INTERNATIONAL JOURNAL of RENEWABLE ENERGY RESEARCH
T.A.Shah et al., Vol.8, No.3, September, 2018

Effect of Alkali Pretreatment on Lignocellulosic Waste Biomass for Biogas Production

Tawaf Ali Shah*[+], Shehbaz Ali*[+], Asifa Afzal*[+], Romana Tabassum*[+][‡]

* National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan
[+] Pakistan Institute of Engineering and Applied Science (PIEAS), Islamabad, Pakistan
[‡]Corresponding author: Romana Tabassum
(tawafbiotech@yahoo.com, romanatabassum@yahoo.com)

[‡] Postal address, National Institute for Biotechnology and Genetic Engineering (NIBGE), Jhang road
3800, Faisalabad, Pakistan Tel: +923444092987,

Received: 21.03.2018 Accepted:14.05.2018

Abstract- Converting lignocellulosic waste biomass into biogas is a multi-step process; the rate limiting reaction is lignin removal. The objective of the current study was to evaluate alkali treatment for lignin removal and potential of biogas yield of pretreated waste biomass compared to untreated one. Three alkali reagents at various dosages: NaOH (1,2,3, and 5%), KOH (1,2,3, and 5%), and $Ca(OH)_2$ (0.5%) were tested at three different heating processes, water bath, autoclave and short time microwave. NaOH with short time microwave heating process had the highest delignification of 70-86% compares to other alkalies and heating processes. However, an opposite effect of high alkali dosage was observed on holocellulose. The highest cumulative biogas of 560 mL/gVS was obtained from 2% NaOH pretreated wheat straw, which was 2-times higher than the cumulative biogas produced from the same untreated substrates. In the present study, 2% NaOH alkali and microwave heating are determined optimum for lignin removal from waste biomass and enhancement in biogas production.

Keywords Anaerobic digestion; alkaline pre-treatment; biogas; delignification; lignocellulosic waste.

1. Nomenclature

MW=Microwave, AUTO= Autoclave, WT= Water bath

NmL/gVS= Normalized biogas in mL per gram volatile solid, CH_4 = Methane

OBA= Online Biogas App, AD = anaerobic digestion, MC= Moisture content, TS= Total solid, VS= Volatile solid.

2. Introduction

Fossil fuels are continuously depleting and their consumption releases greenhouse gases that raise environmental problems. Therefore, interest is developed in looking for alternative energy. Biogas production from lignocellulosic biomass (LB) through anaerobic digestion (AD) is focused on sustainable bioenergy production process [1]. The agriculture waste residue is composed of lignin, hemicellulose, cellulose, and some extractable components. The percentage of these components varies among crop residue, but in general, hardwood biomass contains 40-50% cellulose, 15-25% lignin, 22-35% hemi-cellulose, and 2-7% extractives, whereas softwood biomass contains 20-31% lignin, 24-32% hemicellulose, 40-45% cellulose, and 1-7% extractives [2, 3]. Cellulose is homopolysaccharide chains of glucose units. Hemicellulose is heteropolymers of pentose sugars that is the outer surface layer of the biomass cell wall [4]. Lignin is the most complex hydrocarbon polymer and contains multiple phenylpropane units, crosslinking of these phenylpropane units and hydrophobic nature of lignin make the LB structure more resistant to microbial and enzyme degradation [5]. The degradation resistance of lignin significantly decreases the yield of biogas in AD process [6]. One of the initial rate-limiting steps is to choose a suitable pretreatment method to remove lignin and make the cellulose accessible to hydrolytic enzymes [7, 8]. The second concern is contingent on testing of high concentration of chemicals in biomass pretreatment. Several methods, steam explosion, hydrothermal process, acid treatment, alkalies treatment, ammonia fiber explosion etc, have been reported for biomass pretreatment with low and high solid concentration [9-14].

For lignin removal among all of the listed methods and many others, the preferred selection is alkaline pretreatment method. The lignin can be released by different alkalies, especially, KOH, $Ca(OH)_2$ and NaOH [15]. Treatment of waste biomass with these alkalies decreased the degree of polymerization, remove lignin, and make cellulose more accessible to enzymatic and microbial degradation. However, the high concentration of alkalies increase the cost of treatment than it produces energy and generate volatile fatty acid (VFA) that inhibit digestion process [16]. Therefore, selection of optimum pretreatment method is the crucial step for improving anaerobic digestion from waste biomass for increasing biogas production [17]. Although there are wide-range of reports on the treatment methods, none of the studies has compared the different heating conditions and alkalies reagents on various substrates for an optimum and effective method, the current study emphasized to test different concentration of alkalies coupled with thermal heating for delignification and evaluate the effect on the biogas and methane yield through anaerobic digestion of the pretreated and untreated biomass.

3. Methodology

3.1. Materials and Methods

Chemicals and materials were purchased from Merck group of Chemical company and Fisher Scientific company. A total of 10 different substrates, wheat straw, kallar grass, para grass, paper wastes, almond shell, peanut shell, corn cob, rice straw, bagasse, and pulses peel were collected at National Institute for Biotechnology and Genetic Engineering (NIBGE) Pakistan. The substrates were ground to 20 mm mesh size and stored at room temperature in polyethylene bags. Volatile solid (VS), total solid (TS), ash, moisture, hemicellulose, lignin, and cellulose were measured according to the standard National Renewable Energy Laboratory (NREL) analytical procedure [18]. The degradation effect on biomass contents before and after treatment was calculated.

