

Effect of Thermal Pretreatment on the Yield of Biogas from *Microcoleus Vaginatus*

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ABSTRACT: Biomass is being looked upon as one of the promising renewable energy sources for the future, with growing interest in microalgae conversion into biogas through anaerobic digestion. Recently, the ability of microalgae to treat waste water has doubled its potentials material today. However, in spite of the progress made in that regards, there are still challenges of algae conversion to biofuel, due to the presence of complex cell wall in some algae. Cell wall inhibits bacteria growth during degradation. In this research work 10 grams of *Microcoleus vaginatus* was treated in an oven at varying temperatures of 70, 75 and 80 °C for an hour, out of which 4 g was measured into 250 ml serum bottle for digestion at mesophilic temperature of 37 °C. Based on the results of proximate analysis, 69% increase in carbohydrate was attained with 72.7 – 148% reduction in moisture content. The biogas yield of untreated sample was 4.36 mLg⁻¹ VS, while, pretreated samples at 70, 75 and 80 °C produced 8.39, 9.07 and 9.38 mLg⁻¹VS (volatile solid) of biogas. This corresponds to 92, 108 and 115% higher than that of untreated samples. However, thermal treatment of *M. vaginatus* prior to digestion show positive effect on carbohydrate extraction and enhanced biogas and methane yield as well. Therefore, this makes the substrate a good feedstock for biogas production.

KEYWORDS: Biomass, pretreatment, thermal, anaerobic digestion, degradation, *Microcoleus vaginatus*.

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I. INTRODUCTION

Environmental degradation caused by fossil fuels on human habitat and life had necessitated the need for alternative sources of energy that will be ecofriendly and cost effective. In that regards many research interest have been drawn to renewable energy sources (Chen *et al.*, 2011). Though, it is apparent that urgent need for alternative as well as cheaper sources of energy in developing countries of the world is long overdue, due to hardship posed by militancy, piracy and corruption on fossil facilities. On that note many rural community are forced to use wood as a major source of energy. But, cost, scarcity and government regulations on the wood resources had make access to wood difficult as well. According to Ukono (2011) between 1999 and 2011 the price of petroleum products had skyrocketed to more than 120 % where several efforts by the government to restore normalcy in that sector have not made any appreciable impact, as fuel prices are rising on daily basis.

However, harnessing other forms of energy obtainable from Sun, Wind, Waves, Tidal, Geothermal or Biofuels will go a long way in reducing if not solving the problems of fossils completely. Phunkan *et al.*, (2011) found the use of biofuel over other alternatives more recommendable due to its low capital and technological requirement during establishment. Chen *et al.*, (2009) found promising potential in microalgae when used for biofuel production since it is known for high yield of biogas, low competition with food for arable land during cultivation and can be grown on land even unfit for cultivation. These potentials traced to microalgae brought about research interest to microalgae species with large

amounts of metabolites to improve bioethanol, biodiesel or biogas production (Dragone *et al.*, 2010).

Currently, the quantity of materials used for biogas production is limited. It then calls for new substrates and effective technology to facilitate growth in biogas industry (Horváth *et al.*, 2016). This is more pronounced in developing countries of the world, where consumer's demands are yet to be met. (Aminu *et al.*, 2013). Energy is known as one of the bedrock for socio-economic development of every society. Chisti (2007) stressed the need for microalgae conversion to energy because of its potentials of rapid growth in water bodies, CO₂ bio-fixation (main carbon source for growth), low water demand for cultivation, no competition with food production, can be grown in different environments, the required nutrients for growth may be obtained from environment unfit for normal cultivation such as wastewaters, they have high oil content (20-25% dry matter), they can be used as food or fertilizers and its biochemical composition can be modulated by different growth conditions (Chisti, 2007; Sheehan *et al.*, 1998, Schenk *et al.*, 2008; Phukan *et al.*, 2011; Lakaniemi *et al.*, 2011).

