Chapter 9

Viable Alternatives Study for Reusing Lipids from Microalgae Biomass Present in the Generated Sludge in the Supply Water Treatment Processes

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Additional information is available at the end of the chapter

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Abstract

This chapter aims to evaluate the microalgae species' removal efficiency, using *Moringa oleifera* powder seeds as a natural coagulant with subsequent lipid profile characterization. For the tests were used deionized water artificially contaminated with cell cultures of *Anabaena flos-aquae* and *Chlorella vulgaris*, with a cell density in the order of 10^4 and 10^6 cells mL^{-1}, respectively. Coagulation/flocculation/dissolved air flotation (C/F/DAF) tests were conducted using 'Flotest' equipment, using *M. oleifera* powder seeds in the dosage range of 50–1000 mg L^{-1}. For fatty acid profile analyses, a gas chromatograph equipped with a flame ionization detector was used. Variations of the coagulant dosages showed that there was a difference between dosages and that 100 mg L^{-1} provided the best removal efficiency for *A. flos-aquae* (96.5, 80.5 and 78.1%) and 140 mg L^{-1} for *C. vulgaris* (90.5, 78.34 and 70%) of the tested parameters of chlorophyll, color and turbidity, respectively. In relation to the produced sludge, it was observed that the use of this coagulant in the treatment of water contaminated with microalgae produces a biodegradable sludge, rich in lipids, especially oleic acid (>60%). Thus, these results indicate that the sludge's reutilization could be a good alternative to biodiesel production, as it represents an environmentally viable method for reusing residual biomass produced in the water treatment process.

Keywords: reusing lipids, microalgae, water treatment processes

1. Introduction

Several natural coagulants have been studied for water purification; recently, *Moringa oleifera* Lam comes to stand out because it has good color and turbidity removal and promotes large

bacteria removal, of above 90% [1]. Currently, there are few studies on the coagulant application in eutrophic waters, but in some works, can be seen an excellent microalgae cells removal efficiency [2–4].

The dissolved air flotation has been considered a viable alternative to the sedimentation step when applied to water treatment in the microalgae's presence, since this process is capable of removing whole cells, besides decreasing the time contact between the generated sludge with water treatment. The waste generated removal is performed by mechanical equipment, which is installed in water and has an easy maintenance [5, 6].

The proper disposal of the generated sludge by water treatment plants (WTPs) is essential, according to NBR 10004 [7], is considered a solid residue, which if released without proper treatment in waterways, can cause direct effects in the aquatic environment, damaging receiving fauna and flora. The nonchalance towards the waste generated, causes impacts, such as increase in the amount of solids in water body, water body siltation, increased of color, turbidity and aluminum concentration in the water, decrease of the water's pH, releasing of odors, decreases the amount of dissolved oxygen in the water body and chronic toxicity on aquatic organisms and their vision.

However, this chapter seeking environmentally viable alternatives to microalgae biomass reuse present in the generated sludge by water treatment plants, for example, to characterize the lipid content produced by these microalgae, to consign them to a further biodiesel production.

Thus, this chapter shows the removal of *Anabaena flos-aquae* and *Chlorella vulgaris* cells by coagulation/flocculation/dissolved air flotation (C/F/DAF) processes, using *M. oleifera* as a natural coagulant, with subsequent lipid characterization of the generated sludge in order to check the potential for reusing this biomass.

2. Microalgae

Phylogenetically, microalgae are prokaryotes or eukaryotes organisms, according to the period when they appeared on the planet, belonging to a very heterogeneous group of microorganisms. According to Andrade et al. [8] the microalgae are photosynthetic microorganisms that combining water and atmospheric carbon dioxide with sunlight to produce biomass (polysaccharides, proteins, lipids and hydrocarbons). Which can be used in biofuel production, feeding supplements and also can be used in atmospheric carbon dioxide capture. Microalgae produce more oxygen than all plants in the world, accounting for at least 60% of the Earth's primary production.

The microalgae biomass presents about 50% carbon in its composition, so the supply of this nutrient to these microorganisms' cultures represents an important component of the production costs [9]. However, it is necessary to take care of microalgae culture systems, considering the peculiarities of each species, adaptation to the environment, as well as the availability of nutrients associated with economic viability [10].