3.2. Delignification method

Five grams of each substrate were immersed with 100 mL of alkaline solution of different concentrations: NaOH (1, 2, 3 and 5%), KOH (1, 2, 3 and 5%) and $Ca(OH)_2$ (0.5%) in a 250 ml flask [19]. These alkali-immersed samples were then subjected to autoclave (121 °C for 20 minutes), water bath (80 °C for 30 minutes) and 3-time soakings for 1 minutes with intervals in a general purpose laboratory microwave (C.R.S117 Dawlence) as described previously [20]. The control samples were treated with distilled water under the same conditions. After alkaline heating, the black liquor was filtered. The resulting leftover solid residue was washed thrice with water until the pH reached 7.0. The composition of all the substrates in the given biomasses prior to alkaline delignification was calculated using National Renewable Energy Laboratory (NREL) procedure as described earlier [18]. The amount of lignin in the substrate was determined before and after the treatment and the extent of delignification was calculated by the Eq.1 below described in the by TAPPI T222 om-02 method.

$$\text{Lignin extraction } \% = \frac{\text{weight of lignin extracted}}{\text{acid insoluble lignin in biomass}} \times 100 \qquad (1)$$

The neutralized solid residue was used for SEM, FTIR, and AD process.

3.3. Scanning Electron Microscopy (SEM)

The morphological changes due to pretreatment could be analyzed with scanning electron microscopy (SEM) techniques. The surface morphology of oven dried untreated and pre-treated substrates was imaged using a vacuum-desiccated SEM (S-3700 microscope Hitachi) with the magnification ranges of 1K, 2K, and 3K to visualize the broken and distorted fibrous structure of feedstock.

3.4 Anaerobic digestion of pre-treated biomass

The biomethane potential (BMP) of NaOH (1, 2, 3 and 5%) and KOH (1, 2, 3 and 5%) microwave pretreated biomass was assessed by AD process. The culture volume in each serum bottle was 100 mL. The 100 mL contains, 8 mL of K_2HPO_4 (2.5g/L), 8 mL of 3.5 g/L sodium bicarbonates solution, and 2 mL from stock solutions of vitamin solutions folic acid (0.02 g/L), thymine (0.01 g/L), riboflavin (0.05 g/L), vitamin B_{12} (0.001 g/L), panthonic acid (0.05 g/L), lipoic acid (0.05 g/L). The serum bottles were airtight with rubber cork and aluminum crimp caps. The initial pH of all serum bottles was 7.5. Control samples of inoculum without substrate, water without the substrate, and a substrate without inoculum were included. The untreated and pretreated substrates were run parallel to compare the biogas and methane production. The serum bottles were flushed with N_2 gas for 4 min and were incubated at static condition. The volume of biogas was determined by the water displacement method for 40 days at regular intervals. The CH_4 content of the biogas was analyzed using GFM series gas analyzer.

3.5 Biogas Calculation

Daily volume of biogas, composition of methane (CH_4) and composition of carbon dioxide (CO_2) were determined from each bottle. Cumulative biogas from raw data was summarized using OBA online biogas app. This biogas software (R package) calculated cumulative volume of methane, cumulative volume of biogas, volumetric rate of biogas, and volumetric rate of methane. The R package used Eq.2 to calculate the daily biogas for each bottle based on displaced volume and % CH_4 at each previous and current reading [21].

$$Vb = \frac{P \times Vh \times C}{(RT)} \qquad (2)$$

Where Vb = is the volume of daily produce gas (mL)
P = is the absolute pressure difference (kPa)
Vh = is the volume of head space (mL)
T = is absolute temperature in (K)
C = gas molar volume (22.14 L mol^{-1}) at 273.15 K and 101.325 kPa
R = 8.314 L kPa k^{-1} mol^{-1} is universal gas constant

3.6 Kinetics Model

The solver function in Microsoft Office Excel 2016 was used for modified Gompertz equation (Eq. 3) the minimum methane production (mL/gVS) was calculated of each untreated and pretreated biomass sample by minimizing the least square difference between predicted and experimental values as described previously [22].

$$M\ (t) = P \times\ exp\ \left\{ -\ exp\ \left[\frac{R_{max}{}^{e}}{p(\Delta - t)} \right] + 1 \right\} \qquad (3)$$

Where M (t) is cumulative methane production (mL) during the incubation time t (hours),

P is the methane production (mL),

Rmax is the maximum production rate (mL/h),

Δ (delta) is the lag phase duration (hours) and

e is equal to 2.718282.

3.7 Statistical Analysis

All experiments in the present study were conducted in triplicates. Mean values and standard deviation (SD) among each triplicate were calculated through Microsoft Office Excel 2016. One-way ANOVA and box plot scheme were used to present the data.

4. Results and Discussion

The composition of each biomass was determined as described previously [18]. The lignin composition obtained was 10% in peanut, 15% in raw paper, 23% in almond, 26% in kallar grass, and 18-22% in wheat straw (Table 1). Similarly, higher amounts of hemicellulose and cellulose contents in different substrates have been obtained. The compositions of each substrate is consistent with previously reported data [23]. The lignocellulosic biomass (LB) mainly consist of cellulose, hemicellulose, lignin, small amounts of proteins, minerals, moisture, and other extractable components. However, the composition of the substrates can vary significantly for lignin, hemicellulose, cellulose, and other content depending on whether it is derived from grasses, softwood, or hardwood as has been defined in detail in previous studies [23, 24].

4.1 Alkali treatment to remove lignin from lignocellulosic biomass

An alkali treatment method was used for the measurement of lignin removal percentage as described in (Figure 1). After the composition analysis, NaOH (1,2,3, and 5%), KOH ((1,2,3, and 5%), and 0.5% of $Ca(OH)_2$ solutions were used to remove lignin from the substrates. To find a significant difference among each alkali treatment and heating process a one-way ANOVA was used to find statistical difference in lignin removal. All the tested substrates using one alkali at different concentration were compared, the average result of one factor ANOVA showed p-values of 0.05 and 0.001, indicating that the reactions were significant for most of the conditions. A clear significant difference between increasing value can be seen in (Tables 2 and 3). The difference in the percentage of lignin removal was high in case of 1-2% alkali dosage and was less between 3-5% alkali concentration.