Passos *et al.*, 2014 studied the influence of thermal and chemical pretreatment on microalgae conversion to bioenergy and revealed that, thermal and chemical pretreatment of microalgae biomass gave an improved digestion and bioenergy as well. Andrés *et al.*, (2014) studied the effect of thermal pretreatment on fermentable sugar production of *Chlorella Vulgaris*. In another work, Passos *et al.*, (2015) compared different pretreatment methods of thermal, hydrothermal, microwave and ultrasound to improve microalgae digestion. The results showed that, organic matter solubilisation was

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higher for thermal pretreatment (20 - fold) when compared to hydrothermal (9 - fold), microwave (8 - fold) and ultrasound (7 - fold) pretreatments

These derived benefits from microalgae gave it the status “environmental friendly” for large-scale production of biogas. But, it is imperative to understand the physical and chemical properties of the species involved, since it is one of the critical tools, to the development of an appropriate process for a large scale production. Meanwhile, this work seeks to utilize *Microcoleus vaginatus* as a feedstock for fermentative anaerobic digestion, through critical study of thermal pretreatments for the enhancement of biogas production. The relative ease at which this algae specie can be identified, its abundance in biocrust and diverse method of its isolation and cultivation makes it a preferred choice for this study.

II. MATERIALS AND METHODS

A. Materials

The strain of *M. vaginatus* used was obtained from River Landzun in Bida Local Government Area of Niger State, Nigeria. Glass capillary tube was used for isolation and gently transferred into sterile water for serial dilution. The isolated strain was cultured at the Unit Operation Laboratory, Chemical Engineering Department, Federal Polytechnic, Bida. The culturing medium was set as the natural algae habitats because of its sensitivity to physiological conditions of temperature, pH and salinity Ogbonna, (2011). Each cultured medium was grown for 14 days before it was harvested through filtration on filter cloth and then sun dried for 3 days. The dried samples were kept in an air tight polythene bags before the subsequent stage. 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 for pre-treatment before digestion.

B. Thermal Pretreatment Process

The thermal pretreatment was conducted in an oven at constant time of 1 hour and varying temperature of 70, 75 and 80 °C. Eight grams each of *M. vaginatus* was measured on to metallic containers labelled A1, A2, and A3 then placed in the oven for an hour each at varying temperature. The samples were then removed from the oven and transferred into three digesters for onward digestion as described by Passos *et al.*, (2013).

C. Anaerobic Digestion

A batch process of anaerobic digestion was adopted in 250 ml plastic bottle with the slurry temperature maintained at 37 °C in a water bath. Four grams each of pretreated samples was measured into 250 ml digester. Then, 2 g of fresh cow dung thoroughly mixed with 10 ml of deionized water was then poured into each digester. In order to provide substrate to liquid ratio of 1:10, another 50 ml of deionized water was added to each digester and the remaining headspace in the

digester was for the gas generated. Digesters C was charged with 4 g of untreated *M. Veginatus*, 2 g of cow dung and 60 ml of deionized water, while the reference sample B was charged with 2 g of cow dung and 60 ml of water.

The digester sample was made in duplicate and thoroughly shaken to provide homogenous slurry (Murphy *et al.*, 2014). The deionised water added was added to dissolve substrate and to provide conditions favourable for microorganisms that fed on the substrate. The digesters were properly sealed with adhesive gum to obtain an air free environment (Poole *et al.*, 2014). Proximate, Ultimate, and Scanning Electron Microscope analyses were conducted before, and after pre-treatment on dry basis. The pH and ambient temperature were measured daily. While, daily gas productions were measured through inverted cylinder and the duplicate samples collected in gas bags were analysed with gas sampler connected to a gas chromatography unit.

The proximate composition of *M. vaginatus* was determined according to AOAC methods (AOAC, 1990). For the moisture content the sample was allowed to stay in an oven at 100 - 105 °C for 6 – 12 hrs until a constant weight was obtained. Then, the crucible was placed in a desiccator for 30 mins to cool. The ash was determined after putting the sample in a crucible and placed over a burner until it charred. Then, the crucible was placed in muffle furnace for ashing at 550 °C for 2 – 4 h. Appearance of gray white ashing indicates complete oxidation of all organic matter in the sample. Crude protein content was measured by a standard Kjeldahl method using 5.95 as the conversion factor. The content of lipids in *M. vaginatus* was analysed by the solvent extraction procedure. Total carbohydrates were determined by the difference of all the proximate values. The complete flow process is illustrated in Figure 1.