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The exact number of microalgae species is not yet known, but many species may already grow in cropping systems. The most difficult task, however, is to grow specific species for oil production [10].

Some microalgae species under adverse environmental conditions, such as nutrient stress (lack of nitrogen or phosphorus), may accumulate lipids. The green algae specie, *C. vulgaris*, is widely used in research on the biofuel production from microalgae [11, 12], and in the present study, was chosen for the comparison of the lipid content with the cyanobacteria *A. flos-aquae*.

2.1. Microalgae lipid content

The microalgae biomass contains three main components: carbohydrates, proteins and lipids [13]. In biological systems, the lipids function as membrane components, reserve products, metabolites and as energy sources, with most of them consists of fatty acids. Thus, the lipids are classified in storage lipids (neutral lipids), triacylglycerols and membrane lipids (polar lipids), phospholipids, glycolipids and sterols [14].

Fatty acids are fundamental units most of the lipids. They are short-chain and long-chain organic acids having 4–24 carbon atoms, and short-chain fatty acids are ideal for the biodiesel production [15].

Some fatty acids synthesized by microalgae, such as omega 3 and 6 (ω-3 and ω-6), which are the main precursors of some hormones such as prostaglandins, prostacyclins, leukotrienes and thromboxanes, have a high economic value in the food and pharmaceutical industry and are fundamental for the development and physiological regulators [16].

Fatty acids in microalgae correspond to the largest lipid fraction, and, in some species, polyunsaturates represent between 25 and 60% of total lipids [17].

The polyunsaturated fatty acids from microalgae have a very promising market in biotechnology, especially in the functional food industry [18]. Studies presented by Favaro-Trindade et al.(2008) [48] show that lipids, especially unsaturated fatty acids, have been encapsulated to reduce susceptibility to oxidation.

According to Nelson e Cox [14], fatty acids have a unique carboxyl group and a non-polar hydrocarbon tail, which give lipids their oily and fatty nature, insoluble in water. They occur in cells or tissues in forms covalently attached to different lipids' classes. Different fatty acids have been isolated from lipids of various species.

They differ by the chain extension and its presence, number and double bonds position, and some fatty acids also have methyl-branched groups.

The glycolipids that are composed of glycerol have been found in many organisms, being observed as the main lipid component of microalgae photosynthetic membranes, including cyanobacteria (blue microalgae). Its structure is analogous to that of glycerophospholipids with a sugar molecule, glycosidically attached to glycerol three position and fatty acids esterified in the other two positions.

Among the main glycosylacylglycerols of microalgae and plant photosynthetic membranes is monogalactosyl-diacylglycerol (MGDG), which occurs abundantly in plants and algae, especially in chloroplasts. Contains high proportions of polyunsaturated fatty acids. For *C. vulgaris*, the MGDG presents mainly oleic acid (C18:1) and linoleic acid (C18:2) when cultivated in the dark and 20% of linolenic acid (C18:3) when cultivated in the light [19].

The lipid production estimation by microalgae ranges from 15,000 to 30,000 L.km^2, and its extraction is simple and can be applied to traditional methods used in the chemical industry, including solvent extraction [20].

It is known that among the nutrients that can influence the lipids and fatty acids production are the sources of nitrogen and sulfur, which are used by microalgae in the synthesis of amino acids and fatty acids [20].

The main applications of fatty acids microalgae occur in the enrichment of fish feed, biodiesel production and sources of essential unsaturated fatty acids in the human diet [21].

Although there are many microorganism types capable to accumulating lipids, not all of them have favorable characteristics for the application in the biodiesel production. The microalgae stand out because they present, in some cases, compatibility in the ratio of their oil produced to the vegetable oil used in the transesterification process [22, 23].

According to Schimitz et al. [10], the presence of polyunsaturated compounds produced by microalgae causes a decrease in the stability of produced biodiesel. However, due to the presence of these fatty acids, biodiesel from microalgae presents a high yield at low temperatures, a characteristic that is not presented by conventional oilseed biodiesel, which have little yield at relatively low temperatures.