Among the heating conditions and different concentration of alkalies, the optimum results were observed using 2% of NaOH and KOH. For 2% NaOH, water bath heating treatment displayed an average value of 56.3% lignin removal, whereas autoclave heating 61.6% and the highest lignin removal percentage was 67.9% with microwaving from all the tested substrates (Table 2). Similarly, the 2% KOH removed an average of 40, 47.7 and 55.7 % lignin with water bath heating, autoclave heating, and microwave treatment respectively from all the tested substrates (Table 3).

Groups	Count	Sum	Average	Variance
1%NaOH(WT)	10	322.2	32.2	19.6
1%NaOH(AUTO)	10	390.5	39.0	37.7
1%NaOH(MW)	10	458.5	45.8	33.1
2%NaOH(WT)	10	563.1	56.3	26.7
2%NaOH(AUTO)	10	616.3	61.6	18.9
2%NaOH(MW)	10	679.2	67.9	11.9
3%NaOH(WT)	10	683.7	68.3	60.9
3%NaOH(AUTO)	10	735.6	73.5	47.1
3%NaOH(MW)	10	818.3	81.8	21.1
5%NaOH(WT)	10	704	70.4	56.9
5%NaOH(AUTO)	10	764	76.4	37.8
5%NaOH(MW)	10	858	85.8	24.1

Source of Variation	SS	df	MS	F	P-value
Between Groups	25810.03	10	2581.003	76.26	0.05
Within Groups	3350.459	99	33.84302		
Total	29160.49	109			

Table 1. Estimated percentage composition of different waste biomasses

Substrate	C	H	L	T.S	V.S
Waste Paper	50	25	15	90	80.5
Wheat straw	40	26	18	81.5	65.5
Rice straw	38	24	17	85.3	80

Para grass	29	34	22	65.5	80
Kallar grass	38	23	25	67	74
Bagasse	28	32	19	72	76

Groups	Count	Sum	Average	Variance
1%KOH(WT)	10	216.0	21.6	16.7
1%KOH(AUTO)	10	254.0	25.4	27.0
1%KOH (MW)	10	307.0	30.7	29.2
2%KaOH(WT)	10	407.4	40.7	19.7
2%KaOH(AUTO)	10	470.4	47.0	26.5
2%KaOH(MW)	10	557.2	55.7	25.2
3%KaOH(WT)	10	505.1	50.5	36.6
3%KaOH(AUTO)	10	583.6	58.3	42.6
3%KaOH(MW)	10	650.4	65.0	29.0
5%KOH(WT)	10	525	52.5	32.0
5%KOH(AUTO)	10	609	60.9	29.2
5%KOH(MW)	10	678	67.8	27.0

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	26028.3	11	2366.20	83.19	0.001	1.87
Within Groups	3071.605	108	28.44			
Total	29099.9	119				

Pulses peel	29	24	18	80	65
Peanut shell	31	23	8-10	89	72
Corn cob	42	29	18.5	89	70.5
Almond shell	28	31	23	94.5	90.34

C= Cellulose, H= Hemicellulose, L= Ligin, T. S = total solid, V. S = volatile solid

Table. 2 One-way ANOVA for NaOH delignification of all the tested substrates

MW= microwave, WT= water bath. Auto= autoclave

Table. 3 One-way ANOVA for KOH delignification of all the tested substrates
MW= microwave, WT= water bath. Auto= autoclave

From the temperature effect, the microwave heating was more effective in lignin removal than autoclave and water bath. In addition, it was observed that the potential of lignin removal was higher using NaOH in all the heating conditions than KOH and $Ca(OH)_2$ treatments. The box plot results elucidate that delignification was maximum using microwave compared to the autoclave and water bath heating for each of the 1,2,3, and 5% NaOH and KOH conditions. Increase in lignin removal was observed with increasing concentrations of NaOH and KOH as shown in the box plot (Fig 1 and 2). The results for alkali treatment concluded that alkali treatment significantly reduced lignin from all the biomass tested. In control, untreated samples (treated with distilled water without alkali), no lignin was removed however a negligible amount of weight loss was observed from all the substrates at the same heating conditions (results not shown).

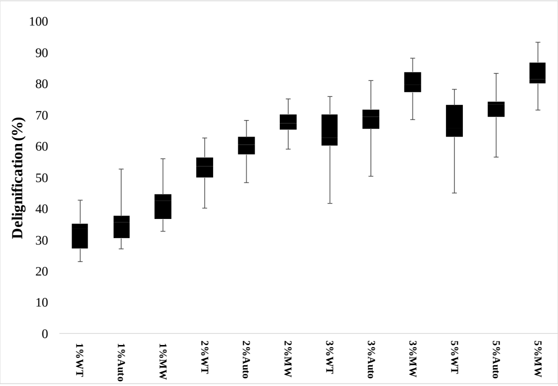

Fig 1. Box plot demonstration of comparative delignification for 1-5% NaOH concentrations. WT= water bath, Auto= autoclaving, MW= microwave

