International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Efficacy of Cellulose Degrading Microbial Strains on the Decomposition of Sewage Water Microalgae for the Production of Biogas

Mekbib Sissay Bekele[a]*, Tebello Samosamo[b], Ayele Abebe Seyoum[c] , Mosotho J. George[d]

[a,b]Department of Biology, [d]Department of Chemistry and Chemical Technology

National University of Lesotho, P. O. Roma 180, Lesotho

[c]Biofuel Research Project, Hydraulics Engineer

[a]Email: sissaybekele@gmail.com / sbmekbib@nul.ls

[b]Email: samosamo.t@gmail.com

[c]Email: abetse2005@yahoo.com

[d]Email: maluti2005@gmail.com

Abstract

Sewage water microalgae are potential sources of feed stock for the production of biofuel. In this study, six treatment combinations of microbial strains (C, N_1 and N_2) in single and combined treatments were used in a 5L plastic container as anaerobic digester. The use of sewage water microalgae alone was used as a negative control; and a separate application of effective microorganisms (EM) were used for comparison. A volume of 3L sewage water suspension containing 300g of microalgae biomass were used in all plastic containers as a feedstock. All treatment applications including the control showed some activity of pressure development in each bag per treatment per cycle except the combined treatment of strain (C, N_1, N_2). Significant volume of biogas production (4-5L/300g)) and pressure development (517.80 and 544.35 Nm^{-2} was observed in the first cycle when using cellulose degrading microorganism (C) alone and the combined treatment with nitrogen fixing strain (N_1), respectively.

---

*Corresponding author,

E-mail address: bekelesissay@gmail.com / sbmekbib@nul.ls

High efficacy of shortening the retention time was observed while using strain C alone and combined treatment of C+N_1 compared to other treatments including the control. Overall, strain C exhibited an increase of the final gas volume by 148.6% utilizing 60% of the microalgae biomass compared to the control. These results highlight the importance of the tested strains on efficiency of converting these algae to biofuel. However, further investigation of strain efficiency in a pilot scale application, outside the laboratory is recommended.

Keywords: Bioenergy; Sewage water; Microalgae; Cellulose degrading microorganisms; Renewable energy

1. Introduction

In the world today, more than 85% of the energy source is derived from burning of fossil fuels, which is a serious predicament to the environment by increasing the Green House Gas [1]. Cutting of trees for domestic fuel worsens the situation in Least Developed Countries by increasing the desertification and eliminating bio-diversity of indigenous and exotic tree species.

While securing energy requirements, establishment of environmentally compatible technologies that can support sustainable use of natural resources to reduce environmental and health impacts of fossil fuels and desertification are important. The use of microalgae as a feed stock either from fresh water [2] or community sewage water [3, 4] is an important biotechnological option to fossil fuels and forest vegetation for the production of biofuel and biofertilizer. Since it doesn't compete with agricultural land and food price, it has the ability to sequester large quantities of carbon dioxide being a carbon sink [5].

In various experimental applications, the use of microbial strains for the production of biofuel from microalgae decomposition have been shown to have great advantage over the application of chemicals and pyrolysis (6-8). The selection and culture maintenance of suitable and efficient cellulose degrading, acidifying and methanogenic microorganisms is therefore an important task towards the successful implementation of the technology for its economic advantage and sustainability in agriculture inclusive project when used fresh water pond system on marginal lands for cultivation of microalgae and aquaculture [9].

The present study was therefore designed to determine the efficacy of selected microbial strains in the decomposition of sewage water microalgae and demonstrate their significant effect in increasing the biogas volume with concomitant decrease of the retention time in simulated laboratory scale anaerobic digester.