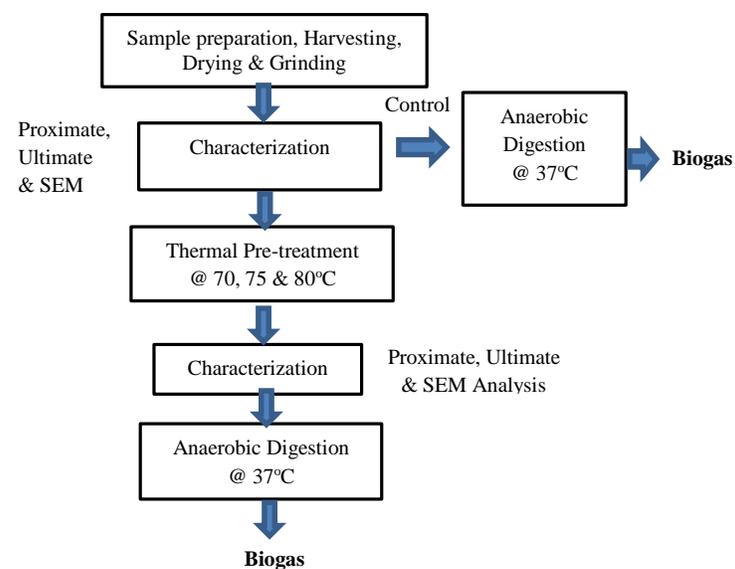


Figure 1: Process flow diagram.

III. RESULTS AND DISCUSSION

A. Characterization of Substrate Biomass

The nutrients and morphological analysis of treated and untreated *M. vaginatus* was carried out through Proximate, Ultimate and SEM analysis of the samples. Likewise, biogas produced was characterized and microbial counts of the slurry digestate before and after the digestion were also observed. Figure 2 reveals the nutritional properties of the substrate. Properties such as fat, ash, moisture, protein, crude fibre and carbohydrate were determined and shown.

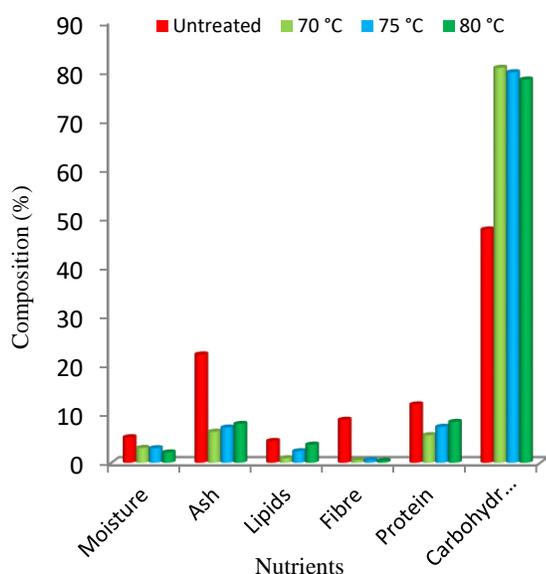


Figure 2: Proximate analysis of *M. vaginatus* before and after thermal pretreatment.

The proximate analysis revealed that, *M. vaginatus* contain 5.2% moisture, 22.1% ash, 4.47% lipids, 8.76% crude fibre, 11.9% protein and 47.6% carbohydrate. The pretreatment at 70, 75 and 80 °C has increased the carbohydrate extraction to between 72.2 – 80.6% and gave rise to 69.3% improvement over control sample. Miranda et al., (2012) reported that variation in biomass concentration can result in variation in the amount of sugar that will be released. The water removed by the microspores of the cellulose in the algae cell wall brought about irreversible changes in the structure to form amorphous area in which the voidage between the elasticity is reduced making the fibre more susceptible to attack by acids that was formed during the digestion process.