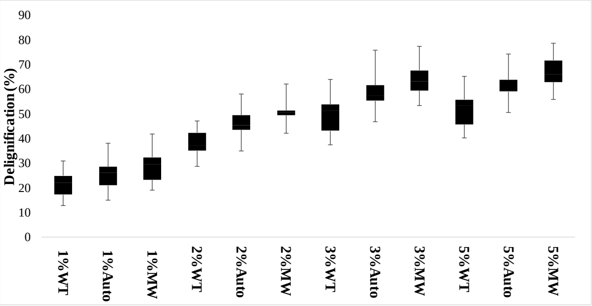

Fig 2. Box plot demonstration of comparative delignification for 1-5% KOH concentrations WT = water bath, Auto= autoclaving, MW= microwave

The results of alkalies treatment verified maximum delignification as the alkalies concentration was increased, however, an opposing consequence of the higher concentration of alkalies was observed on holocellulose. The highest tested dosage of alkalies beside lignin removal also decreased hemicellulose and cellulose composition of the tested biomass. In (Fig 3), the effect of 1,2,3 and 5% NaOH on holocellulose is shown, the reduction of hemicellulose was less in case of 1-2% NaOH dosage and a significantly higher reduction was observed at the maximum dosage of 5% NaOH tested. Similarly, the cellulose content also decreased as the concentration of NaOH increased from 1 to 5% NaOH. A maximum level of 40-50% hemicellulose reduction was observed from pulses peel, corn cob and almond shell and a lower percentage in the wheat straw, rice straw and paper waste when 5% NaOH is tested. Similarly, a maximum of 30-40% reduction in cellulose was observed with 5% NaOH from wheat straw, rice straw and paper waste and lower 25-32% from pulses peel, corn cob and almond shell (Fig 3).

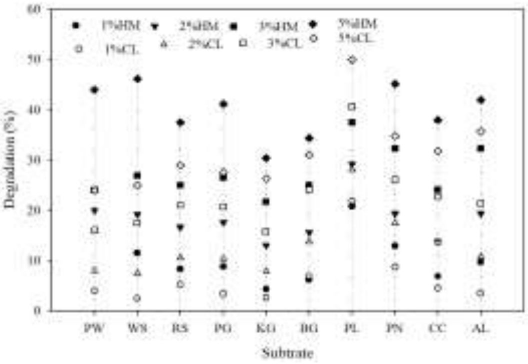

Fig 3. Effect of 1-5% NaOH concentrations on holocellulose degradation. HM= hemicellulose, CL=cellulose, PW =paper waste, WS= wheat straw, RS=rice straw, PG=para grass, KG= kalar grass, BG=bagasse, PP=pulse peel, PN = peanut, CC=corn cob, AL=almond.

4.2 Substrate base lignin removal

In (Fig 4 a, b, c) a comparative delignification of 2% NaOH, 2% KOH, and 0.5% $Ca(OH)_2$ from each tested substrate is shown. Base on the substrate, a different potential of lignin removal was observed. The highest lignin removal was obtained from rice straw, wheat straw, kallar grass, and bagasse respectively with 2% NaOH using the microwave heating as shown in (Fig 4 a). It was observed that the delignification was lower in case of almond, corn cob and paper pulp. A similar pattern of lignin removal was observed for 2% KOH and 0.5% $Ca(OH)_2$ from all the substrates treated by the microwave, autoclave, or water bath, although the lignin reduction was comparatively lower than 2% NaOH treated substrates (Fig 4 b and c).

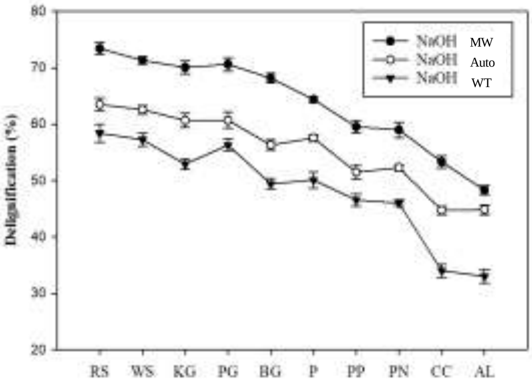

Fig 4A: Comparative delignification of 2% NaOH for microwave (MW), autoclave (Auto), and water bath (WT). P =paper, WS= wheat straw, RS=rice straw, PG=para grass, KG= kalar grass, BG=bagasse, PP=pulse peel, PN = peanut, CC=corn cob, AL=almond.

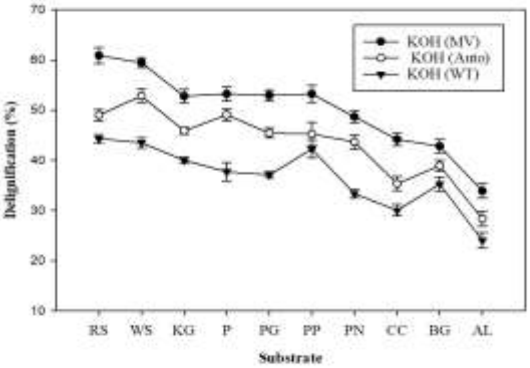

Fig 4B: Comparative delignification of 2% KOH for microwave (MW), autoclave (Auto), and water bath (WT). P =paper, WS= wheat straw, RS=rice straw, PG=para grass, KG= kalar grass, BG=bagasse, PP=pulse peel, PN = peanut, CC=corn cob, AL=almond.

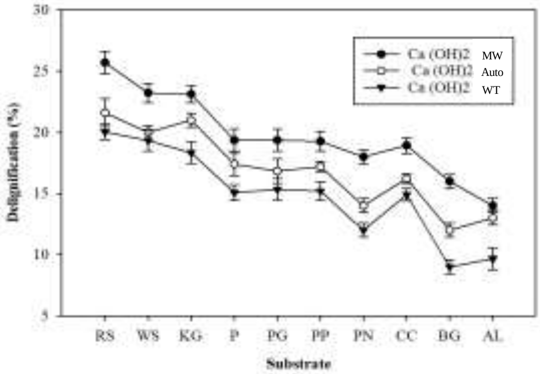

Fig 4C: Comparative delignification of 0.5% $Ca(OH)_2$ for microwave (MW), autoclave (Auto), and water bath (WT). P =paper, WS= wheat straw, RS=rice straw, PG=para grass, KG= kalar grass, BG=bagasse, PP=pulse peel, PN = peanut, CC=corn cob, AL=almond.

