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EFFECTS OF MORINGA EXTRACTS ON SEDIMENTATION AND GROWTH OF *CHLORELLA VARIABILIS*

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ABSTRACT

The effects of extraction solvents, extraction time and extracts from moringa tree parts on the sedimentation and growth of Chlorella variabilis NIES-2541 were investigated. Hot water extract was the most effective in inducing sedimentation of the cells. This was followed closely by cold water extract while ethanol extract was the least effective in inducing cell sedimentation. With all the three solvents tested, the efficacy of the extract in inducing cell sedimentation increased with increase in extraction time. With either hot water or cold water extract from 5g/l seed, more than 80% of the cells sedimented within 30 minutes. This is considered enough for the harvesting of microalgae during repeated batch cultivation. In comparison with seeds, the abilities of leaves, flowers, stem and root bark extracts to induce sedimentation of the cells were very low. Nevertheless, root bark extract was more effective than the leaves, flower and stem bark extracts in inducing cell sedimentation. Low concentrations of moringa seed extract (1~5 g/l) stimulated cell growth but the optimum concentration was 3 g/l. On the other hand, high concentration (6 g/l) of moringa seed extract inhibited cell growth. These results have shown that moringa seed extract can be used for harvesting of Chlorella variabilis cells through sedimentation without adverse effect on the growth of the cells during the subsequent batch.

KEYWORDS: *Cell Growth, Cell Sedimentation, Chlorella Variabilis, Harvesting of Microalgae, Moringa Oliefera, Seed Extract*

Article History

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INTRODUCTION

Microalgae hold a great potential in the present world's energy, food, and environmental issues. Oleaginous microalgae are extensively investigated for biodiesel (Ahmad et al., 2014; Ogbonna and Ogbonna, 2018) while many species are cultivated for single cell protein and for production of various useful metabolites (Ogbonna et al. 2002). Many species are also used for the treatment of various types of wastewater (Ogbonna et al., 2000; Abdel-Raouf et al. 2012; Nwoba et al 2017; Ogbonna et al., 2018). In all these applications, harvesting microalgae is a major technical and economic challenge. Most of the methods employed in harvesting microalgae are energy demanding and economically depriving. Many scientists have investigated the application of different inorganic coagulating agents such as Aluminum sulfate, Aluminum chloride, and Ferric chloride and Ferric sulphate to facilitate economical microalgae harvesting (Ahmad et al., 2014). However, due to the toxicity and polluting nature of these inorganic coagulants, emphasis is now shifting to the use of organic coagulants such as chitosan and other plant-based coagulants (Chen et al., 2014; Moreno et

al., 2015; Trung et al., 2016; Noor et al., 2016; Karanja et al 2017).

Among the various plant-based coagulants, use of *Moringa oleifera* seeds in wastewater treatment has been extensively investigated (Bichi 2013) while interest is growing in exploiting the coagulation properties of Moringa seeds in harvesting microalgae (Teixeira and Teixeira 2017; Hamid et al., 2014; Ogbonna and Ede, 2018). Aside from Moringa seeds, various parts of Moringa plants are also used for various purposes. The leaves and immature pods are used as vegetables while the root and stem are reported to have many medicinal properties (Brihante et al., 2017). However, there is yet no report on the use of these plant parts as coagulants.

In most commercial microalgae cultures, the biomass is harvested in batches whereby only a percentage of the biomass is harvested and the remaining serves as the seed for the next batch. Thus, the method of harvesting should not affect the subsequent growth of the microalgae. Unfortunately, Moringa seeds have been reported to have antimicrobial properties (Oluduro et al., 2010) and its use to inhibit the growth of bloom-forming cyanobacterial species such as *Microcystis aeruginosa* have been reported (Lurling and Beekman, 2010). It is therefore, necessary to investigate the effects of Moringa seeds on the growth of the microalgae to be harvested. In our previous work (Ogbonna and Edeh, 2018) we have demonstrated that although the use of Moringa seed powder resulted in higher percentage sedimentation of *Chlorella variabilis*, cold water extract was also efficient and its use has many potential advantages over the use of seed powder.

