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Original Research Article

Comparative Study of Thermal and Chemical Pretreatment on the Yield of Biogas from *Microcoleus vaginatus* Vauch

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ABSTRACT

The industrial and household energy demand is rapidly outpacing the available fossil resources which has called for alternatives that will be eco-friendly and cost effective. In this work, potentials of thermal pretreatment in an oven at 70, 75, 80 °C and chemical (NaOH) 0.5, 0.6, 0.7 Molar concentration were examined for the enhancement of biogas yield from Microcoleus vaginatus. The results of proximate analysis for untreated sample was 47.6, 5.2, 22.1 4.47, 8.73 and 11.9 % for carbohydrate, moisture, ash, lipids, fibre and protein respectively. The carbohydrates for all treated samples were enhanced. For samples treated at 70, 75 and 80 °C the values were 80.62, 79.7, 78.2% and 75.14, 75.1, 74.9% were obtained from 0.5, 0.6 and 0.7 M (NaOH) treated samples. Other nutrients were all reduced except moisture for chemical treated samples. The optimum reduction in moisture was 148 % for thermally treated samples and 15 % increase for chemically treated. The biogas yield for untreated sample was 4.36 mLg^{-1} VS and 8.39 mLg^{-1} VS, 9.07 mLg^{-1} VS and 9.38 mLg^{-1} VS was obtained at 70, 75 and 80 °C equivalent to 1.9, 2.1 and 2.2-fold increase when compared with the untreated sample against 7.99 mLg^{-1} VS, 7.38 and 7.0 mLg^{-1} VS while, for chemical treated samples which is 1.6, and 1.8 fold for 31 days of retention. In this study thermal pretreatment at 80 °C produced the highest yield of biogas and is thus recommended for biogas production from M. vaginatus.

1. INTRODUCTION

The environmental degradation caused by fossil fuels usage on human habitat and long-term effect on health had attracted many interests to low carbon energy sources on a scale that will compete favourably without negative impact on life and agriculture (Chen *et al*., 2011). It is apparent that urgent need for alternatives and cheaper sources of energy is long overdue in most developing countries, due to problems of militancy, piracy and corruption (Craggs *et al.*, 2011). Our rural communities today rely on wood as major source of energy, and this is not easily sourced due to the cost, scarcity and government regulations on wood resources. For instance, between 1999 and 2011 price of petroleum products in some countries had sky- rocketed by more than 120 % where efforts by government to restore normalcy in the sector have not made any appreciable impact, as fuel prices are dwindling on daily basis (Ukonu, 2011).

Solution to the problems of energy generation and distribution centred on harnessing other forms of energy obtainable from sun, wind, waves, tidal, geothermal or biofuels to complement if not replaced the fossils completely. Microalgae have been suggested to have promising potential due to high yield within short period and less competition for arable land during growth (Schenk *et al.*, 2008); Chen *et al.*, 2011). These reasons had drawn many interests to algae species in the development of biofuel products such as biogas (Dragone *et al.*, 2010). Currently, since the amount of organic materials used for biogas production is limited and new substrates as well as effective technologies are required to facilitate growth in biogas industry worldwide (Horváth, *et al.*, 2016). It is apparent that governments are not meeting up with the consumers demand in most developing countries, resulting to poor living conditions, because energy is the bedrock for socio-economic development of every society (Aminu *et al.,* 2013). Another report by Chisti (2007) had shown microalgae as capable of rapid generation of biomass in water bodies through the use of solar energy, *a*nd nutrients. As a renewable feedstock, microalgae has several benefits when compared to other terrestrial plants. These include high rates of productivity, bio-fixation (main carbon source for growth), lowest water demand for its cultivation, no competition with food production. It can also be cultivated in different environments, and the required nutrients for growth may be obtained from environment unfit for normal cultivation such as wastewaters. Furthermore, they have high oil content (20 - 25 % dry matter) and can be used as food or fertilizers (Chisti, 2007; Schenk *et al*., 2008; Bougrier etal., 2006; Phukan *et al*., 2011). These benefits have given microalgae unique status as environmentally friendly resources for large-scale production of biogas. It is apparent that understanding the physical and chemical properties of microalgae is therefore critical to the development of appropriate processes for commercial biogas production.