The results of the current and previously reported study proved that $Ca(OH)_2$ has less ability for lignin removal as compared to NaOH and KOH, this could be due to solubility reason of $Ca(OH)_{2,}$ it is hardly soluble beyond this concentration and, secondly, it makes a calcium–lignin complex, as calcium ions with two positive charges are inclined to crosslink with negatively charged lignin molecules under alkaline conditions that result in functional group ionization, thus preventing higher amounts of lignin degradation [25, 26].

The finding of the current study is concordant with the previous study, where NaOH has shown with higher lignin degradation rate than any other alkali [27]. It is also proved that high concentration of alkali treatment for longer times and increasing the intensity of treatment has a positive impact on lignin reduction and biomass solubilisation but has a negative effect on the overall treatment process by reducing sugar recovery and degrading useful carbon sources [28]. Among the heating processes, the effect of microwave-assisted alkali treatment in comparison to the conventional heating of autoclaves and water baths, solubilized more lignin than conventional alkali treatment

[29, 30]. It is due to the potential of microwave radiation, which is rapid, efficient, selective, precise, controllable, and produces hot spots on the subjected biomass, while conventional heating is inefficient, non-selective, produces no hot spots, and has less controlled heating [29, 30].

4.3. Scanning Electron Microscopy for the surface degradation of biomass

The surface structures of the untreated and 1,2,3, and 5% NaOH pre-treated wheat straw biomass were compared by analyzing scanning electron microscope (SEM) micrographs. The untreated wheat straw was highly compact with a clear and smooth structure Fig 5 (A). After alkaline pre-treatment, wheat straw was missing its dense structure, and distortion was observed on the surface. Similar observation of degradation after alkali treatment on the surface of straw is also reported earlier [31]. The SEM micrographs demonstrated the impact of pre-treatment removal of lignin and hemicellulose Fig 5 (B), (C), (D), and (E). The severity of the treatment of the surfaces increased with the concentration of NaOH indicating that the pre-treatment promoted the degradation. The images clearly showed ruptures in the silicon waxy structure, broken fibrils, and disrupted wall bundles in the lignin and hemicellulose complex in each sample. The morphologies of the wheat straw and other biomass from the SEM micrographs were very similar in term of the alkaline pre-treatment process.

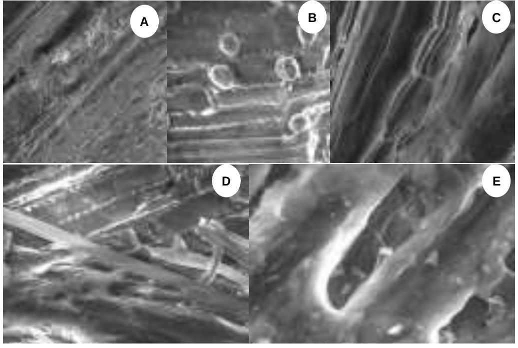

Fig. 5. Morphology of the wheat straw solid fraction after the pre-treatment by SEM micrographs for the untreated and pre-treated with 1-5% NaOH (A) untreated (B) 1% NaOH treated (C) 2% NaOH treated (D) 3% NaOH (E) 5% NaOH treated wheat straw with equal magnification.

4.4. Cumulative biogas and methane (CH_4) potential of alkaline pre-treated agriculture waste biomasses

The biogas potential is often defined as the volume of biogas produced per gram volatile solid (VS) added to the specific substrate. The accumulated final methane production is regarded as the methane potential of the particular substrate. In the current study, an inoculum of a full-scale anaerobic digester with a moisture content (MC) of 87.9%, 4.2% TS, and 2.8% VS was used. For the comparison of anaerobic digestion, the 1,2,3, and 5% NaOH and KOH treated samples of almond shell and wheat straw were tested. The selection of substrate was based on lower delignification (almond shell) and relatively higher delignification (wheat straw) among the tested substrates. The highest methane (CH_4) concentration of the biogas was 65-66% for pre-treated wheat straw and 50-55% for pre-treated almond shell respectively. However, 30-40% methane (CH_4) content was observed from the same untreated substrates.

The average highest daily volumetric biogas rate was between 50.4-70.5 mL from KOH and NaOH treated wheat straw (Fig 6). Similarly, the maximum daily volumetric biogas rate was between 30-45 mL from NaOH and KOH treated almond shell (Fig 7). Correspondingly, the average daily methane yield was 10-12 and 5-7 mL/gVS from wheat straw and almond shell treated with NaOH and KOH respectively (data not shown). It was observed that; the daily volume of biogas and methane rate was 2-2.6 times higher in case of pretreated almond shell and wheat straw comparatively to their untreated substrates. Similarly, the biogas yield of NaOH treated samples were more than the daily biogas and methane yield from KOH treated samples. This could be related to the high lignin removal of NaOH treatment than KOH.

In addition; the daily biogas was high in the first 10 days of anaerobic digestion from 3-5% KOH and NaOH batch assay than 1- 2% treated substrates. This results support that; high delignified substrate yield more biogas at the start of anaerobic digestion process than the less delignified substrate.