The substrate moisture content was reduced from 5.2 to 3.01%, 2.96, and 2.10% at 70, 75 and 80 °C respectively. This implies that, raise in temperature increases the rate at which the moisture can be removed from the sample as evidenced with the findings of (Silva et al., 2008; Zou et al., 2010; & Chen et al., 2011). Anaerobic digestion requires a certain level of moisture in feedstock for efficient substrate conversion as large-scale production requires 8 to 10 % wt moisture in the feedstock. The ash content of all the treated samples were reduced from 22.1% to 7.32, 7.20 and 7.18 at 70, 75 and 80 °C, respectively. According to Bi & He (2013) green microalgae with low ash content of less than 10 % is preferred to brown or mixed brown microalgae with high ash content of 43 %wt;

high level of ash have negative effect when processed for biofuels. Some microalgae species have high level of lipids and, that is why they are used for bio-diesel production (this is not as clumsy as what is there before). High level of lipids in some microalgae species gave rise to the advantage of being used in biodiesel production. Ultimate analysis gives report of elemental composition of the substrate used, elements such as carbon, hydrogen, nitrogen, sulphur and oxygen were determined.

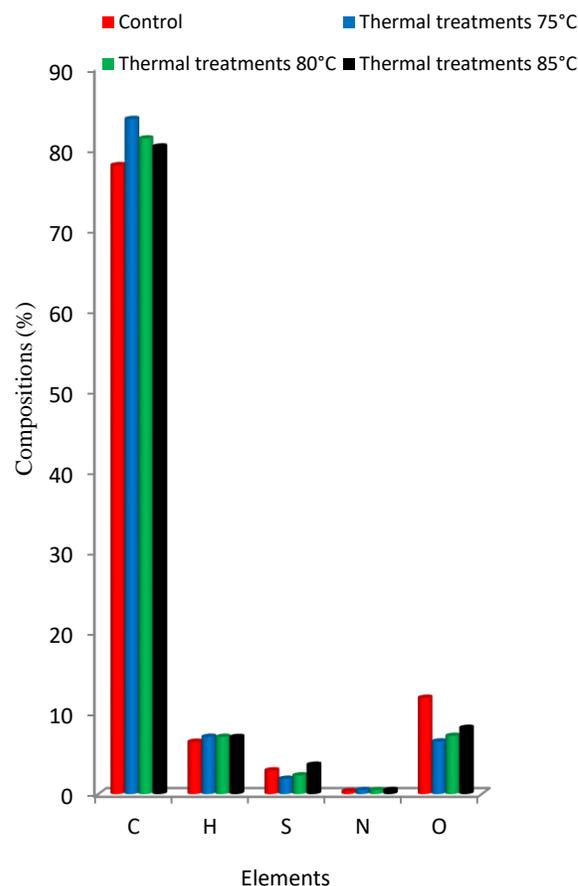


Figure 3: Ultimate analysis of thermal pretreated samples.

Figure 3 revealed that raw sample contains 77% carbon, 6.58% hydrogen, 2.98 % sulphur, 1.36% nitrogen and 12.08% oxygen. The carbon content for thermally treated samples were enhanced from 77% to 82.7, 83.1 and 83.3% at 70, 75 and 80 °C respectively. This increase was due to the fact that, temperature can reduce the binding forces of lignin to release simple sugar from the substrate used. The increase in carbon corresponds with the one noticed in carbohydrate (starch and cellulose) through proximate analysis. The carbohydrate primarily comprised of carbon, hydrogen and oxygen. According to Bi and He (2013) carbon content in biomass correlates proportionally to the heating value of the expected biofuel from the substrate.