This study seeks to utilize *Microcoleus vaginatus* specie of an algae biomass as feedstock for anaerobic digestion through critical study of thermal and chemical pre-treatments influence in the enhancement of biogas yield from the substrate.

2. MATERIALS AND METHODS

2.1. Sample Collection

The strain *M. vaginatus* was obtained from River Landzun in Bida Local Government Area of Niger State, Nigerian. It was then cultured at Unit Operation Laboratory, Department of Chemical Engineering, Federal Polytechnic, Bida. The strain was isolated using glass capillary before they were gently transferred into sterile water in order not to damage the cells. The culturing medium was set to mimic the natural habitat by addition of liquid fertilizer as source of nutrients and to produce algae cells with maximum purity. Ali and Ogbonna, (2011) method were adopted and cells were grown for 14 days each before they were harvested through filter cloth and dried. The dried sample was crushed using mortar and pestle, then screened using Endecott test sieve mounted on sieve shaker. The particles collected on 600 µm mesh size were used in the subsequent stage of this work.

2.2. Pretreatment Process

The thermal pretreatment process was carried out in an oven, at constant time of 1 hour and varying temperature of 70, 75 and 80 °C each. Ten grams each of the raw *M. vaginatus* were measured into three metallic containers and kept in an oven for one hour before the samples were removed and then transferred into digesters for onward digestion. On the other hand, 10 g each of *M. vaginatus* were measured into three beakers charged with 100 ml of 0.5, 0.6 and 0.7 molar concentration of NaOH at room temperature for an hour before the solution was drained and the substrate thoroughly washed with distilled water before it was transferred into digesters.

2.3. Anaerobic Digestion

A batch process of anaerobic digestion was adopted in 250 ml plastic bottle with the slurry temperature maintained at 37 ^{o}C in water bath. Four grams each of thermal and chemical pretreated samples were measured and discharged into various digesters. Fresh cow dung (2 g) was added as seeding agent to facilitate the microbial growth in the medium, while 60 ml of deionized water was added to give substrate to liquid ratio of 1:10 and headspace was allowed on top for gas. Another digester (control) was charged with 4 g of untreated *M. vaginatus,* 2 g of cow dung and 60 ml of deionized water. Each digester was properly mixed in order to form homogenous slurry (Murphy, *et al.*, 2015). Proximate, ultimate, and SEM analyses were conducted before, and after pretreatment on dried basis, while, microbial count of the liquid slurry was carried out. pH was measured daily and inverted cylinder was used to measure the biogas produced.

3. RESULTS AND DISCUSSIONS

3.1. Proximate Analysis of *Microcoleus vaginatus*

The results of proximate analysis of raw, thermal and chemical pretreated *M. vaginatus* before and after pretreatment was shown in Figure 1. The carbohydrate in the raw samples was 47.6 % of total compositions, where 5.2, 22.1, 4.4, 8.76 and 11.9 % corresponded to moisture, ash, lipids, fibre and protein respectively.