2. Materials and Methods

2.1 In vitro treatment application and decomposition of sewage water microalgae

Three potential decomposing strains (C, N_1 and N_2) obtained from the Department of Biology culture collection, at the National University of Lesotho (NUL) were used in this experiment under various treatment combinations on sewage water microalgae decomposition. In total, 11 different treatment applications in single and/or combination were used under laboratory and greenhouse applications. The following treatment applications as single: C, N_1, N_2; combination of two: C+N_1, C+N_2, N_1+N_2; and combination of three: C+ N_1+N_2 were used in

this experiment. Separate application of Effective Microorganisms (EM); and the sewage water and microalgae biomass alone were used as a positive and negative controls, respectively. Treatment applications of EDTA (0.5M) 50ml alone, and combined applications with strain C alone and N_1 alone were used as separate treatments (Data not shown). In each treatment, 5mL of each strain suspension were inoculated as per a given treatment. To agitate and mix samples, treatments with EDTA were placed on an orbital shaker (70rpm) for 7 days at 30°C. Data were collected at regular intervals within the time frame indicated.

2.2 Digester assembly and incubation

Eleven 5L capacity plastic water containers were used as simulated laboratory scale anaerobic digester; and 1L capacity dry infusion bags were fitted as gas collector to each of the digester were used in this experiment (Figure 1). The tight fitting lids of the containers were carefully punctured at the center and connected with a tube to collect the gas produced in the chamber. A series of tubes and needles were used to connect each digester to a 1L capacity infusion bag for gas collection. Careful folding of the tubes was ensured to prevent any kinks which may impede gas transfer. Anaerobic digester containers receiving microbial and/or physical treatments were incubated at the temperature maintained between 27-30°C and daily inspection of gas production were done by observing the swelling of the collector bags fitted.

2.3 Process optimization and methane production

The efficiency and life cycle (retention time) of the system was assessed as the reaction progressed. The temperature was maintained at (27±2°C) throughout the fermentation process under laboratory and glasshouse experimentations [10]. Assessments for production of methane were done by flame test.

3. Results

3.1 Pressure development per cycle per treatment

All treatment applications including the control showed some activity of pressure development in each bag per treatment per cycle except the combined treatment of the three strains (Figure 1 &Table 1). The combined application of strain (C) and (N1) showed the highest activity in the first cycle (Table 1). Treatment application of strain (C) showed successive production of pressure but, in decreasing order (Table 1). Other treatment applications with no gas production are not included.

3.2 Qualitative analysis (flammability tests) of the biogas

The gas volume had to reach a threshold level of 469.4 mL in order to develop pressure as determined in this experiment (Figure 2). Correlation of the volume of gas and the amount of pressure produced per treatment showed a significant correlation (R^2 = 0.9500) as can be seen from Figure 2 and quality of biofuel produced Figure 3.

Figure 1. Simulated lab scale plastic anaerobic digester set and biogas production with various treatments: Control, C, C+N_1, C+N_2 and C+N_1+N_2.

Table 1. Pressure development in each bag per treatment per cycle

Code	Treatments	Pressure generated per cycle (Nm^{-2})			
		Cycle 1	Cycle 2	Cycle 3	Cycle 4
C1	(T1) S_{10} Cellulose degrading strain + Sewage sludge	517.80	371.75	292.09	159.32
N1	(T2) $S_{3\text{-}3}$ Nitrogen fixing strain + Sewage sludge	398.31	345.20	132.77	0
N2	(T3) $S_{4\text{-}4}$ Nitrogen fixing strain + Sewage sludge	451.41	345.20	0	0
CN1	(T4) S_{10} +$S_{3\text{-}3}$ strains + Sewage sludge	544.35	0	238.98	0
CN2	(T5) S_{10} + $S_{4\text{-}4}$ strains + Sewage sludge	424.86	0	345.20	0
CN1N2	(T6) S_{10} + $S_{3\text{-}3}$ + $S_{4\text{-}4}$ strains + Sewage sludge	0	146.05	106.21	0
EM	(T7) S_{EM} (Effective Microorganisms) strains + Sewage sludge	92.94	0	26.55	0
Control	(T8) Sewage sludge only	26.55	0	79.66	0

Legend: The zero values in Table 1 refer to the absence of pressure though there was some volume of gas in the bag.