The aims of the present research were to compare the efficacy of some solvents in extracting coagulants from Moringa seeds and to explore the potentials of different Moringa plant parts as coagulants for harvesting *Chlorella variabilis*. The effects of *M. oleifera* seed on the growth of *Chlorella variabilis* were also investigated.

MATERIALS AND METHODS

Microorganism and Culture Media

Chlorella variabilis NIES-2541 was used in this study. All the media components used in this experiment were purchased from Wako Pure Chemical Industries Ltd. Japan unless otherwise stated. The ***Chlorellavariabilis*** NIES-2541stock culture was re-activated by sub-culturing in BG 11 medium at room temperature for 5 days. The seed culture was used to inoculate a 500 ml main culture in a 1000 ml Erlenmeyer flask. The cultivation was done photo-autotrophically at a light intensity of 100 $\mu molm^{-2}s^{-1}$with a light source from 32-W white bulbs (ASTRA NU-PARK, CHINA).

Preparation of *M. Oleifera* Seed Extracts

Mature and dry *Moringa oleifera* pods were harvested from the Botanical Garden, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Dry *M. oleifera* pods were broken open along the natural dehiscent lines to release the winged seeds. The seed shells were removed by hand and healthy translucent seeds were used for the experiments. The seed extract was prepared according to the method of Ogbonna and Edeh, (2018). The seeds (5g) were ground using a mortar and pestle and 1g of the powder was weighed into 20 ml of one of the following solvents in 100 ml Erlenmeyer flasks: (i) Cold distilled water, (ii) hot distilled water (60^oC) or (iii) 50% ethanol. The mixtures were allowed to extract for 30, 60 or 90 minutes by stirring constantly with a glass stirring rod. The mixtures in the different solvents were filtered through a sterilized muslin cloth at the end of each extraction time.

Extraction of Flocculants from *Moringa Oleifera* Plant Parts

Seeds, leaves, flower, root and stem barks of *M. oleifera* were harvested from the Botanical Garden, University of Nigeria, Nsukka. These components were shredded into fine pieces with a cutter and dried at room temperature for five days and briefly in an oven at 60^{o}C to reduce the moisture content to about 10%. They were ground into powder using mortar and pestle. The powder was sieved through a muslin cloth. One gram of powder from each plant part was weighed into 20 ml of distilled water in a 100 ml Erlenmeyer flask and left to extract for 30 minutes with constant stirring. Each mixture was filtered through a muslin cloth and the supernatant was used to perform the sedimentation experiments.

Flocculation Assay with *Chlorella Variabilis*

Nine labeled test tubes were arranged and 1 ml of the extract was added into each test tube, 10 ml of 14 days old *C. variabilis* cells with an optical density of 3.47 was added to each test tube and the contents were mixed by inverting severally. The sedimentation was allowed to proceed for 30 minutes. One milliliter of the broth was withdrawn from the upper layer of each test tube, diluted with water and the optical density was read at 680nm.

Effects of *Moringa Oleifera* Seed Extract Concentration on the Growth of *C. Variabilis*

Various volumes (4, 6, 8, 10, and 12 ml) of *M. oleifera* seed extract from 50 g/l stock solution was added into each 250 ml - Erlenmeyer flask. A corresponding volume of BG 11 medium containing Chloramphenicol (5ug/ml) was added to make 100 ml. The flasks were sterilized by autoclaving at 121^{o}C for 20 minutes. Each flask was inoculated with active *C. variabilis* cells and incubated under a light intensity of 100 $\mu molm^{-2}s^{-1}$ from 32-W white bulbs (ASTRA NU-PARK, CHINA). The flasks were manually shaken twice daily for 14 days. At the end of cultivation, the cells were harvested by centrifuging at 15,000 rpm for 30 minutes. The cell pellets were transferred into pre-weighed Petri dishes and dried in an oven at 80^{o} C for 24h. The Petri dishes were weighed and the dry cell weights were determined by subtracting the empty plate weights from the total weights.