2.2. *Moringa oleifera* Lam

M. oleifera Lam is a tropical tree that grows naturally in India, South Saharan Africa and South America [24]. The leaves, flowers, seeds, roots and bark may be used as food or for medicinal and therapeutic purposes [25], especially in developing countries [24, 26]. In addition, other applications were pointed out for cosmetics preparation, mechanical lubricants and even for potential biodiesel elaboration [27].

According to Ndabigengesere and Narasiah e Talbot [28], *M. oleifera* seeds contain about 37% protein, 35% lipids and 5% carbohydrates (oligosaccharides). The carbohydrate content is very low whereas the high lipid content explains why the seeds can be used as a source of vegetable oil. This oil resembles olive oil in its composition, being rich in oleic acid, which makes it suitable for edible purposes [29].

The *M. oleifera* seeds are also very useful as a coagulant in drinking water clarification and effluent treatment since 1979, due to the presence of a water soluble cationic coagulant protein capable of reducing the turbidity of the treated water. The seeds can be used in the form of powder, such as aqueous or saline extracts [24, 30, 31].

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2.3. Removal of microalgae from water using *Moringa oleifera*, as natural coagulant

Coagulation/flocculation (C/F) followed by dissolved air flotation (DAF) is suitable for the treatment of naturals and synthetic eutrophic waters [5, 32]. When it comes to the removal of cyanobacterial cells, DAF is an effective alternative, as shown by some studies in the literature [5, 6]. However, to achieve good efficiency, water treatment plants (WTPs) use a series of auxiliary products in the process, especially the use of inorganic coagulants, usually based on metals such as aluminum, as well as pH control. However, these coagulants do not generate biodegradable sludge, causing problems in terms of disposal and treatment; this may be also related to some diseases, such as Alzheimer's disease, due to residual aluminum in treated water [33, 34]. Thus, the search becomes necessary for alternative natural coagulants that are biodegradable and safe to human health [35].

The *M. oleifera* (MO) seeds can be used for efficient clarification of water [26, 36]. This efficiency has been shown by high values of color, turbidity and bacteria removal [2, 37] and even cyanobacteria cells in the water treatment process [38], as well as some economic and environmental advantages related to decreasing the costs of synthetic products to correct the pH of water and produce a sludge without metals.

The water treatment processes in WTPs produce residues, mostly water used for washing the filters and sludge from sedimentation tanks/floaters [39]. Particularly, in WTPs with cyanobacteria problems, the sludge generated is composed of microalgae biomass. Knowing that such biomass has a relatively high amount of lipids in their composition [40], which could be used for biodiesel production.

Firstly, by evaluating the results obtained (**Figure 1**), one can observe the percentages of the removal of the parameters color, turbidity and chlorophyll-a and compounds with absorp-

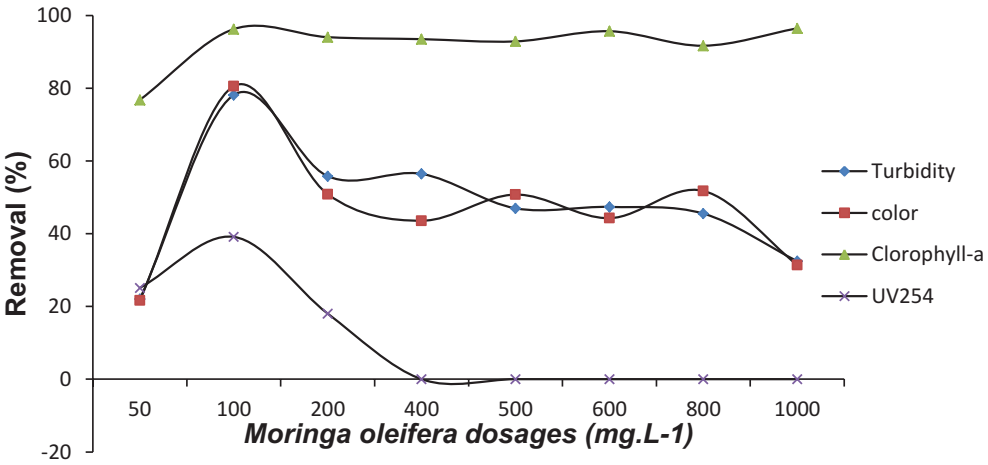

Figure 1. Color, turbidity, compounds with absorption in $UV_{254\ nm}$ and chlorophyll-a removal in relation to the dosage of *Moringa oleifera* powder seeds for *Anabaena flos-aquae*.

tion in $UV_{254\,nm}$, indicating the optimum dosage of the *M. oleifera* powder seeds used in water contaminated with cyanobacteria species (*A. flos-aquae*).