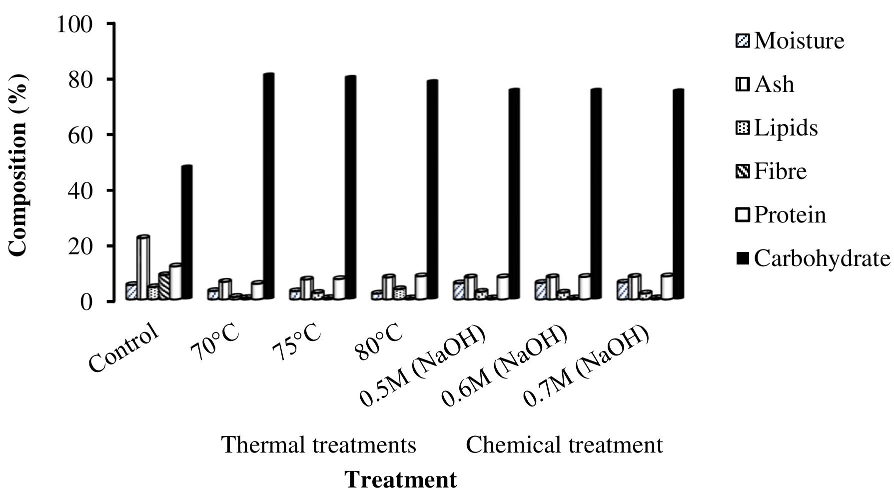


Figure 1: Proximate analysis of control, thermal and chemical treated samples

Figure 1 showed an increase in carbohydrate for all pretreatments. There was increase from 47.6 % to 80.62, 79.7 and 78.2 % for 70, 75 and 80 ^{o}C treated samples. While 75.14, 75.06 and 74.81 % are obtained from NaOH treated samples of 0.5, 0.6 and 0.7 M. All pretreatments showed influence on carbohydrate extraction. For thermal pretreatment, 70 ^{o}C produced the highest while the lower concentration 0.5 M NaOH gave more yield of carbohydrates. This corresponds to Cantarella *et al.* (2004) who found that, higher concentration of NaOH removes not only the lignin but also the hemicellulose part of the substrate. Andrés *et al.* (2014) also observed higher carbohydrate extraction at 75 ^{o}C in *Chlorella vulgaris* with approximately 8 % moisture as compared to other pretreatment methods with lower moisture values. Rise in temperature weakened the 1-4, D glycosidic bonds in either the starch or cellulose. According to Chen *et al*. (2013) to efficiently utilize microalgae-based carbohydrates, it is necessary to identify cleavage method of β-1,4-glycosidic bonds between the hydroglucose subunits of cellulose molecules, and that of α-1,4-glycosidic linkages in starch. When these bonds are weakened cellulose are freed from lignin.

The moisture content was reduced after pretreatment at 70, 75 and 80 ^{o}C from 5.2 % to 3.01, 2.96 and 2.1 % which led to (73 – 148 %) reduction in moisture. By implication, moisture content was reduced as pretreatment temperature increased. In line with this, Studer, (2012) reported that, increase in temperature above 20 ^{o}C is capable of breaking down the hydrogen bond between crystalline structure of cellulose that will lead to loss of water and volatile solutes in substrates. The chemical treated samples at 0.5, 0.6 and 0.7 M (NaOH) had 5.81, 5.93 and 6.1 % moisture which was approximately 12, 14 and 17 % increments compared to untreated sample and this was attributed to substrate swelling caused by NaOH. Chang (2014) noticed that substrate swelling can assist in delignification and deacetylation of anaerobic substrate by removing parallel barriers to hydrolytic enzymes. However, to achieve optimal conversion of substrate into valuable products about 8 % moisture is required, where sample treated with 0.7 M NaOH had the maximum value of 6.1 % moisture.

Meanwhile, other parameters such as ash, lipids, fibre, and protein were all reduced after pretreatments. The ash contents were reduced from 22.1 % to 7.93, 8.0, 8.13 % for treatment with 0.5, 0.6, 0.7 M (NaOH) and 6.32, 7.18, 7.93 % for 70, 75 and 80 ^{o}C. These reductions are natural for biomass, since most elements absorbed during growth are decomposable at certain concentration and temperature. Microalgae often contain both macro and micro nutrients that are capable of ash formation (Dragone *et al*., 2010). Lipids were also reduced from 4.47 % to 2.27, 2.45, 2.14 % and 0.92, 2.32, 3.05 % for both chemical and thermal treated samples respectively. For fibre, the reduction was significant, that is from 8.73 % to 0.39, 0.40 and 0.42 % for 0.5, 0.6 and 0.7 M (NaOH) and 0.51, 0.5 and 0.39 % at 70, 75 and 80 ^{o}C respectively. While, proteins were reduced to 7.98, 8.12, 8.31 % and 5.62, 7.33, 8.33 % as against 11.9 % for untreated from chemical and thermal pretreated samples. Reduction in protein which happened to be the major source of nitrogen in the substrate was helpful in reducing ammonia and hydrogen sulphide inhibition within the digester system.

