

Effect of Dilute Alkaline Pretreatment of Lignocellulosic Biomass on the Production of Biogas

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Abstract

The study appraised the effects of dilute NaOH pretreatment on biomass composition and the consequential effects on biogas production. The three biomass were subjected to compositional analysis before and after pretreatment to ascertain the influence of pretreatment. A 1.5 litre digester was used to subject the co-substrates to digestion and biogas was collected and measured using an airtight Hamilton syringe after every 24 hours. Data were analysed using Analysis of Variance and Microsoft Excel Analysis Tool Pack. Findings revealed that the 5, 6 and 7% NaOH solution have demonstrated substantial depolymerisation and delignification of the three biomass used in the study. It was also found that when these substrates were used as co-substrates in an anaerobic digestion with cow dung and domestic food waste, significantly higher biogas production was obtained when all the 3 pretreated substrates were used. Moreover, for all the biomass, pretreatment with 5% NaOH solution appeared to be the best treatment.

Keywords: Pretreatment, biogas, biomass, maize cob, soybean straw, rice straw.

Introduction

To attain the firm objective of minimizing out fossil fuel and balancing the large share of other renewable sources in the next few years to come, increased production of biogas is of high importance. Countries around the globe are now making exceptional plans in the development of energy production from biogas. Accordingly, increased use of biogas refineries plays a significant role towards achieving this goal. This is thought to relax the anticipated energy crises likely to occur soon because of the discrepancy between oil consumption and the discovery of new oil reserves (Darvekar, 2016). The design and operation of digester for biogas production is hinge on factors such as physical and chemical characteristics, structural complexity and quantity of feedstock, type of digester and condition of operation (Garffi *et al.*, 2016; Kothari *et al.*, 2014). Furthermore, the effective implementation of a gigantic biogas facility largely relied on availability of appropriate feedstock. Animal manure is readily accessible substrate for large scale production of biogas in most parts of the globe and its potentials for biogas production is enhanced when co-digested with high suitable organic wastes (Abouelenien *et al.*, 2014; Aragaw *et al.*, 2013). Moreover, agro-industrial biomass (lignocellulosic waste) is cheap, renewable, and abundant which provides an exceptional natural resource for large-scale and cost-effective bio-energy collection.

To enlarge the range of natural bio-resources, the rapidly evolving tools of biotechnology could lower the conversion costs and enhance target yield of the product of interest (Anwar *et al.*, 2014). Additionally, biogas production has been identified as one of the cost-effective method for decreasing environmental pollution linked to manure management, reduces greenhouse gas emissions, improves hygiene and reduces the use of synthetic fertilisers (Cuong *et al.*, 2012). However, biochemical transformation processes and deconstruction of lignocellulosic substrates into its components (cellulose, hemicellulose, and lignin) for succeeding microbial conversion have been a major challenge because of recalcitrance of the biomass. This could be attained through cost-effective pre-treatment processes which was a prerequisite of efficient hydrolysis. The cost for such treatment is currently about a third of the overall processing expenses in a cellulosic bio-refinery (Biswas *et al.*, 2015). Most likely, such pretreatment are not sustainable for biofuel production. The need therefore, for the evaluation of new and environmental friendly technology that could be accomplished with minimal energy input and expenditure became very essential.

Investigation by He *et al.* (2008) explored the influence of solid-state NaOH pretreatment on rice straw during biogas production. Fourier transform infrared, hydrogen-1 nuclear magnetic resonance spectrometry, x-ray diffraction and gas permeation chromatography were used to assess changes in chemical and physical characteristics of lignin, hemicellulose and cellulose. Results showed that biogas produced by rice straw treated with 6% NaOH increased by 27.5%. Degradation of 16.4% cellulose, 36.8% hemicellulose and 28.4% lignin were observed and water-soluble substances were by 122.5%. Moreover, the ester bond of lignin-carbohydrate complexes was destroyed, releasing more cellulose. Linkages of interunit and functional groups of lignin, cellulose and hemicellulose were broken down, leading to significant changes of chemical structures. Pretreatment reduces large molecular weight and 3-dimensional network of lignin to small molecular weight and linear structures. The changes in chemical compositions and structures, and physical characteristics made the biomass more susceptible to biodegradation and were responsible for optimisation of biogas production (He *et al.*, 2008).