The results indicate that the *M. oleifera* powder seed added directly to cell suspensions was effective in removing cells, color and turbidity, reaching up to 96.4, 80.5 and 78.1%, respectively, for the dosage of 100 mg L^{-1}, which was considered ideal for this study.

Regarding the removal of $UV_{254\,nm}$, it was observed that *M. oleifera* didn't obtain very satisfactory results, reaching 39.1% removal in the dosage of 100 mg L^{-1}. There was a drop in the removal efficiency as the *M. oleifera* dosage was increased. This result can be attributed to the fact that *M. oleifera* is an organic coagulant, basically composed of proteins, lipids and carbohydrates, responsible for the organic residual in the treated water.

The optimum *M. oleifera* dosage for the *C. vulgaris* species, which is a unicellular microalga, was also evaluated in order to verify if the different morphology of the microalgae interferes in the parameters of removal efficiency. In this way, it can be observed that the optimum coagulant dosage was different among the species.

For *C. vulgaris*, the optimum coagulant dosage was 400 mg L^{-1}, verified by the removal efficiency for color (78.34%), turbidity (70%), chlorophyll-a (90.5%) and $UV_{254\,nm}$ (16%) absorption compounds as shown in **Figure 2**.

Thus, it was observed that the C/F/DAF processes used together with *M. oleifera* as a coagulant had an excellent efficiency for both species that were tested.

In relation to the microalgae lipid profile analysis, the fatty acids and esters microalgae were first identified without the *M. oleifera* treatment.

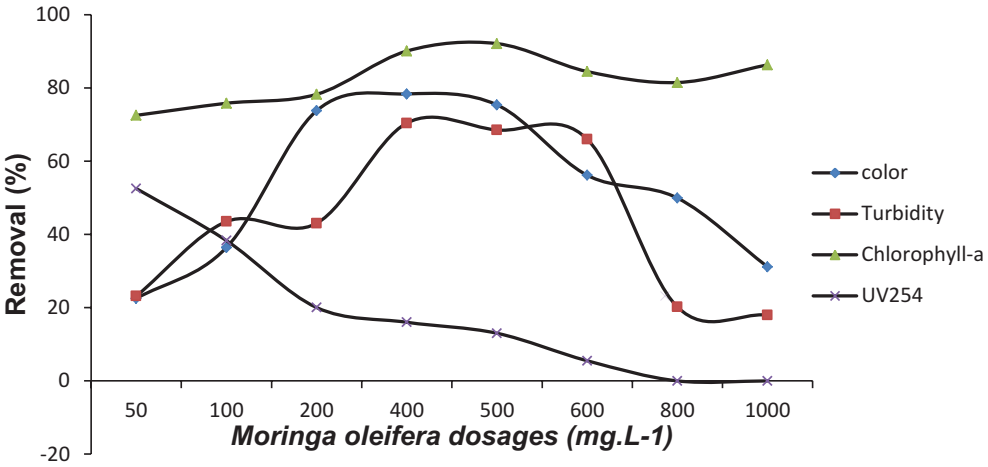

Figure 2. Color, turbidity, compounds with absorption in $UV_{254\,nm}$ and chlorophyll-a removal in relation to the dosage of *Moringa oleifera* powder seeds for *Chlorella vulgaris*.

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It can be verified that the saturated fatty acids corresponded to 40.4% composition of *C. vulgaris* and 35.85% of *A. flos-aquae*, whereas the unsaturated ones presented values of 39.58 and 40.1%, respectively.

Among the acids with the highest values in *C. Vulgaris* were C20:0 (arachidic acid) with 21.15%, C18:1n9 (oleic acid) with 18.85% followed by C16:0 (palmitic acid) and C18:2n6 (linoleic acid) with about 15% each. Already *A. flos-aquae*, the highest percentages are due first to C16:0 (palmitic acid) with 30.55%, then C18:2n6 (linoleic acid) presented 17% and, finally, C18: 1n9 (oleic acid) presented 7.4% of its composition, shown in **Table 1**.