Statistical Analysis

Each experiment was repeated 3 times and the data were subjected to Analysis of Variance (single classification). Where there were significant differences, the means were separated using Least Significant Difference (LSD). The mean values ± Standard Error were used for the plots.

RESULTS AND DISCUSSIONS

In our previous study, we have demonstrated the potentials of using Moringa seeds to induce sedimentation of microalgae for harvesting and showed that using the extract in place of the seed powder has many advantages (Ogbonna and Ede, 2018). In the present study, the effects of using cold water, hot water and ethanol as solvents on the ability of the extracts to induce sedimentation of *Chlorella variabilis* were compared as shown in Figure 1. The results showed that hot water extract gave the highest percentage sedimentation, followed by cold water extract. The percentage sedimentation obtained with ethanol extract was significantly lower than the values obtained with cold and hot water extracts ($P < 0.05$). The coagulating components of Moringa seeds have been attributed to either a protein or polyelectrolytes (Okuda, 1999; Bichi, 2013) and the present results have shown that it can be efficiently extracted by both cold and hot water. On the other hand, ethanol is a poor solvent for the extraction of the coagulants.

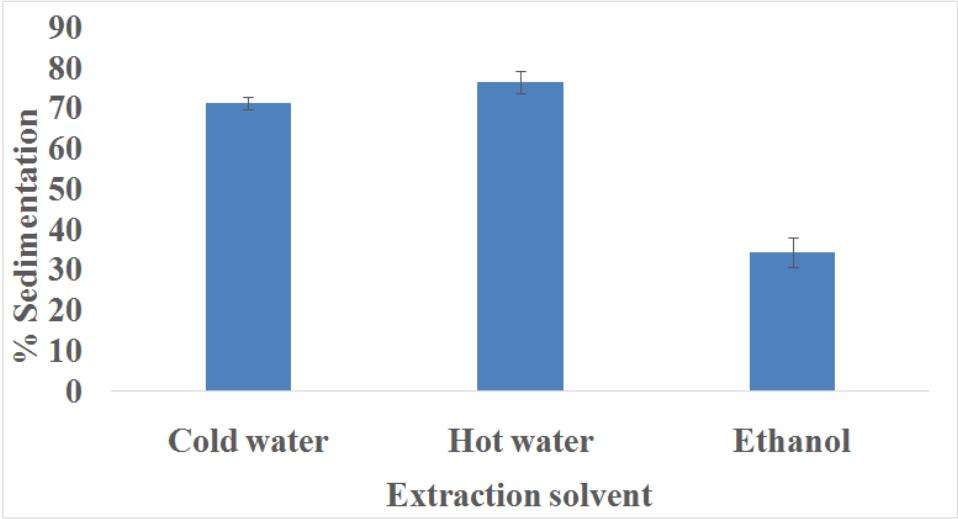

Figure 1: Effect of Extraction Solvent on the Flocculating Ability of Moringa Seed Extract. The Moringa Seed Powder (1 g) was Suspended in 20 mL of the Solvent and Shaken for 30 Minutes. The Extract was Filtered through Muslin Cloth and 1ml was used for Sedimentation of 10 ml of *C. Variabilis*

The effects of the period of extraction with the three solvents on the percentage of sedimentation are shown in Figure 2. With all the three solvents, the percentage of sedimentation increased as the period of extraction was prolonged from 30 minutes to 90 minutes. Regardless of the length of extraction period, the effectiveness of the extracts to induce sedimentation of *Chlorella variabilis* was ranked as hot water > cold water > ethanol. The optimum length of time for extraction therefore, depends on the desired percentage of cells to be harvested. In the present study, 20 ml of solvent was used to extract 1 g of the seed (50 g/l) and 1 ml of the extract was added to 10 ml of the *Chlorella* culture, giving a final concentration of Moringa seed per *Chlorella* culture broth of 5 g/l. For most practical microalgae cultures, less than 80% of the cells are harvested at a time, leaving the remaining 20% cells to serve as a seed for the next batch. Over-harvesting the cells will lead to the lag phase and poor light utilization. The optimum concentration of the Moringa seed to achieve 80% sedimentation therefore, depends on the solvent and the length of extraction. Using cold water (for convenient and costs) and extracting for 1 hour is sufficient to achieve more than 80% sedimentation using a low extract concentration of 5 g-moringa/l-*Chlorella* culture.