In support of these findings, similar study by Su *et al.* (2015) using alkaline hydrogen peroxide, report high lignin deconstruction, cellulose and hemicellulose depolymerisations and improved biogas production. On the contrary, Mahdy *et al.* (2014) evaluated low temperature auto-hydrolysis at 50°C incubated at 24 and 48-hour with (0.5, 2 and 5% w/w NaOH) doses. It was found that improvement in methane production was not significant because organic matter solubilised were more of exopolymers released during pretreatment than intracellular materials. In support of Mahdy, Monlau *et al.* (2015) indicate that alkaline pretreatment fails to improve methane yield and, hemicellulose was more effectively digested than the cellulose, while lignin was not affected.

This inconsistency is probably due to the differences in the pretreatment temperatures and doses of NaOH/other alkali used in the studies. These studies show that any alkali could be used in pretreatment, NaOH being the most popular. There are many undesirable

processes such as raise of substrates pH and lately during digestion, salt build-up may be significant and probably inhibit methanogens due to imbalance of ammonium and ammonia (Montgomery and Bochmann, 2014). Another major weakness of alkali pretreatment is the destruction of lignin instead of separation, which allows lignin to be used as biopolymers. Moreover, cost may limit the use of alkali as pretreatment substance. However, it may be beneficial in the treatment of high lignin and acidic substrates (Montgomery and Bochmann, 2014), in contrast to the assertion of Singh *et al.* (2015). This argument suggests that pretreatment procedures using NaOH should be carefully evaluated and selected for effectiveness and to make it cost-effective, dilution of the alkali solution may be beneficial in the reduction of severe salt-build, amount of chemical needed for pretreatment and the resulting destruction of polymers. The purpose of this article is to determine the effects of application of dilute alkaline pretreatment of lignocellulosic biomass on biogas production when used as co-substrates.

Materials and Methods

Sample collection and preparations of raw biomass

Samples which include soybean straw (SBS), maize cob (MC) and rice straw (RS) were collected from The Polytechnic Crop Farm immediately after harvest in Bali Local Government Area of Taraba State. The biomass were air-dried in an open space (average temperature of $35 \pm 2^\circ\text{C}$) for three days (8 h per day). The sample size were reduced to about 2 cm in length for effective milling. Size reduction was carried out by a mill and the milled biomass was used for the compositional analysis before and after the dilute acid/alkali pretreatment. A portion of the materials was screened into different sizes using a set of sieves and shaker.

Biomass analyses

Compositional analysis of the raw and pretreated lignocellulosic materials were conducted as detailed below:

Extractives

About 2.5 g of dried raw biomass was loaded into the cellulose thimble. 150 mL of acetone was used as solvent for extraction using Soxhlet. Residence times for the boiling and rising stages were carefully adjusted to 70°C and 25 min respectively on the heating mantle for a 4 h run period. After extraction, the sample was air-dried at room temperature for few minutes. Constant weight of the extracted material was achieved in a convection oven at 105°C . The % (w/w) of the extractives content was evaluated as the difference in weight between the raw extractive-laden biomass and extractive-free biomass (Ayeti *et al.*, 2015).

Lignin, hemicellulose and cellulose

Exactly, 1 g of extracted dried biomass was transferred into a 250ml flask. 150 ml of 500 mol/m³ NaOH was added. The mixture was boiled for 3.5 h with distilled water and filtered

after cooling through vacuum filtration and washed until neutral pH. The residue was dried to a constant weight at 105°C in a convection oven. The difference between the sample weight before and after this treatment is the hemicellulose content (%w/w) of dry biomass (Ayeni *et al.*, 2015).

Precisely, 0.3 g of dried extracted raw biomass will be weighed in glass test tubes and 3 ml of 72% H₂SO₄ was added. The sample was kept at room temperature for 2 h with careful shaking at 30 minutes intervals to allow for complete hydrolysis. After the initial hydrolysis, 84 ml of distilled water was added. The second step of hydrolysis was made to occur in an autoclave for 1 h at 121°C. The slurry was then cooled at room temperature. Hydrolysates was filtered through vacuum using a filtering crucible. The acid insoluble lignin was determined by drying the residues at 105°C and accounting for ash by incinerating the hydrolysed samples at 575°C in a furnace (Ayeni *et al.*, 2015).

The cellulose content (%w/w) was determined by the difference. This assumes that extractives, hemicellulose, lignin, ash, and cellulose are the only components of the entire biomass (Ayeni *et al.*, 2015).