		% Fatty acids means present in microalgae without treatment	
Fatty acids		*Chlorella vulgaris*	*Anabaena flos-aquae*
Saturated	**10:00**	0.2	0.25
	12:00	0.35	0.35
	14:00	0.55	0.6
	15:00	0.55	-
	16:00	15.35	30.55
	17:00	0.2	-
	18:00	1.1	2.3
	20:00	21.15	0.1
	21:00	0.2	0.25
	22:00	0.6	1.45
	24:0	0.15	-
Subtotal		40.4	35.85
Monounsaturated	**16:01**	0.8	1.2
	20:01	1.45	2.15
	24:1	0.3	0.55
Subtotal		2.55	3.9
polyunsaturated	**15:1n5**	1	0.95
	18:1n9	18.85	7.4
	18:2n6	15.13	17
	18:3n6	0.95	2.35
	20:3n3	0.8	1.9
	20:3n6	0.3	-
	20:5n3	0.2	4
	22:02	-	2.6

	% Fatty acids means present in microalgae without treatment	
Fatty acids	*Chlorella vulgaris*	*Anabaena flos-aquae*
Subtotal	37.23	36.2
Not identified	19.8	23.95
Total	100	100
Total lipids	5%	3.05%

Table 1. Microalgae chromatographic profile without *Moringa oleifera* treatment.

The species *C. vulgaris* presented a lipid content of 5% of its dry weight, a value higher than the cyanobacteria studied (*A. flos-aquae*), which was 3.05%, but this result can be reversed, since the medium and conditions as potentiated as lipid productions by microalgae, as light [41], carbon dioxide concentration, temperature [42, 43], nitrogen source concentration [43], among other nutrients. The value obtained for *C. vulgaris* is in agreement with the results obtained by Radman and Costa [20], which presented concentrations of approximately 5.97% of lipid content for the same microalgae under the same culture conditions.

Embora haja poucos trabalhos relatando o perfil lipídico de *Anabaena flos-aquae*, os resultados obtidos neste estudo apresentam valores próximos aos apresentados por Nichols e Wood (1967) em que apresentam C16:0 (39,5%), C16:1 (5,5%), C18:1 (5,2%) C18:2 (36,5%) como os principais ácidos graxos pertencentes da maioria da composição desta alga.

Although there are few studies reporting the *A. flos-aquae* lipid profile, the results obtained in this chapter present values close to those presented by Nichols and Wood [44] in which they present C16:0 (39.5%), C16:1 (5.5%), C18:1 (5.2%) and C18:2 (36.5%) as the main fatty acids belonging to the majority of this algae composition.

After treatment with the *M. oleifera* optimal dosages, the analysis was repeated, and it was observed that results presented in **Table 2** demonstrate that after the treatment with the coagulant optimal dosages, the total lipid percentages of each sample suffered an increase, seen by the values of 16.4% of the total lipids for *C. vulgaris* and 6.2% for *A. flos-aquae*.

This increase is probably related to the residual *M. oleifera* coagulant in the sludge because, according to the *M. oleifera* seeds' physicochemical characterization from Aracaju–SE, they present approximately 37% of lipids in their composition. Therefore, most of the fatty acids present in the samples for both microalgae species were unsaturated fatty acids.

The acid responsible for this increase was C18:1n9 (oleic acid), presenting 69.5% in the *C. vulgaris* sample against 61.7% in the *A. flos-aquae* sample. These results are in agreement with those reported by Silva et al. [45] in which the oil extracted from *M. oleifera* is characterized and with the presence of 78% of oleic acid. Rashid et al. [46] also presented more than 70% of oleic acid in its *Moringa* samples. According to him, some oscillations in the fatty acids' values can occur related to the conditional variations used by the farmers such as fertilizers, soil and the seed variety.