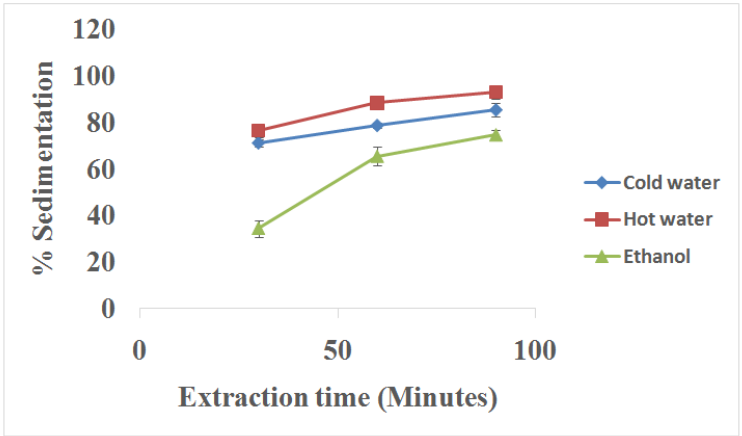

Figure 2: Effect of Extraction Time on the Flocculating Ability of Moringa Seed Extract. The Extraction Procedure was as Described for Figure 1 Except that the Extraction Period was Varied from 30 Minutes to 90 Minutes

Various parts of Moringa plants have been investigated for various applications. In the present study, therefore, the ability of extracts of various parts of the Moringa plant to induce sedimentation of *Chlorella variabilis* cells were compared (Figure 3). In comparison with seed extract, the percentage sedimentation obtained with other parts (leaves, stem bark, flowers, and root bark) was very low. Statistical analysis ranked the effectiveness of these plant parts in inducing sedimentation as seed > root > [leave, flower, bar]. On the whole, the percentage sedimentation achieved with plant parts, except the seed, was too low for practical application.

In commercial microalgae cultures, only a part of the cells is harvested at a time, leaving sufficient cells to serve as inoculum for the subsequent culture cycle. Any useful coagulant for microalgae harvesting should therefore not have an adverse effect on the growth of the microalgae. However, antimicrobial activities of Moringa seed have been reported (Oluduro et al., 2010). Caceres et al. (1991) reported that although cold water extracts inhibited the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, extraction temperatures above 56°C inhibited this activity. In other words, antimicrobial components are deactivated by high temperature. This contrasts our previous results which showed that autoclaved extracts retained their coagulation properties (Ogbonna and Edeh, 2018).