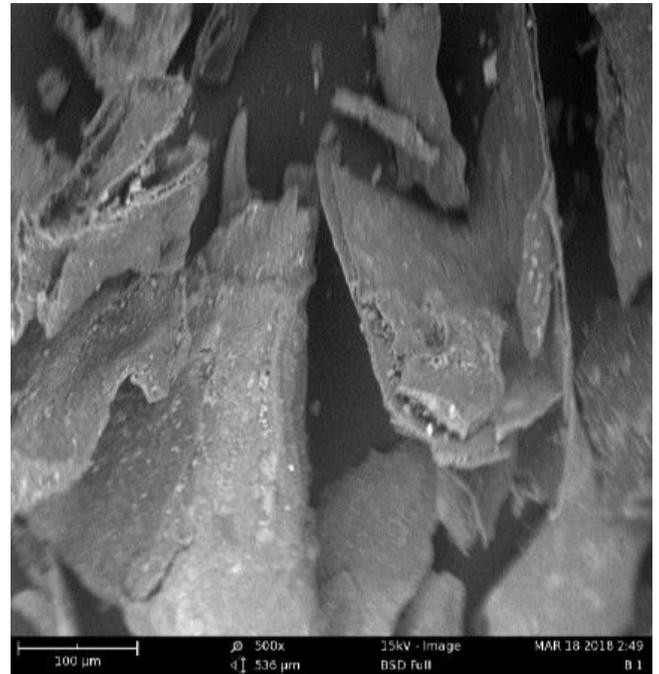
Also shown in Figure 3 is that hydrogen was 6.58% before pretreatment, this was close to 7.1 % hydrogen found in *Chlorella vulgaris* as reported by Bi and He (2013). It was then increase to 7.21, 7.20 and 7.18% after treatment at 70, 75 and 80 °C. An indication that, increase in temperature caused a

decrease in hydrogen content. Nielfa and Cano (2015) reported that, substrates with high hydrogen and nitrogen content promotes toxic concentration of ammonia and hydrogen sulphide in the digester. The initial content of sulphur was 2.98 % then reduced to 1.92 % at lower pretreatment temperature of 70 °C, and as the pretreatment temperature increases there was an increase to 2.35 % and 3.69 % at 75 and 80 °C.

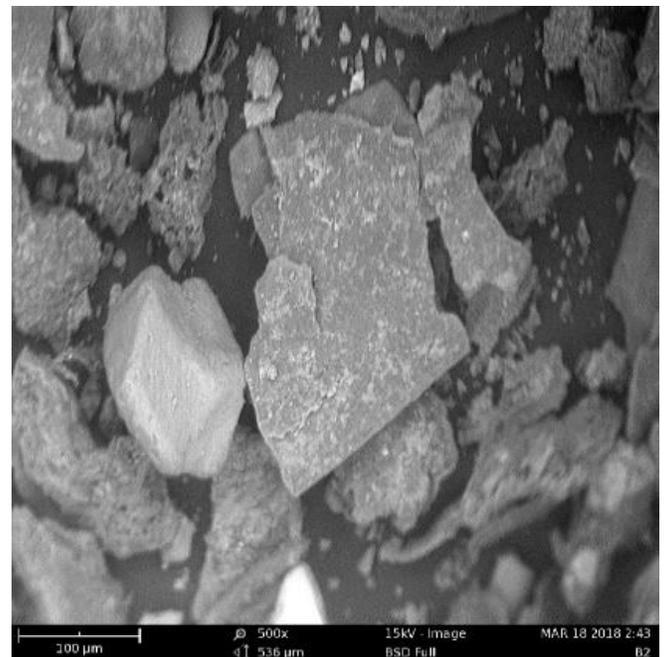
High protein content in anaerobic substrates implied high nitrogen content that was not desirable in biogas production. However, low nitrogen and sulphur is more desirable for environmental friendly biofuels (Bi and He, 2013). Untreated sample of *M. vaginatus* had 1.36 % nitrogen and increase with pretreatment temperature to 1.51 %, 2.32 % and 2.87 % at 70 °C, 75 °C and 80 °C respectively. This increase in nitrogen after pretreatment was beneficial in stabilizing C:N ratio during digestion. According to Jard *et al.*, (2013) optimum levels of C:N ratio for biogas production are in the range of 20:1 to 30:1. While, enzymatic activities on nitrogenous substrates with C:N ratio less than 15 can cause formation of ammonia in the digester thereby raise the level of ammonia in digester system to cause microbial inhibition (Allen *et al.*, 2013). Protein is the primary source of nitrogen concentrations and is low in brown seaweeds but, high in red and green seaweeds (Jard *et al.*, 2013). Thus, it is therefore better to use either red or green sea weed for efficient biogas production.