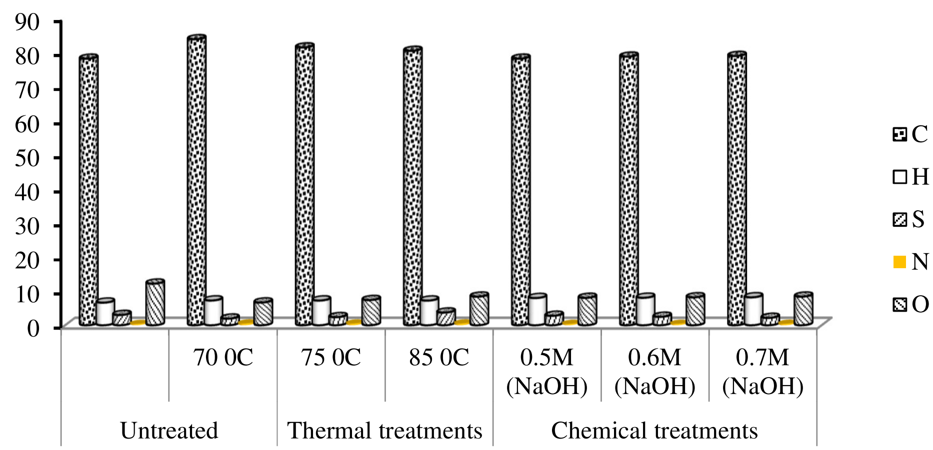


Figure 2: Ultimate analysis of untreated, thermal and chemical treated *M. vaginatus* samples

3.2. Ultimate Analysis

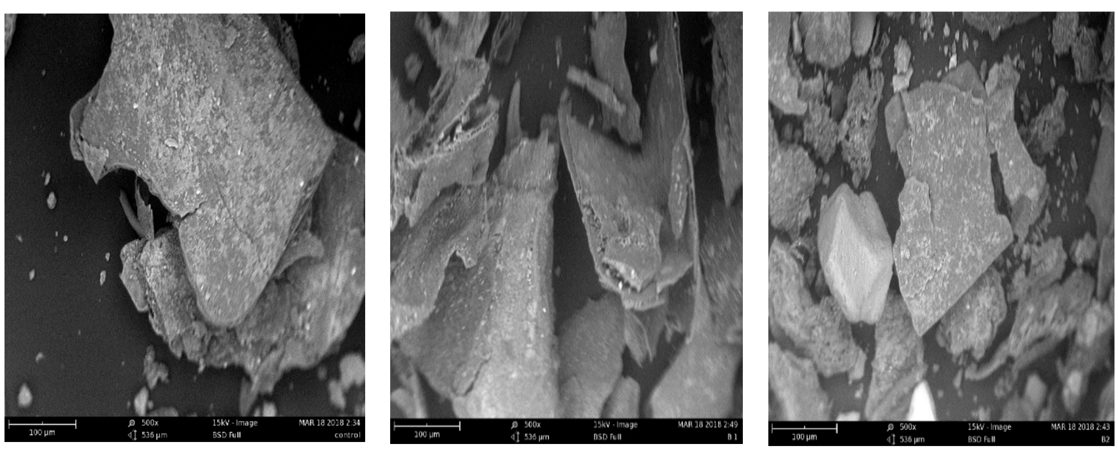
Ultimate analysis revealed the elemental composition of the substrate used and was reported on dry basis otherwise moisture content will be shown as additional hydrogen or oxygen in the results. Elements such as carbon, hydrogen, nitrogen, sulphur and oxygen are determined. The raw sample had 77, 6.58, 2.98, 1.36 and 12.08 % carbon, hydrogen, sulphur, nitrogen and oxygen respectively. These after pretreatment carbon, hydrogen, nitrogen and oxygen were enhanced and only sulphur was reduced. The carbon was enhanced from 76.0 to 77.6, 78.0, and 78.2 % for 0.5, 0.6 and 0.7 M NaOH where 82.7, 83.1 and 83.3 % were obtained for 70, 75 and 80 ^{o}C. This increase in carbon corresponds with the increase seen for carbohydrate which composed primarily of carbon, hydrogen and oxygen. The fact that, carbon in biomass correlates proportionally to the heating value of the biofuel (Bi and He, 2013) then, increase in it will be beneficial in digestion process since most of the required nutrient by the microbes for conversion to methane are of this origin.