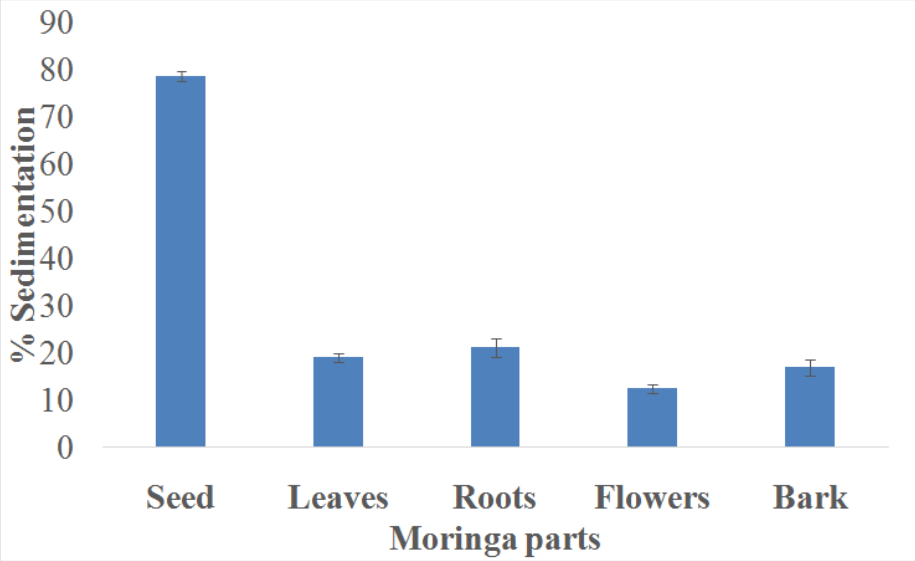

Figure 3: Comparison of the Abilities of Different Parts of Moringa to Sediment *Chlorella Variabilis* cells. The Parts of Moringa Were Dried, Pulvarized and the Powder (1g) was Suspended in 20 mL of the Solvent and Shaken for 30 Minutes. The Extract was Filtered through Muslin Cloth and used for Sedimentation Experiments

It was therefore necessary to investigate the effects of Moringa seeds on the growth of *Chlorella variabilis*. The effects of various concentrations of Moringa seed extract on the growth of the cells are shown in Figure 4. It is interesting to note that low concentrations of Moringa seed extract stimulated the growth of *Chlorella variabilis*. The cell concentration obtained in a culture containing 3 g/l of Moringa seed extract was significantly higher than the value obtained in the control culture (without Moringa seed) ($P < 0.05$). The stimulatory effects of addition of Moringa can be due to the various active components of the seed (Anwar et al., 2007), but can also be due to improved light penetration into the culture. In a typical microalgae culture, light reaches only a small depth from the illumination surface and the greater part of the culture is without light (Ogbonna et al, 1996, Ogbonna and Tanaka, 2001). The depth of light penetration decreases with increase in the standing biomass concentration. However, with Moringa seed powder, most of the cells sedimented, leaving the low concentration of suspended cells. Under this condition, light penetrated deep into the culture

and the non-sedimented cells had enough light for active growth. However, when the concentration of Moringa was higher than 3 g/l, the cell growth decreased. Addition of 6 g/l of Moringa extract was inhibitory to cell growth as the final cell concentration was significantly lower than the value obtained in the control culture ($P < 0.05$). Saadabi and Zaid (2011) noted that the antimicrobial activity of Moringa seed extract was more on Gram-negative bacteria than on fungi and yeasts.