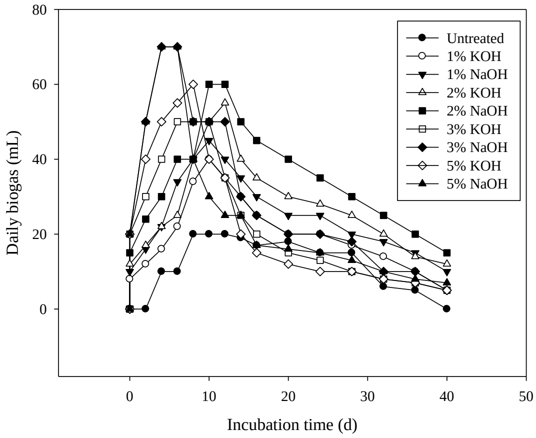

Fig 6. Daily volumetric biogas of 1,2,3 and 5% NaOH and KOH pretreated wheat straw.

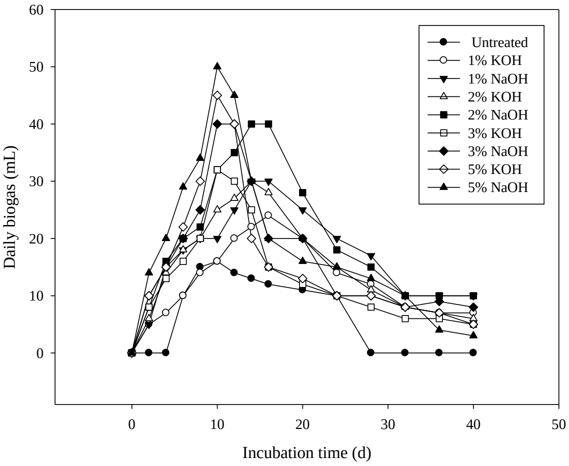

Fig 7. Daily volumetric biogas of 1,2,3 and 5% NaOH and KOH pretreated almond shell.

The cumulative biogas obtained from the alkali treated substrate was 2-times higher than the untreated substrates.

The highest cumulative biogas was 560.6 NmL/gVS from 2% NaOH treated wheat straw (Fig 8). Similarly, the highest cumulative biogas was 310.2 NmL/gVS from 2% NaOH treated almond shell (Fig 9). The total cumulative biogas and methane yield was minimum in case of 3 and 5% batch assay than 1 and 2% NaOH and KOH samples. This could be related to the hemicellulose and cellulose degradation from 3 and 5% delignified substrates (Fig 4). The total biogas yield was less in case of KOH treated samples than the NaOH treated substrate. This observation is also supported from the Gompert kinetic calculation for cumulative methane yield, a comparison of 2% NaOH and KOH is shown in (Table 4).

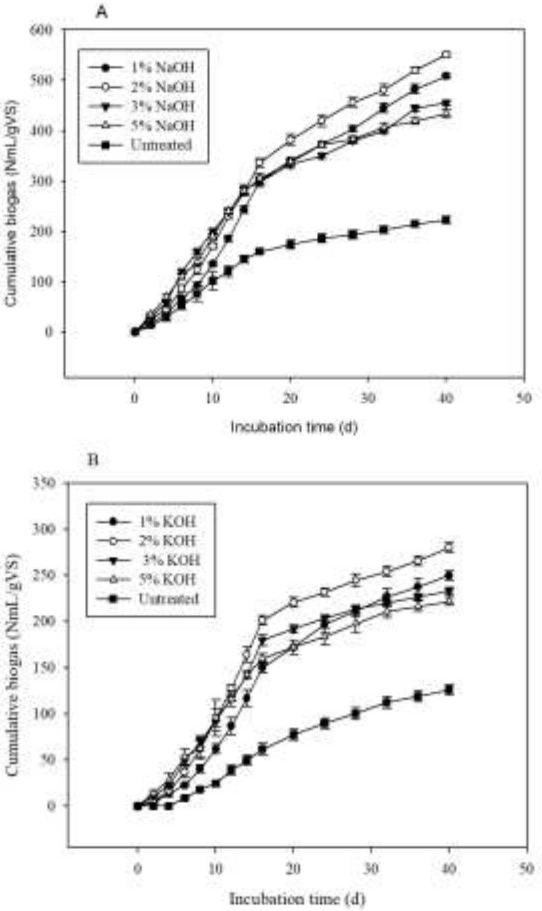

Fig 8. Cumulative biogas of 1,2,3 and 5% NaOH and KOH pretreated wheat straw.

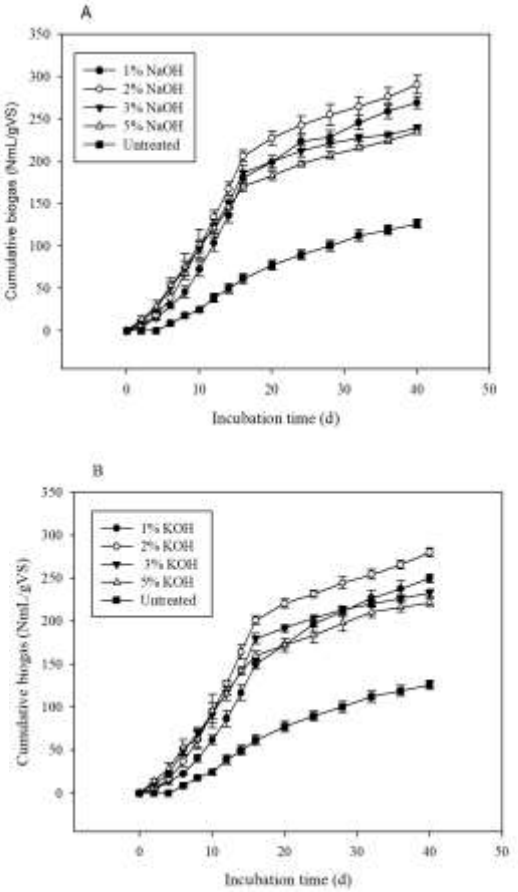

Fig 9. Cumulative biogas of 1,2,3 and 5% NaOH and KOH pretreated almond shell.