The hydrogen values were all increased from 6.58 % to 7.21, 7.20 and 7.18 % for 70, 75 and 80 ^{o}C that is, temperature increase led to hydrogen reduction. Nielfa *et al.* (2015) reported that, if substrates with high content of hydrogen and nitrogen are reduced before digestion, it will go a long way in reducing toxic concentration of ammonia and hydrogen sulphide that are capable of inhibitory formation in anaerobic digestion. Likewise, chemical treated samples there were increased to 7.18, 7.20 and 7.21 % for 0.5, 0.6 and 0.7 M NaOH, meaning that, as treatment temperature increases hydrogen content in the substrates were reduced but increased with concentration.

The sulphur was also reduced to 1.92 % at lower pretreatment concentration of 0.5 M NaOH and as pretreatment concentration increases there were corresponding increase in sulphur to 2.35 and 3.69 % while 1.92 % at lower pretreatment temperature of 70 ^{o}C and as the treatment temperature increases to 75 and 80 ^{o}C there was corresponding increase to 2.35 and 3.69 %. Meanwhile, low nitrogen and sulphur in biomass are desirable for environmentally friendly biofuels production (Bi and He, 2013).

3.3. Scanning Electronic Microscope (SEM)

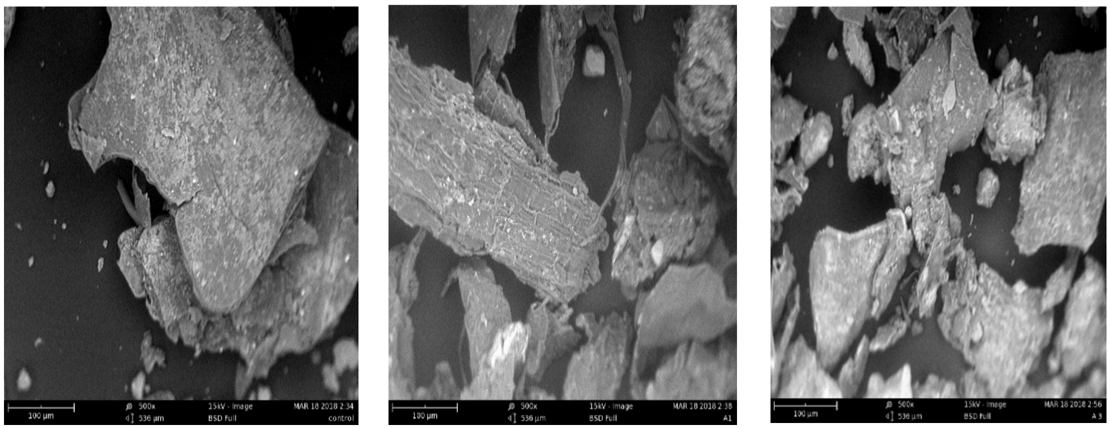
SEM images provide qualitative information on morphological distortion that might have been caused on the substrate cells composition. In this study only the minimum and maximum pretreatment conditions were considered.



(a) (b) (c)

Figure 3: SEM results of control (a) and thermal treated *M. vaginatus* at 70 ^{o}C (b) and 80 ^{o}C (c)

Figure 3 above shows the SEM visualization of thermally pretreated samples of *M. vaginatus* species of microalgae. The SEM images show that thermal pretreatment had significantly affected the substrate and hence changed the cell morphology. The thermal treatment at 80 ºC had more effect on the substrate than other treatment observed. The images taken at 100 × magnifications for control and samples treated at 70 and 80 ºC only. The SEM images shows that there was a significant morphological change after each pretreatment with more being observed on sample treated at 80 ºC. The SEM image of control sample revealed that, cells structures remained intact as the binding forces exhibited by lignin on cellulose and hemicellulose were presence. Likewise, as pretreatment temperature increased, the cell surface was disrupted with wider pore size.