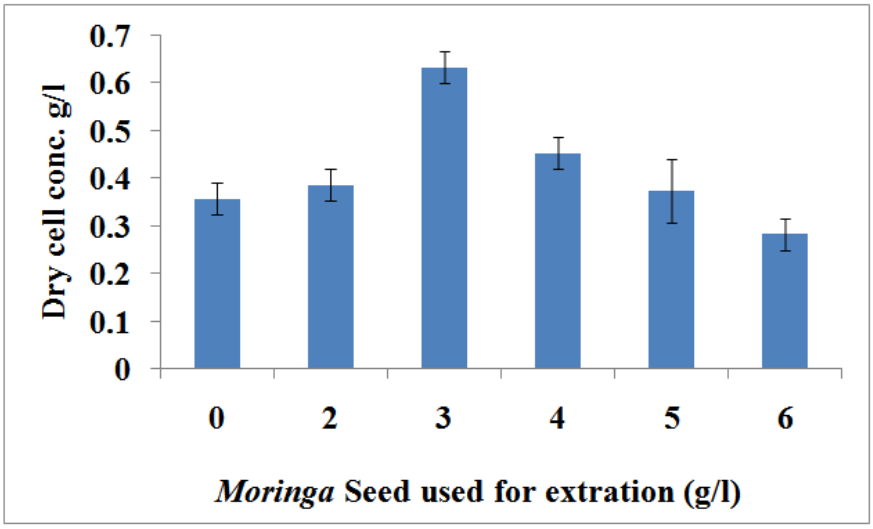

Figure 4: Effect of Moringa Seed Extract on the Growth of *Chlorella Variabilis*

CONCLUSIONS

In comparison with Moringa seed extract, other parts of the plant (leaves, stem bark, roots, and flowers) are not good flocculants for the harvesting of *Chlorella variabilis*. Hot water was more effective than cold water and ethanol as solvents for extraction of the flocculants from the seeds. Although the efficacy of the seed extracts in inducing cell sedimentation increased with increase in the extraction time, 60 minutes extraction at a final concentration of 5 g/l was sufficient to induce 80% sedimentation within 30 minutes. Low concentrations of Moringa seed extracts stimulated cell growth but high concentrations inhibited cell growth. These results have demonstrated that Moringa seed extract can be used to induce sedimentation and thus harvest microalgae during repeated batch cultures without adverse effect on cell growth.

REFERENCES

1. *Abdel-Raouf, N., Al-Homaidan, A. A., Ibraheem, I. B. M. 2012. Microalgae and wastewater treatment. Saudi Journal of Biological Science. 19(3): 257- 275.*
2. *Ahmad, A. L., Mat Yasin, N. H., Derek, C. J., Lim, J. K. 2014. Comparison of harvesting methods for microalgae Chlorella sp and its potential use as a biodiesel feedstock. Environmental Technology. 35(17-20): 2244 – 2253.*
3. *Anwar, F., Latif, S., Ashraf, M., Gilani, A. H. 2007. Moringa oleifera: A food plant with multiple medicinal uses. Phytotherapy Research. 21: 17– 25.*
4. *Bichi, M. H. 2013. A review of the applications of Moringa oleifera seed extracts in water treatment. Civil and Environmental Research. 3(8):1-10.*
5. *Brihante, R. S. N., Sales, J. A., Perewa, V. S., Castelo-Branch, D. S. C. M., Cordeiro, R. A., Sampaio, C. M. S.,*

Paiva, M. A. N., Santos, J. B. F., Sidrim, J. J. C., Rocha, M. F. G. 2017. Research advances on the multiple uses of Moringa oleifera: A sustainable alternatives for socially neglected population. Asian Pacific Journal of Tropical Medicine. 10(7): 621 – 630.

6. *Caceres, A., Cabrera, O., Morales, O., Mollinedo, P., Mendia, P. 1991. Pharmacological properties of Moringa oleifera. 1: Preliminary screening for antimicrobial activity. Journal of Ethnopharmacology. 33 (3): 213-216.*
7. *Chen, G., Zhao, L., Qi, Y., Cui, Y. L. 2014. Chitosan and its derivatives applied in harvesting microalgae for biodiesel production: An Outlook Journal of Nanomaterials Article ID 217537, 9 pages http://dx.doi.org/10.1155/2014/217537*
8. *Hamid, S. H., Lananan, F., Din, W. N. S., Lam, S. S., Khatoon H., Endut, A., Jusoh, A. 2014. Harvesting microalgae, Chlorella sp. by bio-flocculation of Moringa oleifera seed derivatives from aquaculture wastewater phytoremediation. International Biodeterioration and Biodegradation. 95 (Part A): 270-275.*
9. *Karanja, A.,Fengting, L., Ng'ang'a, W. 2017. Use of cactus opuntia as a natural coagulant: Water treatment in developing countries. International Journal of Advanced Research 5(3): 884 – 894.*
10. *Lurling, M., Beekman, W. 2010. Anticyanobacteria activity of Moringa oleifera seeds. Journal of Applied Phycology. 22(4): 503-510.*
11. *Anjalai. K, Revathi. K, Vidhya. G, Kirubakaran. R & Babu. M, Effect of Dietary Supplementation of Chlorella Vulgaris (Green Microalgae) on Serum Biochemical Parameters of Japanese Quail, IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS), Volume 6, Issue 5, May 2018, pp. 111-116*
12. *Moreno, L. M., Muñoz Prieto, E., Casanova, H. 2015. Flocculating with chitosan of microalgae native of the Colombian Plateau. Cienciaen Desarrollo. 6(1): Tunja Jan./June*
13. *Noor, N., Shuhada, N., Enche, D., Muhad, B., Basiran, N., Sohif, H. N., Karim, A., Azhria, W., R al- Obaidi, J. 2016. Marine microalgae flocculation using plant: The case of Nannochloropsis oculata and Moringa oleifera. Pakistan Journal of Botany. 48(2): 831 – 840.*
14. *Nwoba, E. G., Moheimani, N., Ubi, B. E., Ogbonna, J. C., Vodiveloo, A., Pluske, J. R., Huisman, J. M. 2017. Macroalgae culture to treat anaerobic digestion piggery effluent (ADPE). Bioresource Technology. 227: 15 - 23.*
15. *Ogbonna C. N., Edeh, I., 2018. Harvesting of Chlorella variabilis Biomass Using Moringa oleifera Seed-Induced Sedimentation. Journal of Advances in Biology & Biotechnology. In press*
16. *Ogbonna J.C., Yoshizawa, H., Tanaka, H. 2000. Treatment of high strength organic wastewater by a mixed culture of photosynthetic microorganisms. Journal of Applied Phycology.* ***12:*** *277 - 284.*
17. *Ogbonna, I. O., Ogbonna, J. C. 2018. Effects of carbon source on growth characteristics and lipid accumulation by microalaga Dictyosphaerium sp with potential for biodiesel production. Energy and Power Engineering. 10(2): 29 – 34.*