Preparation of digesters

Inoculation of substrates in the digester

Inoculum was cultured from fresh cow dung prepared according to the method of Lawal *et al.* (2015). About 500 g of cow dung was collected from Animal Farm of the Federal Polytechnic Bali and was taken to the laboratory. A sample of 200 g was diluted in distilled water to 10% dry matter and transferred into 2 litre glass bottles. The headspace of the diluted cow dung was flushed with nitrogen gas and closed with a rubber cork and resin. The solution was incubated at 35°C. During the incubation, the solution was degassed by allowing the build-up gas to escape through valve control tube. The completely degassed solution was used as inoculum.

Biogas analysis

Biogas production was quantified using positive displacement technique.

Pretreatment of the biomass

The lignocellulosic biomass samples was mechanically pulverized until particle size of <2 mm was attained. The milled biomass was pretreated in 5% (w/v) NaOH at 20% (w/v) Solid/Liquid ratio in a tightly capped bottle placed in an autoclave at 121°C (Gonzales *et al.*, 2016). The same dilution was implemented for the pretreatment.

Determination of total solids in biomass

To determine total solids in biomass sample, the procedure of LAP NREL/TP-510-42621 for the Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid process Samples was followed as presented by Sluiter *et al.* (2008). Aluminium weighing dishes was pre-dried by placing them in a 105 ± 3°C drying oven for four hours and the dish was cooled

in a desiccator. Using gloves to handle the dish, the pre-dried dish to the nearest 0.1 mg. The weight of the dish was recorded. Sample was thoroughly mixed and an appropriate amount to the nearest 0.1 mg was placed into the weighing dish. This measurement was noted. The sample was placed into a convection oven at $105 \pm 3^{\circ}\text{C}$ for a minimum of four hours. Sample was removed from the oven and allowed to cool to room temperature in a desiccator. The weight of the dish containing the oven-dried sample to the nearest 0.1 mg was measured and recorded. The oven-dried sample was placed back into a convection oven at $105 \pm 3^{\circ}\text{C}$ and dry to a constant weight. Constant weight was defined as $\pm 0.1\%$ change in the weight percent solids upon one hour of re-heating the sample (Sluiter *et al.*, 2008). The total solid in biomass sample is computed according to equation 3.3.

$$\%Total\ Solids = \frac{Weight_{dry\ pan\ plus\ dry\ sample} - Weight_{dry\ pan}}{Weight_{sample\ as\ recieved}} \times 100$$

Determination of fixed and volatile solids

These are carried out in accordance with the METHOD 1684 EPA-821-R-01-015 of the United State Environmental protection Agency (USEPA). Biomass sample was heated for one hour, and allowed to cool to a balance temperature in a desiccator and weighed. The heating, cooling, desiccating and weighing procedure was repeated until the weight change was less than 4% or 50 mg and the final weight was recorded as W_{total} .

The evaporating dish containing the dried sample was transferred to a cool muffle furnace and then heated to 550°C and ignited for 2 hours. The sample was cooled in a desiccator to balance the temperature and the sample was weighed. The process of igniting for 30 minutes, cooling, desiccating and weighing was repeated until the weight change was less than 4% or 50 mg and the final weight was recorded as $W_{volatile}$. Values for fixed and volatile solids were computed according Equations 3.4 and 3.5 respectively (USEPA, 2001).

$$\%fixed\ solids = \frac{W_{volatile} - W_{dish}}{W_{total} - W_{dish}} \times 100$$

$$\%volatile\ solids = \frac{W_{total} - W_{volatile}}{W_{total} - W_{dish}} \times 100$$

Results and Discussion

Effects of pretreatment on some physicochemical properties of biomass

To determine the effects of subjecting biomass to dilute NaOH pretreatment, SBS, RS and MC were subjected to NaOH pretreatment under different dilutions which were 5, 6 and 7% (w/v) NaOH. These treatments have substantially affected the physico-chemical parameters of the biomass. The information in Table 1 is the results of some physico-chemical properties of SBS and the results showed significant shift in the volatile solids (VS) of the biomass where VS increased from 38.5% in the raw biomass to 75, 60 and 51% when treated with 5, 6 and 7% NaOH respectively. This evidence suggested that higher VS was achieved with 5% NaOH and as dilution decrease, the VS contents decreased. Although,

the values of hemicellulose and cellulose in the untreated SBS were consistent with values reported by Kovačić *et al.* (2017) in their excellent review, it was however much lower than the results reported recently (Adebowale *et al.*, 2022).