(a) (b) (c)

Figure 4: SEM results of control (a) and chemical treated *M. vaginatus* at 0.5 M NaOH (b) and 0.7 M NaOH (c)

The SEM result for chemical pretreated sample also shows that, as the concentration of NaOH increases there was increase in morphological changes of the substrate. The pore sizes where increased thereby cause an equal increment in the surface area were increase in microbial accessibility to the nutrient therein are made available to the microbes. This is because, most of the substrate properties such as cellulose crystallinity had been lost, surface area increased due to lignin elimination and degree of hemicellulose acetylation.

3.4. Cumulative Biogas Yield from Untreated and Chemical Treated Samples

Figure 5 shows the cumulative biogas produced measured through the use of inverted cylinder on daily basis. The cumulative biogas yield obtained from chemically treated samples as shown in Figure 5 revealed that, sample treated with 0.5 M NaOH produced the highest volume of 7.99 mLg^{-1} VS biogas, whereas samples treated with 0.6 and 0.7 M NaOH produced cumulative biogas of 7.38 and 7.0 mLg^{-1} VS for 31 days. Increase in pretreatment concentration to 0.7 M NaOH shows higher affinity for lignin reduction from cellulose, and hemicelluloses but produced lower biogas compared to other lower concentrations tried. This could possibly be due to the loss of more volatile solids during washing to neutralize NaOH effect in digestion step. It was noted that, increase in pretreatment concentration facilitated the initial stage of hydrolysis, where more substrate broken down without equivalent methanogen formation. This could lead to an imbalanced system between hydrolytics and methanogenesic enzymes. This was in line with the report of Dai e*t al.* (2014) who noted highest yield of biogas 18 720 mL kg^{-1} from 6 % NaOH pretreated rice straw followed by 8 % NaOH 15 057 $mLkg^{-1}$, 4 % NaOH 12 103 $mLkg^{-1}$, and 2 % NaOH 10 754 $mLkg^{-1}$. This is an indication that, the higher the concentration of NaOH the lower the yield of biogas but, after certain concentration. At relatively low alkali concentrations (<4 %), structural changes for cellulose are insignificant, as glycosidic β (1,4) linkages are alkali-stable under these conditions (Knill and Kennedy, 2003). It was worth noting that as pretreatment concentration increases, more volatile solids are dissolved. Sreekrishnan *et al.* (2004) found similar results when plant residue and cattle dung are treated with alkali (1 % NaOH for 7 days) the result was almost 2 fold increase in biogas and methane as well.

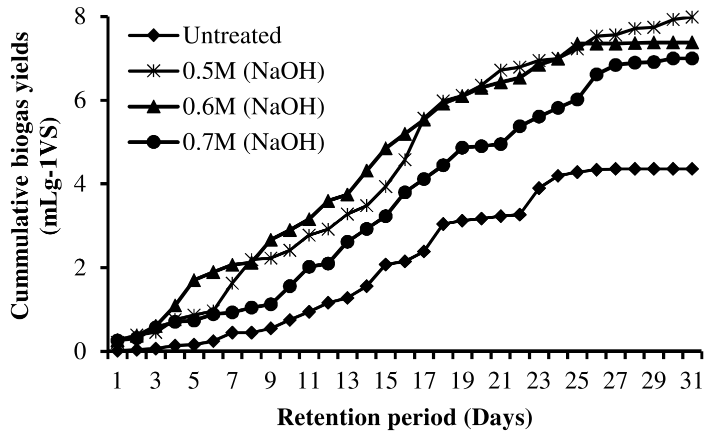


Figure 5: Cumulative biogas yield of untreated and chemical treated samples

3.5. Cumulative Biogas Yield from Untreated and Thermally Treated *M. vaginatus*

The cumulative biogas yield for untreated and thermal pretreated samples is shown in Figure 6 for the hydraulic retention period of 31 days.