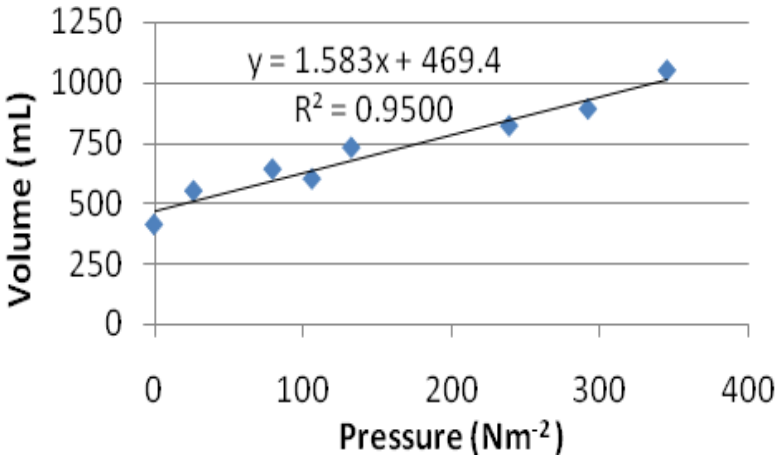

Figure 2. The correlation between pressure and gas volume.

Figure 3. Flame test for biogas production.

3.3 Determination of biogas volume

The volume of biogas produced was determined after five cycles (each cycle having 5 days of duration) and expressed as cumulative effect for the period of 25 days fermentations process (Figure 4). The application of cellulose degrading strain (C) showed the highest volume of gas production compared to other treatments (Figure 4).

3.4 Algae biomass decomposition rate

All treatment applications including the control showed some activity of microalgae biomass reduction in the process of decomposition (Figure 5). Treatment application with the use of cellulose degrading strains (C) showed the highest biomass reduction percentage (60.9%) compared to other treatments (Figure 5).

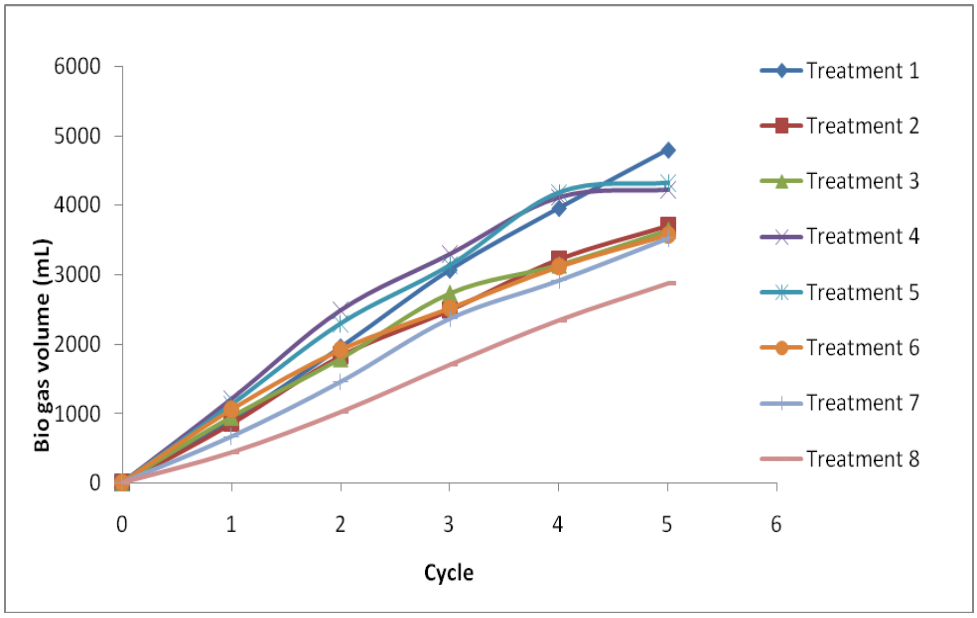

Figure 4. Cumulative biogas production as a function of time. For treatment designations refer Table 1.