Table 4. Methane yield (NmL/gVS) of the 2% NaOH and KOH treated biomass

	Untreated WS	Untreated AL	2% NaOH WS	2% NaOH AL	2% KOH WS	2% KOH AL
(P)	131.00	108.00	430.60	275.50	308.10	220.90
Rmax	35	30	64.5	49.9	40.3	30.5
(λ)	94.6	96.5	48.1	51.2	49.2	52.5
R^2	96.1	97.3	98.2	98.2	97.5	97.5

(P)= Methane production, Rmax= maximum production rate,

(λ)=lag phase, WS=wheat straw, AL=almond shell

The presence of lignin decreases the biodegradability of the substrate and is inversely related to the biogas yield. The biomass having more lignin has less biodegradability and yield lower biogas. The current study also has shown that the substrate almond shell with high lignin content and low cellulose composition produced less biogas compared to wheat straw having low lignin and high cellulose in their composition. These observations are in line with literature studies as reported earlier [32]. Further, the untreated wheat straw and almond shell produced less biogas, because the lignin was not removed from these substrates, whereas in case of the treated samples where lignin was removed, the biogas yield was increased.

The results of the current study proved that, pretreatment is necessary to include for increasing biogas production from agriculture biomass. Our data from different pretreatment conditions unfold that low alkali dosage for short time heating process can decrease lignin up to more than 50% without degrading useful carbon sources (hemicellulose and cellulose). Beside this, the short heating process with low dosage of alkali has shown significant results in term of cumulative biogas yield. In addition, it is also proved that the diluted alkali pretreatment release more soluble sugar than concentrated alkali treatment [33]. Although, the difference in delignification with dilute and concentrated alkali treatment has a direct impact on the biomass hydrolysis and biogas yield. But to move forward from lab research to commercial scale development, it is important to consider a short thermochemical heating process for lignocellulosic biomass pretreatment, as the pretreatment is the crucial step, where it needs to be less expensive and effective enough to increase biogas production [9]. Considering, the results of the current study, it would be practically ideal to use 2% NaOH treatment for delignification of agribiomass, to be less expensive for industrial scale development of anaerobic digestion process.

5. Conclusion

Three alkali reagents at different concentrations were compared at three heating conditions to evaluate the impact of treatment on agriculture waste biomass. Delignification from the waste residue of agribiomass increased as the concentration of alkali reagent was increased. However, the high dosage of alkali has shown adverse effect on holocellulose, removing hemicellulose and cellulose, which shown a negative effect on net biogas yield during BMP batch assay. The results of lignin removal and anaerobic digestion batch experiments shown that, the optimum results were displayed by 2% NaOH treatment that removed a significant level of lignin from all the tested substrates. Similarly, the biogas obtained with 2% NaOH has also shown more total biogas yield than 2% KOH and even from the concentrated alkali dosage treated samples. Among the heating processes, short time microwave heating was the most effective treatment for lignin reduction. Notably, wheat straw batch samples displayed highest cumulative biogas production compare to almond shell.

Acknowledgment

This work is supported by the Pak-US project entitled “solid waste management for bioenergy production”.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] N. Halder, "Thermophilic biogas digester for efficient biogas production from cooked waste and cow dung and some field study", *IJRER*, 7(3), p. 1062-1073, 2017. (Article)

[2] R. Kumar, G. Mago, V. Balan, C.E Wyman, "Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies". *Bioresource Technol* 100(17), 3948-3962, 2009. (Article)

[3] I. Syaichurrozi, R. Rusdi, S. Dwicahyanto, Y.S. Toron, "Biogas production from co-digestion vinasse waste and tofu-prosessing waste water and knetics". *IJRER* 6(3), 1057-1070, 2016. (Article)

[4] S.R Paudel, S.P. Banjara, O.K. Choi, K.Y. Park, Y.M. Kim, J.W. Lee, "Pretreatment of agricultural biomass for anaerobic digestion: current state and challenges". *Bioresource Technol* , 2017. (Article)

[5] J. Pérez, J. Munoz-Dorado, T. de la Rubia, J. Martinez, " Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview". *Intl Microbiol* 5(2), 53-63, 2002. (Article)

[6] D.D Guta, " Assessment of biomass fuel resource potential and utilization in Ethiopia: sourcing strategies for renewable energies". *IJRER* 2(1), 131-139, 2012. (Article)

[7] S. Igoud, F. Soahi, A. Sebti, N. Mansouri, and A. Chouikh,"Potential of renewable energies generated from organic waste in algeria*". IJRER*,. 3(1): p. 109-113. 2013. (Article)

[8] P. Kullavanijaya, P. Thongduang, "Enhanced biogas production in anaerobic digestion of cassava wastewater though supplementation of biodiesel waste as co-substrate". *IJRER* 2(3), 510-515, 2012. (Article)

[9] M. Galbe and G. Zacchi, "Pretreatment: the key to efficient utilization of lignocellulosic materials," Biomass and Bioenergy, vol. 46, pp. 70-78, 2012. (Article)

[10] G. Turker,O. Ince, E. Ertekin, C. Akyol, B. Ince, "Changes in performance and active microbial communities due to single and multiple effects of mixing and solid content in anaerobic digestion process of OTC medicated cattle manure". *IJRER*,3(1), 144-148,2013. (Article)

[11] A.M. Patel, S.K. Singal," Economic analysis of renewable energy system for electrification of remote rural area having scattered population". *IJRER* 8(1), 523-539, 2018. (Article)