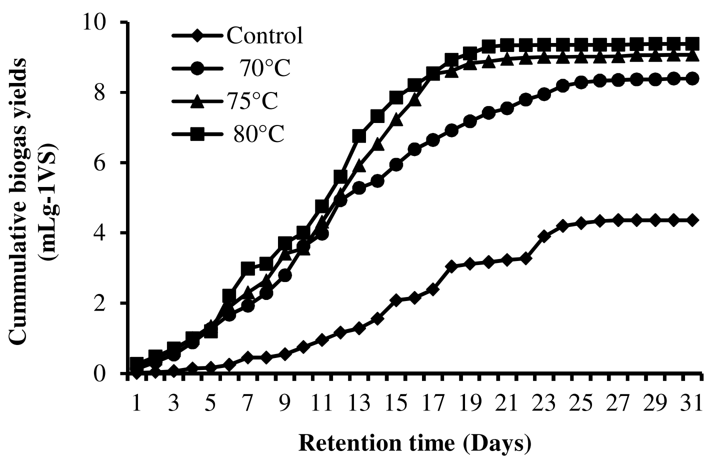


Figure 6: Cumulative biogas yields from untreated and thermal treated samples

Figure 6 presented the cumulative biogas yield for untreated 4.36 mLg^{-1} VS, and thermal pretreated samples as 8.39 mLg^{-1} VS, 9.07 mLg^{-1} VS and 9.38 mLg^{-1} VS at 70, 75 and 80 ºC equivalent to 92 %, 108 %, and 115 % higher than untreated sample. These translated into 1.9 and 2.2-fold increase compared to untreated sample. The figure showed that, more than half of the biogas produced was in the first fifteen days of digestion and more was produced by 75 ºC and 80 ºC pretreated sets. Similar study conducted on digested *Chlorella sp.* for 13 days by Wang *et al.* (2016) resulted into 87 % biogas yield. However, there are large variations in methane yields as published by many literatures due to, high dependence of the processes on microalgae species used and growth conditions.

4. CONCLUSION

The potentials of thermal and chemical pretreatment on *M. vaginatus* for an enhanced biogas production were studied. As a general conclusion, the results showed that, *M. vaginatus* can be good substrates for anaerobic fermentation. It was noted that, the two pretreatments adopted had positively affected the biogas yield and methane generation as well. Thermally pretreated sample at 80 °C gave accelerated hydrolysis, thereby resulting into more biogas yield of 9.38 mLg^{-1} VS as against 4.36 mLg^{-1} VS for untreated sample, this translated into 2.2 fold increase when compared with untreated sample. This increase could be attributed to the enhanced carbohydrate extraction of 83.7 % evidenced at 80 °C as against 47.6 % for untreated sample. This was also supported by 81% substrate biodegradation achieved at 80 °C when compared with 19 % for untreated sample. Thermal pretreatment was excellent method for the enhancement of biogas and methane yield as well from *M. vaginatus* since other pretreatment temperatures of 70 °C and 75 °C also resulted in an improved biogas production over chemical and raw samples studied. For chemically treated samples the maximum yield of 7.99 mLg^{-1} VS biogas was obtained from 0.5 M NaOH which gave rise to 1.9 fold over untreated sample. The substrate treated with 0.5 M NaOH resulted in more biogas and methane yield as well. The volatile solids conversion to methane and carbon dioxide from 0.5 M NaOH pretreated sample was found to be 64.7 % higher than that of untreated substrate. However, each concentration of NaOH used affect in many ways to create opportunities for an enhanced process of biogas production. That is change in reagent concentration and temperature played significant role in changing the substrate structure thereby improved access of enzymatic hydrolysis to cellulose and hemicellulose. Therefore, effective pretreatment of biomass is characterized by reduction in particle size, increase in surface area, disruption of cellulose and hemicelluloses crystallinity, lignin redistribution and changes in compositional morphology to give an enhanced biogas yield at reduced retention period.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

REFERENCES

Ali, F. U. and Ogbonna, J. C. (2011). Suitability of Nigerian Weather Conditions for Cultivation of Microalgae. *Nigerian Journal of Biotechnology*, *22*, pp. 60-65.

Aminu, N. M., Tijjani, N. U. and Aladire, Y. Y. (2013). Overview of Biodiesel Production from Algae in Nigeria and Some Developing Countries. *International Journal of Scientific & Engineering Research*, *4*(1), pp. 277-286.

Bi, Z. and He, B. B. (2013). Characterization of microalgae for the purpose of biofuel production. *Transactions of the ASABE*, *56*(4), pp. 1529-1539.