The degradation or deconstruction of hemicellulose and cellulose also decreased with decrease in dilution of NaOH with 7% NaOH exhibited the highest depolymerisation or deconstruction of these polymers. Lignin was substantially degraded as the concentration of NaOH increased but may have swapped to formation of pseudo lignin and could be responsible for higher content of acid insoluble lignin which was about 9.2% higher than the acid insoluble lignin content of the untreated or raw SBS.

Table 1. Some physico-chemical properties of pretreated and raw soybean straw

Parameters	Soybean Straw treated with <u>NaOH</u> (w/v)			
	5%	6%	7%	Untreated
Total solids (%)	93.0	94.8	94.9	94.7
Volatile solids (%)	75.0	60.1	51.6	38.5
Hemicellulose (%)	22.8	20.5	18.1	29.3
Cellulose (%)	34.1	27.8	22.0	42.3
Acid soluble lignin (%)	6.1	5.9	4.6	8.0
Acid insoluble lignin (%)	17.9	16.8	20.2	18.5

Source: Laboratory results.

The results in Table 2 showed the effects of dilute NaOH pretreatment on RS where significant rise in the VS was observed for all treatments. Hemicellulose and cellulose deconstruction followed similar pattern as observed with SBS where increasing concentration resulted in increased degradation of these polymers.

Table 2. Some physico-chemical properties of pretreated and raw rice straw

Parameters	Rice straw treated with <u>NaOH</u> (w/v)			
	5%	6%	7%	Untreated
Total solids (%)	96.5	98.3	98.4	98.2
Volatile solids (%)	77.8	62.3	53.5	39.9
Hemicellulose (%)	23.7	21.2	18.7	30.4
Cellulose (%)	35.4	28.8	22.8	43.9
Acid soluble lignin (%)	6.3	6.1	4.7	8.2
Acid insoluble lignin (%)	18.5	17.4	20.9	19.2

Source: Laboratory results.

Table 3. Some physico-chemical properties of pretreated and raw maize cob.

Parameters	Maize cob treated with NaOH (w/v)			
	5%	6%	7%	Untreated
Total solids (%)	86.6	86.8	85.2	85.4
Volatile solids (%)	58.2	51.8	67.5	29.1
Hemicellulose (%)	24.4	16.0	14.2	30.4
Cellulose (%)	34.2	25.0	21.4	39.2
Acid soluble lignin (%)	1.9	2.6	2.1	3.9
Acid insoluble lignin (%)	4.4	4.5	4.7	4.7

Source: Laboratory results.

Influence of dilute NaOH pretreatment of SBS on biogas production

One of the main purposes of this work is to determine the influence of pretreatment with different concentrations of NaOH on lignocellulosic biomass structure and compositions. The data in Figure 1a is the results of cumulative biogas production from co-digestion of cow dung, food waste and SBS pretreated with 5% NaOH solution. Highest daily biogas of 30.2 ml was obtained on the 12th day and a lag phase of 4 day and generally, incubation period was in the range of 2 to 4 days.

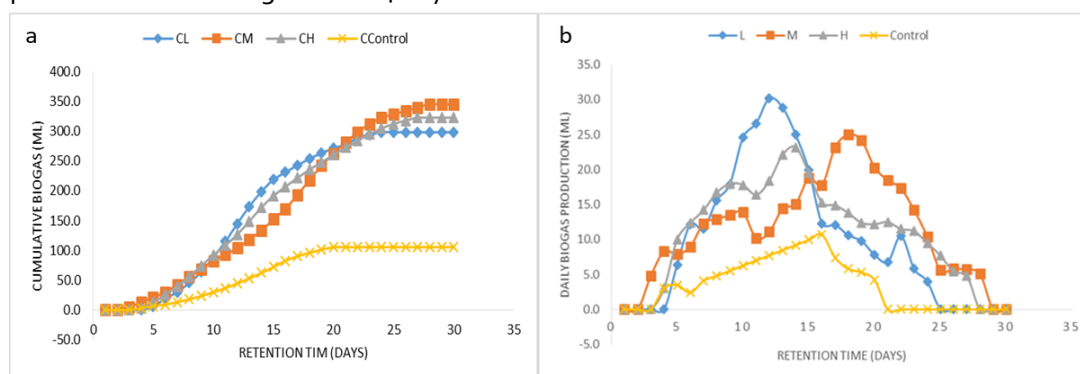


Figure 1. Cumulative (a) and daily (b) biogas production from co-digesting cow dung, food waste and soybean straw pretreated with 5%, 6% and 7% NaOH solution (Source: Laboratory results).