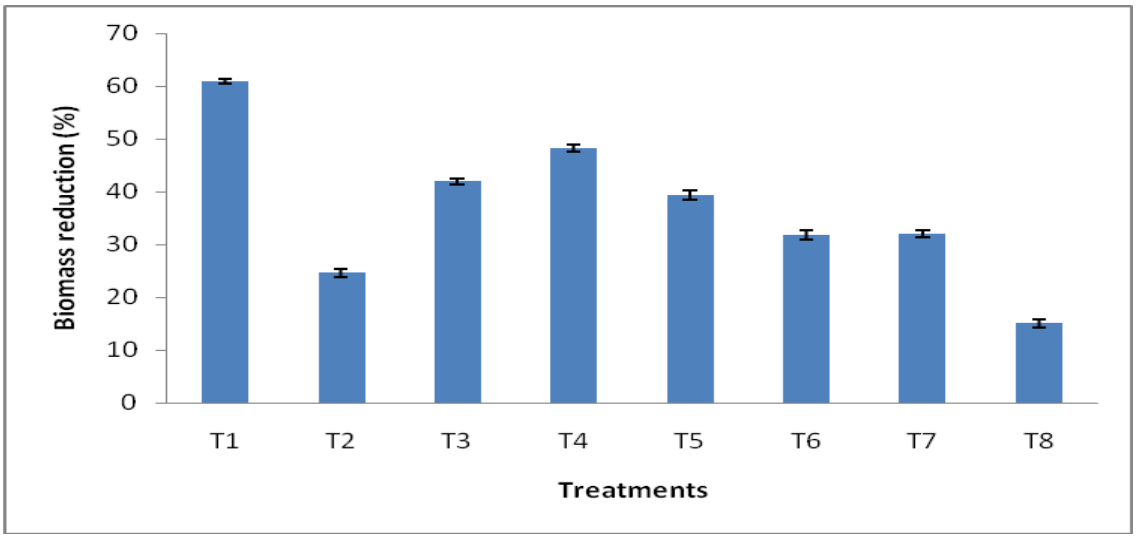

Figure 5. Percentage biomass decomposition in the anaerobic digester. For designation refer Table 1.

4. Discussion

In this study, the efficacy of microbial strains with application of different treatment combinations in the process of sewage water microalgae decomposition in simulated lab scale anaerobic digester is reported. A single strain treatment application of cellulose degrading microorganism (C) showed the highest biogas production (1.34L) by the end of the fifth (5^{th}) cycle (Figure 5) and pressure development in the first cycle, which of course decreased later in succeeding cycles unlike N_1, that dropped to zero at the second cycle (Table 1). This indicates that strain S_{10} has a great potential to decompose the algal biomass and played a leading role in the conversion of

the algal biomass into methane. According to the reviewed documents [11], the high proportion of proteins in microalgae in the system would result in low C/N ratio, which can affect the performance of the decomposing microorganisms in the anaerobic digester.

However, it has been evident from this study that the use of strain (C) has shown a better performance in the decomposition of algal biomass and increase biogas volume compared to an increase made by the addition of waste paper to algal biomass [12]. The high productivity of methane may have also been associated directly with the high contents of carbohydrate and lipid in the microalgae [13]. From the reports made by [14, 15], it has also been documented that the addition of exogenous microbial sources during the process of anaerobic digestion may have actively involved in ammonium remediation and high methane production.

The rates of gas production by the different treatments were also accompanied by reduction in the total biomass of the algae. The wet biomass analysis shows correlation between the volume of biogas produced by each treatment and enhanced degradation of biomass (Table 1 and Figure 5). Treatment one showed the highest biomass degradation than treatment four despite higher biogas production, which could be influenced by microbial synergism that have enhanced the production of more methane while hindering its utilization [16]. The efficiency of algal decomposition by the microbial strains introduced is an indication for the presence of all the enzyme systems in the cell, which shorten the retention time of the algal biomass in the anaerobic digester.