Bougrier, C., Albasi, C., Delgenès, J. P. and Carrère, H. (2006). Effect of ultrasonic, thermal and ozone pre-treatments on waste activated sludge solubilisation and anaerobic biodegradability. *Chemical Engineering and Processing: Process Intensification*, *45*(8), 711-718.

Cantarella, M., Cantarella, L., Gallifuoco, A., Spera, A. and Alfani, F. (2004). Effect of inhibitors released during steam-explosion treatment of poplar wood on subsequent enzymatic hydrolysis and SSF. *Biotechnology progress*, *20*(1), pp. 200-206.

Chang, S. (2014). Anaerobic membrane bioreactors (AnMBR) for wastewater treatment. *Advances in Chemical Engineering and Science*, *4*(1), pp. 56-61.

Chen, C. Y., Yeh, K. L., Aisyah, R., Lee, D. J. and Chang, J. S. (2011). Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. *Bioresource technology*, *102*(1), pp. 71-81.

Chen, C. Y., Zhao, X. Q., Yen, H. W., Ho, S. H., Cheng, C. L., Lee, D. J. and Chang, J. S. (2013). Microalgae-based carbohydrates for biofuel production. *Biochemical Engineering Journal*, *78*, pp. 1-10.

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology advances*, *25*(3), pp. 294-306.

Park, J. B. K., Craggs, R. J. and Shilton, A. N. (2011). Wastewater treatment high rate algal ponds for biofuel production. *Bioresource technology*, *102*(1), pp. 35-42.

Dai, B. L., Zhu, A. F., Mu, F. H., Xu, N. and Wu, Z. (2014). Experiments on anaerobic digestion of rice straw for biogas production under NaOH pretreatment. *Advanced Materials Research*, 953, pp. 220-223.

Dragone, G., Fernandes, B., Vicente, A. A. and Teixeira, J. A. (2010). Third Generation Biofuels from Microalgae, pp. 1355–1366.

Horváth, I. S., Tabatabaei, M., Karimi, K. and Kumar, R. (2016). Recent updates on biogas production - a review. *Biofuel Research Journal*, *10*, pp. 394–402.

Knill, C. J. and Kennedy, J. F. (2003). Degradation of cellulose under alkaline conditions. *Carbohydrate Polymers,* 51(3), pp. 281-300.

Murphy, J. D., Drosg, B., Allen, E., Jerney, J., Xia, A. and Herrmann, C. (2015). *A perspective on algal biogas* (pp. 5-35). IEA Bioenergy.

Nielfa, A., Cano, R., and Polanco, M. F. (2011). Theoretical methane production generated by the co-digestion of organic fraction of municipal solid waste and biological sludge. Biotechnology Reports. 2015; 5: 14–21. PMid: 28435805 PMCid: PMC5374264. *DEStech Publications, Inc*, *18*, 60-68.

Phukan, M. M., Chutia, R. S., Konwar, B. K. and Kataki, R. (2011). Microalgae Chlorella as a potential bio-energy feedstock. *Applied Energy*, *88*(10), pp. 3307-3312.

Schenk, P. M., Thomas-Hall, S. R., Stephens, E., Marx, U. C., Mussgnug Posten J.H.C., Kruse, O. and Ben Hankamer, B (2008). Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production. *Journal of BioEnergy Research,* 1(1), pp. 20-43.

Sreekrishnan, T. R., Kohli, S. and Rana, V. (2004). Enhancement of biogas production from solid substrates using different techniques—a review. *Bioresource technology*, *95*(1), pp. 1-10.

Studer, A. (2012). *Encyclopedia of radicals in chemistry, biology and materials*. John Wiley and Sons.

Ukonu, C. U. (2011). *Optimization of biogas production using combinations of saw dust and cow dung in a batch anaerobic digestion bioreactor*. Masters thesis, University of Nigeria Nsukka.

Wang, M., Lee, E., Dilbeck, M. P., Liebelt, M., Zhang, Q., and Ergas, S. J. (2017). Thermal pretreatment of microalgae for biomethane production: experimental studies, kinetics and energy analysis. *Journal of Chemical Technology & Biotechnology*, *92*(2), pp. 399-407.