The results also indicated that highest cumulative biogas production of 345.3 ml was obtained with digester where SBS pretreated with 6% NaOH was used as co-substrates while lowest cumulative biogas production of 298.5 ml was attained in digester with incorporation of 5% NaOH-pretreated SBS as co-substrates and 7% NaOH was used, the biogas output was higher than the output from 5% NaOH but lower than that of 6% NaOH pretreated SBS. Although, highest maximum production rate was identified with L-digester, this did not translates to highest cumulative yield because of lag phase delay and production ceased earlier, probably due to raise in digestate pH or salt build-up as pointed

out by Clarkson (2018). The results obtained in this study supported the findings of Zhurka *et al.* (2019) who reported significant delignification and cellulose depolymerisation and resulted in higher biogas output. It is also consistent with the results documented by Stamatelatou *et al.* (2012). Although 8% NaOH was utilised attained a relatively lower output than the one reported in this study.

Figure 1b is the results of daily biogas production obtained by incorporating SBS under the tree treatments and the control and it was found that maximum production rate was obtained in L-digester with about 14.9 ml which was 64% higher than the control, output from M-digester was 53% higher than control while H-digester which was the digester with moderate biogas output was also 54% higher than the control digester in which untreated SBS was incorporated as co-substrates. These evidences suggest that application of 5% NaOH solution in the pretreatment of SBS for possible use as co-substrate involving CD and FW is more appropriate than the higher percentage. ANOVA analysis revealed that the differences in effect between the pretreatments is not significant $P > 0.05$ at 95% confidence level. Interestingly, the effects of these treatments on biogas production were substantially higher ($P < 0.05$) than the output from control experiment at 95% level of confidence. Therefore, use of additional NaOH beyond 5% may certainly not be justified by the differences in biogas production.

Influence of dilute NaOH pretreatment of rice straw on biogas production

Rice straw was also pretreated with 5, 6 and 7% NaOH solution and their results were compared with the control as presented in Figure 2. The result illustrated that pretreatment with 5% NaOH demonstrated highest biogas potentials of 506.4 ml, highest maximum biogas production rate of 48.4 ml and the highest average biogas production rate of 22 ml during the active production phase. Obviously, the treatments have relatively no effect on lag phase when compared with that of control. Moreover, gas production ceased towards the end of the experiment but in the control digester, production stopped 22 days into the experiment. Analysis of variance revealed that the difference between the treatments and the control digesters biogas out was statistically different ($P < 0.05$) at 95% level of confidence. Also, the effect identified with the L-digester was significantly higher ($P < 0.05$) than the M- and H-digesters for rice straw and this can be seen on the cumulative curve in Figure 2a. Again, the diluted solution of 5% NaOH in the pretreatment of rice straw for biogas production as co-substrate is more appropriate than higher contents.

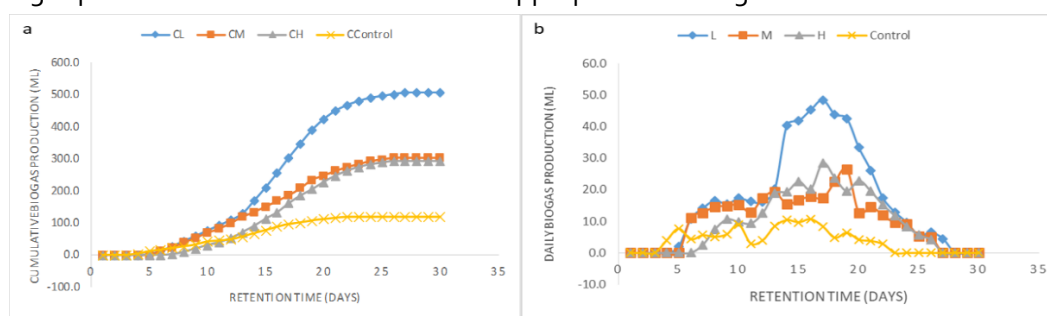


Figure 2. Cumulative (a) and daily (b) biogas production from co-digesting cow dung, food waste and rice straw pretreated with 5%, 6% and 7% NaOH solution (Source: Laboratory results).