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		% Fatty acids means presented in microalgae with MO treatment	
Fatty acids		*Chlorella vulgaris*	*Anabaena flos-aquae*
Saturated	**10:00**	0.02	0.15
	12:00	0.03	0.4
	14:00	0.11	0.2
	15:00	0.03	-
	16:00	5.83	10.8
	17:00	0.1	-
	18:00	1.2	5.9
	20:00	5.4	0.2
	21:00	0.2	3.85
	22:00	0.1	0.2
	24:0	0.8	0.95
Subtotal		13.82	22.65
Mono-unsaturated	**16:01**	0.1	1.4
	20:01	0.05	1.75
	24:1	0.03	0.1
Subtotal		0.18	1.85
poli-unsaturated	**15:1n5**	1.01	1
	18:1n9	69.5	61.7
	18:2n6	0.04	0.2
	18:3n6	0.1	0.2
	20:3n3	0.1	0.25
	20:3n6	0.1	-
	20:5n3	0.25	3.4
	22:02	-	0.15
Subtotal		71.1	66.9
Not identified		14.4	8.6
	total	100	100
Total lipids		16.4%	6.20%

Table 2. Microalgae chromatographic profile after treatment with *Moringa oleifera*.

According to Qu et al. [47], oils with high oleic acid values (>70%) improve the biodiesel properties, such as cold flow, cloud point and pour point, in this way, the sludge produced after

treatment with the *M. oleifera* optimum dosage presented high oleic acid values in its composition, data that make interesting the use of this residue to a future production of biodiesel.

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References

[1] T. Nkurunziza, J. B. Nduwayezu, E. N. Banadda, I. Nhapi. The effect of turbidity levels and *Moringa oleifera* concentration on the effectiveness of coagulation in water treatment. Water Science & Technology. 2009;**59**:1551-1558. doi:10.2166/wst.2009.155

[2] F. P. Camacho, M. Bongiovani, F. S. Arakawa, A. M. S. Vieira, R. Bergamasco. Advanced processes of cyanobacteria and cyanotoxins removal in supply water treatment. Chemical Engineering Transactions. 2013;**32**:421-426. doi:10.3003/CET1332071

[3] L. O. R. Moreti, P. F. Coldebella, F. P. Camacho, M. C. Bongiovani, A. H. P de Souza, A. K. Gohara, M. Matsushita, M. F. Silva, L. Nishi, R. Bergamasco. Removal of *Anabaena flos-aquae* in water treatment process using *Moringa oleifera* and assessment of fatty acid profile of generated sludge. Environmental Technology. 2015;**37**(11):1408-1417. doi:10.1080/09593330.2015.1117144

[4] M. S. Carvalho, B. R. R. Alves, M. F. Silva, R. Bergamasco, L. A. Coral, F. J. Bassetti. $CaCl_2$ applied to the extraction of *Moringa oleifera* seeds and the use for *Microcystis aeruginosa* removal. Chemical Engineering Journal. 2016;**304**:469-475. doi:10.1016/j.cej.2016.06.101

[5] M. R. Teixeira, M. J. Rosa. Comparing dissolved air flotation and conventional sedimentation to remove cyanobacterial cells of *Microcystis aeruginosa*: Part II. The effect of water background organics. Separation and Purification Technology. 2007;**53**(1):126-134. doi:10.1016/j.seppur.2006.07.001

[6] P. A. P. do Amaral, L. A. Coral, M. E. Nagel-Hassemer, T. J. Belli, F. R. Lapolli. Association of dissolved air flotation (DAF) with microfiltration for cyanobacteria removal in water supply. Desalination and Water Treatment. 2012;**51**(7-9):1664-1671. doi:10.1080/19443994.2012.715128

[7] Brazilian Association of Technical Standards. NBR 10004: Solid wastes-classification. Rio de Janeiro. 2004:71.

[8] M. R. Andrade, J. A. V. Costa. Cultivation of Spirulina platensis microalgae in alternative sources of nutrients. Science and Agrotechnology. 2008;**32**(5):1551-1556. doi:10.1590/S1413-70542008000500029

[9] A. Vonshak, editor. Spirulina Platensis (arthrospira) Physiology, Cell-biology and Biotechnology. London: Taylor & Francis; 1997. 252 p.