Usually, oxygen affect heating value in an inverse manner, an indication that high amount of oxygen is not desirable when biofuels with higher energy density is required. Anaerobic digestion is a complex process that involves biochemical reaction by several types of microorganisms that requires no oxygen to survive. The oxygen inhibits the methanogens which convert other intermediate compounds formed into methane. Therefore, it is essential to maintain an oxygen reduced or free environment for an enhanced methanogenic growth.

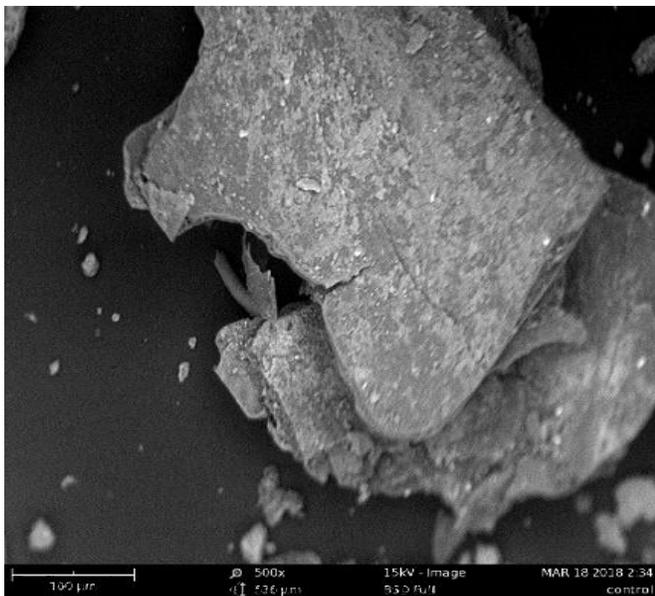
SEM analysis uses a focused beam of high energy electron to generate signal at the surface of solid particle. The carbohydrate which housed the cellulose and hemicellulose are decomposed from lignin by thermal pretreatments and resulted into substrate weaken and inter wall cracks provide an improved biomass degradation. Therefore, in order to ascertain this thermal effect on the samples, SEM analysis of the minimum and maximum pre-treatment temperature was conducted to compare the effect on composition and substrate morphology before and after pretreatment as shown in Figure 4.



(b)



(c)



(a)

Figure 4 shows the SEM visualization of thermally treated *M. vaginatus*. The images revealed that thermal treatment had significant effect on the nutrients and cell morphology of the substrate. The structural distortion at 80 °C was more evident over that of 70 °C as observed. The images were taken at 100 × magnification for all samples. There were significant structural changes observed after each pretreatment process than untreated, with more dispersion observed on sample pre-treated at 80 °C. The cells structures of control sample remained intact, since the lignin effect in binding cellulose and hemicellulose together were still effective. While in contrary, increase in pretreatment temperature lead to an increase in cell disrupted resulted into a wider pore sizes. According to Cooney *et al.*, (2011) temperature destroyed surface crystallinity to form amorphous material. It therefore, suggests that the pretreatment temperatures used on *M. vaginatus* had significantly affected the cell structure since more disintegration are observed as temperature increases between 70 °C and 80 °C. Therefore, it is worth of note that treatment at 80 °C shown more tendencies for breaking the glycosidic bond in cellulose, hemicellulose and lignin to allowed bacteria access to the nutrient contained in the substrate via wall pores.