On the other hand, the inefficiency of strains in combined treatment could be attributed to the likely phenomena of the interference competition between the nitrogen fixing strains, which may have increased the ammonium concentration in the system [14, 15]. Competition among microorganisms for resources in a habitat may be intense, with the outcome dependent on several factors, including rates of nutrient uptake, inherent metabolic rates, and ultimately, growth rates [17]. Shortage in biomass substrate toward the end of the fermentation process decreased in biogas yield which can be attributed to the utilization of methane as a carbon source. The presence of little amount of substrate as a carbon source is suitable for good amount of methane production in the system [18]. Under anaerobic condition, the effects of various anaerobic bacteria on degrading, multi molecular organic substances into simple, chemically stabilized compounds – mainly of methane and carbon dioxide involves the process of liquefaction and hydrolysis of insoluble compounds and gasification of intermediate products. The syntrophy between cellulose degrading and ammonium remediating strains (CN_1) has shown to be more efficient in the reduction of the total biomass than the activity of cellulose decomposing strains alone. Similar studies on a non-symbiotic nitrogen-fixing bacterium (*Azospirillum*) and cellulose degrading bacterium (*Cellulomonas*) showed relatively low or negligible performance when grown alone [19].

From this study therefore, it can be concluded that the two treatments can be used for different purposes depending on the primary objective (s) of the fermentation process. For effective methane production, strain (C) was found to be a potential candidate, while strain (CN_1) remains the prime option for rapid reduction of biomass. The combined treatments although have shown relatively low methane production; they have reduced the biomass to greater extent in the system. This effect could have a positive influence in reduction of the biological oxygen demand (BOD) in a system to a safe level before discharging the sewage water into the nearby river. The reduction of microbial diversity in anaerobic digesters as progressing with further

fermentation was evident with the flourishing and dominancy of *Actinomycetes spp*. [9]. In this study, the raw sewage water was confirmed to have *Escherichia coli*, and mycelial fungi belonging to the genus *Fusarium* (data not presented), which was consistent observation with Omenwa, *et al*. [20]. The application of raw sewage water as it is for irrigation will cause serious health problems to the community. The application of selected cellulose degrading microbial strains in the anareobic digester besides significant increase of the biogas volume with less retention time, they also have impacted the reduction of pathogenic strains such as *E. coli* and *Fusarium spp* as has been observed in this study (data not presented). The integration of these application with agro-energy policy would become a cross cutting strategy for future energy, food security and waste management practice in Lesotho and Africa at large.

5. Conclusion

The present study clearly indicates that strain type and treatment combinations have shown different level of efficacy in the production of methane and biomass reduction. A single strain treatment application of cellulose degrading microorganism (C) has shown the highest biogas production (1.34L) by the end of the fifth (5^{th}) cycle while strain (CN_1) showed low methane production with high biomass reduction. It can be concluded that the two treatments can be used for different purposes depending on the primary objective(s) of the fermentation process. For effective methane production, strain (C) has shown to be a potential candidate while strain (CN_1) remains the prime option for rapid reduction of biomass. The combined treatment applications of the two strains have shown relatively low methane production, which implies that the syntrophy between the two strains would be more efficient in the reduction of the total biomass rather than high volume of methane production. Therefore, the separate application of the two strains in the respective treatment applications will have a positive influence in reduction of the biological oxygen demand (BOD) in pond systems for safe discharging of the sewage water and pathogen free sludge that can be used as biofertilizer.

Acknowledgments

Global Environment Facility Small Grants Programme (GEF-SGP) of the UNDP Lesotho and the National University of Lesotho are acknowledged for their support of this project.

References

[1] D. L. Klass. “A critical assessment of renewable energy usage in the USA.” *Energy Policy*, vol. 31, pp. 353-367, 2003.

[2] P. M. Slegers, M.B. Lösing, R. H. Wijffels, G. van Straten, A. J. B. van Boxtel. “Scenario evaluation of open pond microalgae production.” *Algal Research*, vol. 2, pp. 358-368. 2013.

[3] E. D. Kathryn, G. W. Crystal, J. M. Patrick. “Nutrient remediation rates in municipal wastewater and their effect on biochemical composition of the microalga *Cenedesmus sp*. AMDD.” *Algal Research*, vol. 2, pp. 127-134, 2013.

