-bonded O-H stretching vibrations of hydroxyl 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).

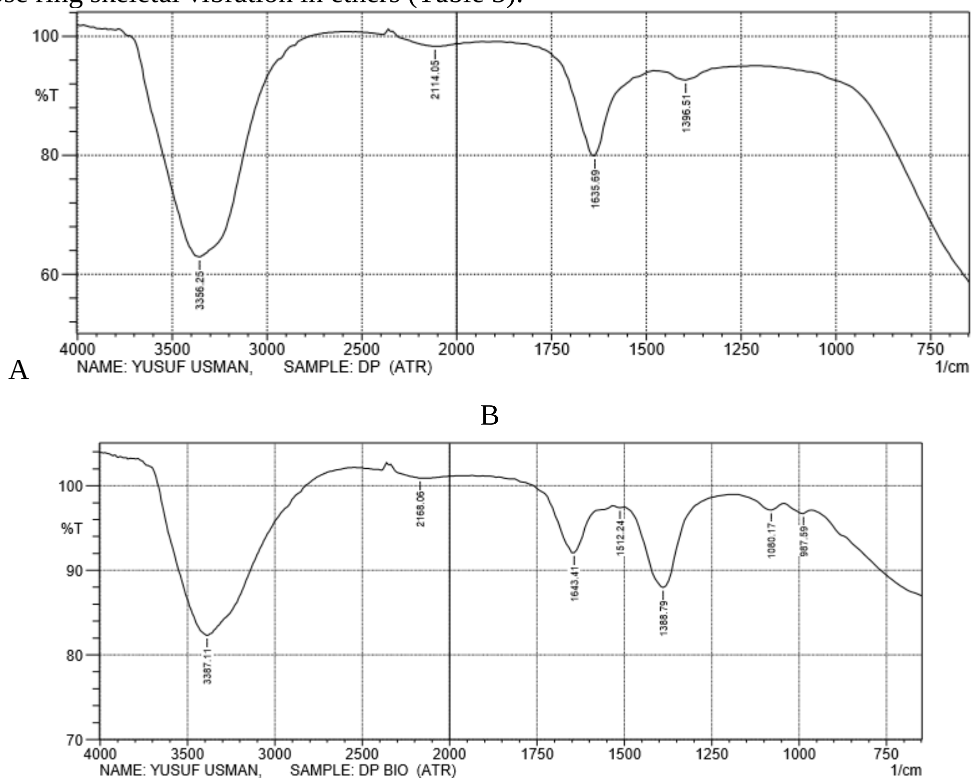

Fig. 2. FTIR spectrogram of DPSP Hydrolysate for (A) Inorganic KOH and (B) BioKOH Hydrolysate

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)

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

	Factor Settings			Reducing Sugar	Yield (mg/g)
Runs	Conc (%)	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 Minitab 16 statistics software, each value for Red. Sugar yield using BioKOH and Inorganic KOH is an average of 3 trials and the standard deviations given.

4. Conclusion

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

References

1. J. Goldemberg, The ethanol program in Brazil. Environmental Research Letters. 1(2006) 014008, 2006.

2. A. C. O'sullivan, Cellulose: the structure slowly unravels. Cellulose. 4 (1997) 173-207.

3. A. Zaid, E. Arias-Jiménez, Date palm cultivation. FAO: Rome (2002).

4. Y. Al-Yousef, R. Belyea and J. Vandepopuliere, Sodium hydroxide treatment of date pits. In: Proceedings of the Second Symposium on the Date Palm in Saudi Arabia. (1986) 197-206.

5. A. Nasser, M. Z. Salem, S. Hiziroglu, H. A. Al-Mefarrej, A. S. Mohareb, M. Alam and I. M. Aref. Chemical analysis

of different parts of date palm (*Phoenix dactylifera L.*) using ultimate, proximate and thermo-gravimetric techniques for energy production. Energies. 9 (2016) 374.

6. D. Fengel, G. Wegener, Wood: chemistry, ultrastructure, reactions. Walter de Gruyter. 613 (1984 1960-1982.

7. S. PÉrez, D. Samain, Structure and engineering of celluloses. Advances in carbohydrate chemistry and biochemistry. 64 (2010) 25-116.

8. L. Yahaya, A. Ajao, C. Jayeola, R. Igbinadolor, F. Mokwunye. Soap production from agricultural residues-a comparative study. American J. Chem. 2(1) (2012) 7-10.

9. C. E. Onyegbado, E. Iyagba and O. Offor. Solid soap production using plantain peel ash as source of alkali. J. Applied Sc. Environ. Manag. 6 (2002) 73-77.

10. C. Onyekwere, Cassava peels ash: An alternative source of alkali in soap production . B. Eng. Thesis Department of Chemical Engineering, University of Port Harcourt. (1996).

11. J. O. Babayemi, K. T. Dauda, D. O. Nwude, J. A. Ajiboye, E. R. Essien, O. O. Abiona, Determination of potash alkali and metal contents of ashes obtained from peels of some varieties of Nigeria Grown Musa species. BioResources 5 (2010) 1384-1392.

12. L. Uyigue, E. Viele, F. Chukwuma, A Preliminary assessment of the potash biocatalyst potential of Empty-Oil-Palm-Bunch (EOPB) residues for biodiesel production. Journal of Emerging Trends in Eng. Applied Sc. (JETEAS) 4 (2013) 446-450.

13. R. Sharma, V. Palled, R. R. Sharma-Shivappa, J. Osborne, Potential of potassium hydroxide pretreatment of switchgrass for fermentable sugar production. Applied Biochem. Biotechn. 169 (2013) 761-772.

14. S. S. M Sukri, R. A. Rahman, R. M. Illias, H. Yaako, Optimization of alkaline pretreatment conditions of oil palm fronds in improving the lignocelluloses contents for reducing sugar production. *Rom Biotechnol Lett*. 19 (2014.):9006-9018.

15. A. Thanapimmetha, K. Vuttibunchon, M. Saisriyoot, P. Srinophakun, Chemical and microbial hydrolysis of sweet sorghum bagasse for ethanol production. In: World Renewable Energy Congress - Sweden; 8-13 May; 2011; Linköping; Sweden. Linköping University Electronic Press. (2011)_389-396.

16. G. L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chem. 31 (1959. 426-428.

17. I. O Olabanji, E. A. Oluyemi, O. S. Ajayi, Metal analyses of ash derived alkalis from banana and plantain peels (Musa spp.) in soap making. African J. Biotechn. 11 (2012) 16512-16518.

18. M. K. Pedersen,. S. Johansen, A. S. Meyer, Low temperature lignocellulose pretreatment: effects and interactions of pretreatment pH are critical for maximizing enzymatic monosaccharide yields from wheat straw. Biotechnology for Biofuels. 4 (2011) 11.