[12] T. Poespowati, "Optimization of acid hydrolysis process on macro-alga ulva lactuca for reducing sugar production as feedstock of bioethanol". *IJRER* 8(1), 466-475, 2018.3(Article)

[13] N. Tippayawong, and N. Chanhom, "Conversion of bamboo to sugars by dilute acid and enzymatic hydrolysis", *IJRER*, 1(4), p. 240-244. 2011. (Article)

[14] M.Z. Alam, M. Moniruzzaman, S. Sujan, M. Hossain, and M.S. Jamal,"Enzymatic saccharaification of bagasse, effects of different pre-treatment methods", *IJRER*, 3(2), p. 230-234. 2013. (Article)

[15] B.I. Dai, X.J. Guo, D.H. Yuan, J.M. Xu,"Comparison of different pretreatments of rice straw substrate to improve

biogas production. *Waste Biomass Valorization,* 1-10, 2017. (Article)

[16] S.E. Reza,T.A. Nitol, I. Abd-Al-Fattah, "Present scenario of renewable energy in Bangladesh and a proposed hybrid system to minimize power crisis in remote areas". *IJRER,* 2(2), 280-288, 2012. (Article)

[17] K. Sreekanth, S. Jayaraj, N. Sudarsan, " Achieving certified emission reduction in rural domestic energy sector through alternative fuel replacement". *IJRER* 2(1), 38-43, 2012. (Article)

[18] A. Sluiter, J. Sluiter, and E.J. Wolfrum, "Methods for biomass compositional analysis", NREL, Golden, CO. 2013. (Protocol)

[19] N. Mosier, C. Wyman, B. Dale, R. Elander, Y. Lee, M. Holtzapple, M. Ladisch, "Features of promising technologies for pretreatment of lignocellulosic biomass". *Bioresource Technol* 96(6), 673-686, 2005. (Article)

[20] M. Anjum, A. Khalid, T. Mahmood, I. Aziz," Anaerobic co-digestion of catering waste with partially pretreated lignocellulosic crop residues". *J. Cleaner Prod* 117, 56-63.2016. (Article)

[21] X.F. Lou, J. Nair, G. Ho, "Influence of food waste composition and volumetric water dilution on methane generation kinetics". *Intl J Environ Prot* 2, 22-29, 2012, (https://biotransformers.shinyapps.io/oba1/). (Article)

[22] C.N. Etuwe, Y.O.L. Momoh, E.T. Iyagba, "Development of mathematical models and application of the modified gompertz model for designing batch biogas reactors. *Waste Biomass Valorization* 7(3), 543-550, 2016. (Article)

[23] Y. Sun, J. Cheng, "Hydrolysis of lignocellulosic materials for ethanol production: a review". *Bioresource Technol* 83(1), 1-11, 2002. (Article)

[24] C. Álvarez,F.M. Reyes-Sosa, B. Díez, "Enzymatic hydrolysis of biomass from wood". *Microbial Biotechnol* 9(2), 149-156. 2016. (Article)

[25] J.K. Weng, X. Li, N.D. Bonawitz, C. Chapple, "Emerging strategies of lignin engineering and degradation for cellulosic biofuel production". *Curr Opinion in Biotechnol* 19(2), 166-172. 2008. (Article)

[26] J. Xu, J.J. Cheng, R.R. Sharma-Shivappa, J.C. Burns, "Lime pretreatment of switchgrass at mild temperatures for ethanol production". *Bioresource Technol* 101(8), 2900-2903. 2010. (Article)

[27] C.A. Rezende, M.A. de Lima, P. Maziero, E. Ribeiro deAzevedo, W. Garcia, I. Polikarpov, "Chemical and morphological characterization of sugarcane bagasse submitted to a delignification process for enhanced enzymatic digestibility". *Biotechnol Biofuels* 4(1), 54, 2011. (Article)

[28]J.C. Frigon, P. Mehta, S.R. Guiot, "Impact of mechanical, chemical and enzymatic pre-treatments on the methane yield from the anaerobic digestion of switchgrass". *Biomass Bioenerg* 36(Supplement C), 1-11, 2012. (Article)

[29]Z. Zhu, C. A. Rezende, R. Simister, S. J. McQueen-Mason, D. J. Macquarrie, I. Polikarpov "Efficient sugar production from sugarcane bagasse by microwave assisted acid and alkali pretreatment," Biomass and Bioenerg, vol. 93, pp. 269-278, 2016. (Article)

[30]Q. Li, C. Guo, and C.-Z. Liu, "Dynamic microwave-assisted alkali pretreatment of cornstalk to enhance hydrogen production via co-culture fermentation of Clostridium thermocellum and Clostridium thermosaccharolyticum," Biomass and Bioenerg, vol. 64, no. Supplement C, pp. 220-229, 2014/05/01/ 2014. (Article)

[31]D. J. Shetty, P. Kshirsagar, S. Tapadia-Maheshwari, V. Lanjekar, S. K. Singh, and P. K. Dhakephalkar, "Alkali pretreatment at ambient temperature: A promising method to enhance biomethanation of rice straw," Bioresource Technol, vol. 226, pp. 80-88, 2017. (Article)

[32]S. Ali, T.A. Shah, A. Afzal, R. Tabassum, "Exploring lignocellulosic biomass for bio-methane potential by anaerobic digestion and its economic feasibility," Energy & Environment, p. 0958305X18759009, 2018 (Article)

[33]S. McIntosh and T. Vancov, "Optimisation of dilute alkaline pretreatment for enzymatic saccharification of wheat straw," Biomass and Bioenerg, vol. 35, no. 7, pp. 3094-3103, 2011. (Article)