18. *Ogbonna, I. O., Okpozu, O. O., Ikwebe, J., Ogbonna, J. C. 2018. Utilization of Desmodesmus subspicatus LC 172266 for simultaneous remediation of cassava wastewater and accumulation of lipids for biodiesel production. Biofuels, 2018;1-8*

19. *Ogbonna, J. C., Ichige, E., Tanaka, H. 2002. Regulating the ratio of photoautotrophic to heterotrophic metabolic activities in photoheterotrophic culture of Euglena gracilis and its application to α-tocopherol production. Biotechnology Letters. 24: 953 - 958.*

20. *Ogbonna, J. C., Yada, H., Masui, H., Tanaka, H. 1996. A novel Internally Illuminated Stirred Tank Photobioreactor for large-scale cultivation of Photosynthetic cells. Journal of Fermentation and Bioengineering. 62: 61- 67.*

21. *Ogbonna J.C., Tanaka H. 2001. Light requirement and photosynthetic cell cultivation- development of processes for efficient light utilization in photobioreactors. Journal of Applied Phycology. 13: 395 - 402.*

22. *Okuda, T., Baes, A. U., Nishijima, W., Okada, M. 1999. Improvement of extraction methods of coagulation active compounds from Moringa oleifera seeds. Water Research. 33(15): 3373-3378.*

23. *Oluduro, O. A., Aderiye, B. I., Connolly, J. D., Akintayo, E. T., Famurewa, O. 2010. Characterization and antimicrobial activity of 4-(β-D-glucopyranosyl-1→4-α-L -rhamnopyranosyloxy)-benzyl thiocarboxamide; a novel bioactive compound from Moringa oliefera seed extract. Folia microbiologica 55: 422-426.*

24. *Saadabi, A. M., Zaid, I. E. A. 2011. An In vitro Antimicrobial activity of Moringa oleifera L. seed extracts against different groups of microorganisms. Australian Journal of Basic and Applied Sciences. 5(5): 129-134.*

25. *Teixeira, C. M. L., Teixeira, P. C. N. 2017. Evaluation of the flocculation efficiency of Chlorella vulgaris mediated by Moringa oleifera seed under different forms: flour, seed cake and extracts of flour and cake. Brazilian Journal of Chemical Engineering. 34 (1): http://dx.doi.org/10.1590/0104-6632.20170341s20150470*

26. *Trung, T. S., Huyen, N. T., Minh, N. C., Trang, T. T., Han, N. T. 2016. Optimization of harvesting of microalgal Thalassiosira pseudonana biomass using chitosan prepared from shrimp shell waste. Asian Journal of Agricultural Resources.10 (5): 162-174.*