C. Biogas Yield

From Figure 5, the biogas yield of untreated sample was 4.36 mLg⁻¹ VS. The pretreated samples at 70 , 75 and 80 °C resulted in biogas yields of 8.39 mLg⁻¹ VS (92 %), 9.07 mLg⁻¹ VS (108 %) and 9.38 mLg⁻¹ VS (115 % higher than untreated) respectively. More than 5 mL of biogas produced for all the treated samples was in the first 10 days of digestion, while, the remaining were obtained in the remaining period of retention. Thermally treated sample at 80 °C produced biogas with a higher methane (CH₄) content. The CH₄ content of the biogas from digestion of the untreated microalgae was 44 %, which was similar to the study conducted by Wang *et al.*, 2013.

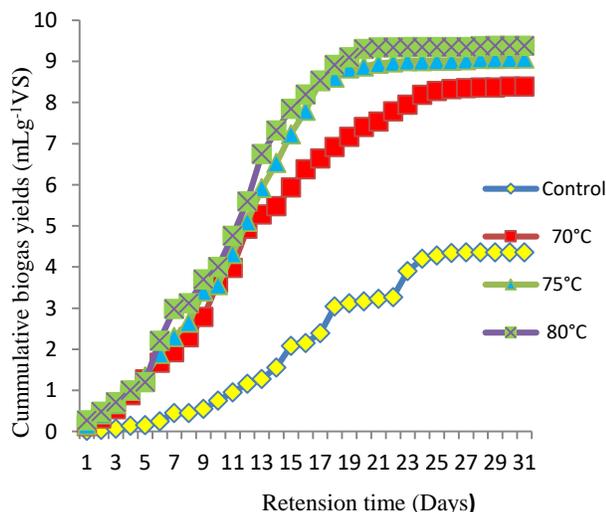


Figure 5: Biogas yield from untreated and thermal pretreated *M. vaginatus*.

D. Biogas Characterization

Biogas obtained after fermentation is usually a mixture of gases among which methane (CH₄) is more useful as energy sources. Generally, biogas consist 55 - 80 % methane and 20 - 45 % carbon dioxide (CO₂), with other gases such as hydrogen sulfide (H₂S) 0-3% and 0 - 1 % hydrogen, nitrogen and ammonia. However, depends on the substrate type and process management involved of the organic matter and the management of the anaerobic digestion process, small amounts of other gases such as ammonia (NH₃), hydrogen sulfide (H₂S) and water vapour (H₂O).

Table 1: Characterization of biogas produced from *M. vaginatus*.

S/N	Compositions	Control	Pretreatment temperatures (°C)		
			70	75	80
1.	CO ₂	39.72	27.90	21.48	14.72
2.	CH ₄	19.34	65.80	71.90	79.81
3.	H ₂	14.83	3.05	2.92	1.23
4.	Others	26.11	3.25	3.70	4.24

Table 1 revealed that, sample treated at 80 °C produced the highest volume of methane 79.81 % CH₄, and this was followed by 71.9 % and 65.8 % CH₄ for 75 °C and 70 °C, respectively.

V. CONCLUSION

In this work, 10 grams of *Microcoleus vaginatus* was treated in an oven at varying temperatures of 70, 75 and 80 °C for an hour. Of this, 4 g was measured into 250 ml serum bottle for digestion at mesophilic temperature of 37 °C. The following conclusions were made;

- (i.) Thermal pretreated *M. vaginatus* showed positive effect on carbohydrate extraction and it was observed that an increase in temperature resulted in enhanced extraction.
- (ii.) Hydrolysis during substrate biodegradation was also eased and enhanced, leading to increased biogas yield. The highest value of biogas was obtained at 80 °C with methane production rate of 73 mL/VVS. The biogas produced has an average methane content of 70.25 % mol.
- (iii.) If the potentials of algae biogas is properly harnessed, it could serve as a viable option to other sources of energy such as petrol, coal and natural gas can be found.
- (iv.) The use of *M. vaginatus* for biogas production contribute to sustainable development as algae cultivation required almost equal volume of CO₂ emitted during combustion for its growth. The digestate obtained after digestion should be used for algae cultivation to reduce nitrogen formation during digestion. Blend or co-digestion of *M. vaginatus* with other carbon rich material can also be helpful.

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