[4] J. M. Patrick, E. D. Kathryn, C. P. Kyoung, G. W. Crystal, P. M. Q. Scott, J. B. Frank, F. Jean-Claude, R. G. Serge, J. B. O. Stephen. "Assessment of the bioenergy and bioremediation potentials of the microalga *Scenedesmus sp*. AMDD cultivated in municipal wastewater effluent in batch and continuous mode." *Algal Research*, vol. 1, pp. 155-165, 2012.

[5] S. J. P. Jegathese and M. Farid. "Microalgae as a renewable source of energy: A niche opportunity." *Journal of Renewable Energy*, Article ID 430203, 2014.

[6] M. Ras, L. Lardon, S. Bruno, N. Bernet and J. P. Steyer. "experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris*." *Bioresource Technology*, vol. 102, pp. 200-206, 2011.

[7] P. H. Chen, and W. J. Oswald. "Thermochemical treatment for algal fermentation." *Environment International*, vol. 24, no.8, pp. 889-897, 1998.

[8] R. H. Wijffels, and M. J. Barbosa. "An outlook on microalgal biofuels." *Science*, vol. 329, no. 5993, pp. 796- 799, 2010.

[9] M. Cauley. "*Benchmark soils of Lesotho: their classification, interpretation, use and Management*." Office of Soil Survey, Soil Conservation Division Ministry of Agriculture: Maseru, Lesotho, 1986.

[10] Brennan, and P. Owende. "Biofuels from microalgae-a review of technologies for production, processing, and extraction of biofuels and co-products." *Renewable and Sustainable Energy Reviews*, vol.14, no.2, pp. 557-577, 2010.

[11] H.W. Yen, and D.E. Brune. "Anaerobic co-digestion of algal sludge and wastepaper to produce methane." *Bioresource Technology*, vol. 98, pp.130-134, 2007.

[12] K. E. Dickinson, C. G.Whitney, and P. J. McGinn. "Nutrient remediation rates in municipal wastewater and their effect on biochemical composition of the microalgae *Scenedesmus* sp." *AMDD, Algal Research*, vol. 2, pp. 127-134, 2013.

[13] G. Giordano. "Interactions between C and N metabolism in *Dunaliella salina*cells cultured at elevated CO_2 and high N concentrations." *Journal of Plant Physiology*, vol. 158, pp. 577-581, 2001.

[14] J. H. Mussgnug, V. Klassen, A. Schluter, and O. Kruse. "Microalgae as substrates for fermentative biogas production in a combined biorefinary concept." *Journal of Biotechnology*. Vol. 150, pp. 51-56, 2010.

[15] M. Ras, L. Lardon, S. Bruno, N. Bernet, and J. Steyer. "Experimental study on a coupled process of production and an aerobic digestion of *Chlorella velgaris*." *Bioresource Technology*, vol. 102, pp. 200-206, 2011.

[16] M. T. Madigan, and J. M. Martinko. "*Brock Biology of Microorganisms."* 13th Edition. Pearson Education, Inc1301 Sansome Street, San Francisco, CA 94111, 673, 2012.

[17] H. G. Jung, F. R. Valdez, R. D. Hatfield, and R. A. Blanchette. "Cell wall composition and degradability of forage stem following chemical and biological treatment," *Journal of Science and Food Agriculture*, vol. 58, pp. 347–355, 1992.

[18] D. H. Halsall, and A. H. Gibson. "Comparison of two cellulomonas strains and their interaction with *Azosprillum brasilense* in degradation of wheat strains and associated nitrogen fixation." *Applied Environmental Microbiology*, vol. 51, no. 4, pp. 855-861, 1986.

[19] Y. Lu, B. Young, D. Gregg, J. N. Saddler, and S. D. Mansfield. "Cellulase adsorption and an evaluation of enzyme recycle during hydrolysis of steam-exploded softwood residues." *Applied Biochemistry and Biotechnology*, vol. 98, pp. 641–654, 2002.

[20] V. C. Omenwa, E. J. Ansa, O. E. Agokei, A. UKa, and O. S. George. "Microbiological quality of raw and processed farm-reared periwinkles from Brakish water earthen pond Buguma, Nigeria." *African Journal of Food, Agriculture, Nutrition and Development*, vol. 11, no. 2, 4623-4631, 2011.