Incorporation of rice straw in the co-digestion of CD and FW has substantially improved biogas production. A pretreatment with 5% has shown 72% higher biogas output than the control. Even the treatment with the lowest gas output was 57% higher output than when untreated rice straw was incorporated.

Influence of dilute NaOH pretreatment of MC on biogas production

The impact of NaOH on maize cob documented in Table 3 indicated increased cellulose and hemicellulose depolymerisation as the concentration of NaOH in the pretreatment solution increased. However, moderate concentration of 6% solution was the treatment that yielded highest cumulative biogas as shown in Figure 3a and 3b. Although, H-digester has the highest average biogas production (18.5 ml) compared with L-digester (15.7 ml) and M-digester (17.5), gas production ceased earlier in the H-digester and this might have been an explanation of the lower cumulative biogas production relative to other treatment digesters (374.4 ml for M-digester, 298 ml for L-digester).

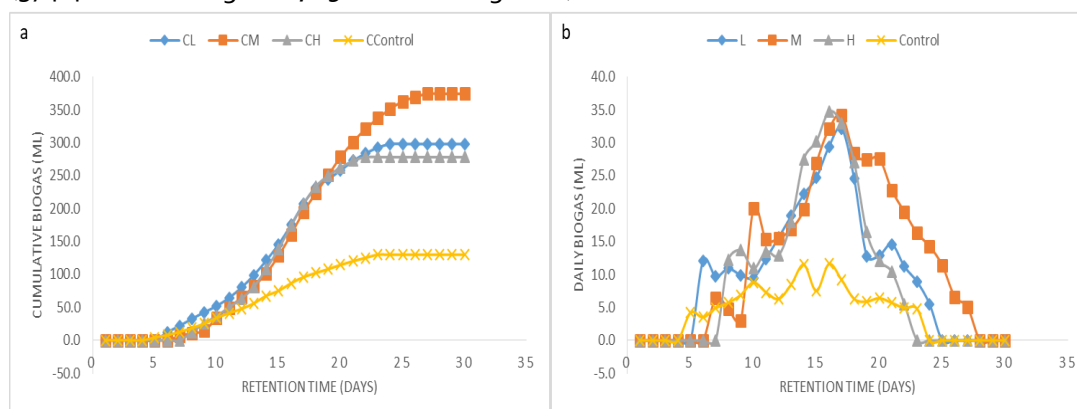


Figure 3. Cumulative (a) and daily (b) biogas production from co-digesting cow dung, food waste and maize cob pretreated with 5%, 6% and 7% NaOH solution (Source: Laboratory results).

The output from H- M- and H-digester where NaOH pretreated biomass was used in co-digestion has illustrated 64, 63 and 58% higher biogas production rates than the control experiments. In support of the findings obtained in this study, Surra *et al.* (2018) found that pre-treatment allows to overcome the recalcitrance characteristic of MC to digestion, thus, increasing the biogas and methane yields by percentages of 46.0% and 36.6%, respectively, with a biogas composition of 66.8% v/v CH₄ and 32.2% v/v CO₂. From the above evidences, it could be deduced that with these disparities, and looking at the cumulative gas production from the treatments, the recommended treatment is the pretreatment with 5% NaOH solution because the differences in rates and cumulative production may not justify the use of higher doses of concentration.

Conclusion

The study aimed to determine the impacts of dilute NaOH pretreatment on biomass composition and the consequential effects on biogas production. It was found that the 5, 6 and 7% NaOH solution have demonstrated substantial depolymerisation and delignification of the three biomass used in the study. It was also found that when these substrates were used as co-substrates in an anaerobic digestion with cow dung and domestic food waste, significantly higher biogas production was obtained when all the 3 pretreated substrates were used. Moreover, for all the biomass, pretreatment with 5% NaOH solution appeared to be the most economically viable pretreatment option compared with 6 and 7% NaOH solution.

Recommendations

Based on the findings of this investigation, the following recommendations have been outlined.

- Prior to any large scale applications of NaOH in the pretreatment of biomass for possible, application in biofuel production, pilot scale on specific recalcitrance or susceptibility of biomass to pretreatment solution is necessary.
- For the biomass under study, 5% NaOH is appropriate in the pretreatment of the biomass for use as co-substrates with CD and FW.

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