[10] R. Schimitz, C. D. Magro, L. M. Colla. Aplicações ambientais das microalgas. Revista CIATEC. 2012;4(1):48-60. doi:10.5335/ciatec.v4i1.2393

[11] A. Scragg, J. Morrison, S. Shales. The use of a fuel containing *Chlorella vulgaris* in a diesel engine. Enzyme and Microbial Technology. 2003;**33**(7):884-889. doi:10.1016/j.enzmictec.2003.01.001

[12] C. M. L. L. Teixeira, F. V. Kirsten, P. C. N. Teixeira. Evaluation of *Moringa oleifera* seed flour as a flocculating agent for potential biodiesel producer microalgae. Journal of Applied Phycology. 2012;**24**(3):557-563. doi:10.1007/s10811-011-9773-1

[13] V. Gressler, N. S. Yokoya, M. T. Fujii, P. Colepicoloc, J. M. Filhod, R. P. Torresd, E. Pinto. The microalgae biomass contains three main components: Carbohydrates, proteins and lipids. Food Chemistry. 2010;**120**(2):585-590. doi:10.1016/j.foodchem.2009.10.028

[14] David L. Nelson, Michael M. Cox, editors. Princípios de Bioquímica de Lehninger. 5th ed. Porto Alegre: Editora Artmed; 2011. 1304 p.

[15] Y. Christi. Biodiesel from microalgae. Biotechnology Advances. 2007;**25**:294-306. doi:10.1016/j.biotechadv.2007.02.001

[16] C. M. P. Pereira, C. B. Hobuss, J. V. Maciel, L. R. Ferreira, F. B. Del Pino, M. F. Mesko, E. Jacob-Lopes, P. C. Neto. Renewable biodiesel derived from microalgae: advances and technological perspectives. New Chemistry. 2012;**35**(10):2013-2018. doi:10.1590/S0100-40422012001000022

[17] A. Richmond. Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Oxford: Blackwell Publishing; 2003. 566 p.

[18] F. C. Bertoldi, E. Sant'Anna, J. L. B. Oliveira. Review: Microalgae biotechnology. Food Processing Research Center Bulletin. 2008;**26**:9-20.

[19] M. I. Gurr, J. L. Harwood, K. N. Frayn. Lipid Biochemistry: An introduction. 5th ed. Wiley-Blackwell; 2002. 340 p.

[20] E. M. Radmann, J. A. V. Costa. Lipid content and fatty acid composition of Microalgae exposed to the gases CO_2, SO_2 and NO. New Chemistry. 2008;**31**(7):1609-1612.

[21] M. G. Morais, J. A. V. Costa. Profile of fatty acids from microalgae grown with dioxide of carbon. Science and Agrotechnology. 2008;**32**(4):1245-1251.

[22] X. Meng, J. Yang, X. Xu, L. Zhang, Q. Nie, M. Xian. Biodiesel production from oleaginous microorganisms. Renewable Energy. 2009;**34**(1):1-5. doi:10.1016/j.renene.2008.04.014

[23] G. Huang, F. Chen, D. Wei, X. Zhang, G. Chen. Biodiesel production by microalgal biotechnology. Applied Energy. 2010;**87**(1):38-46. doi:10.1016/j.apenergy.2009.06.016

[24] S. A. A. Jahn. Using Moringa seeds as coagulants in developing countries. Journal of American Water Works Association. 1988;**80**(6):43-50.

[25] F. Anwar, S. Latif, M. Ashraf, A. H. Gilani. *Moringa oleifera*: A food plant with multiple bio-chemical and medicinal uses- a review. Phytotherapy Research. 2007;**21**(1):17-25. doi:10.1002/ptr.2023

[26] A. Ndabigengesere, K. S. Narasiah. Quality of water treated by coagulation using *Moringa oleifera* seeds. Water Research. 1998;**32**(3):781-791. doi:10.1016/S0043-1354(97)00295-9

[27] K. A. Yongabi, D. M. Lewis, P. L. Harris. A *Moringa oleifera* disinfectant-sand filter integration: A review of an alternative sustainable technology for household treatment. Journal of Environmental Science and Engineering. 2011;**5**(9):1100-1108.

[28] A. Ndabigengesere, K. S. Narasiah, B. G. Talbot. Active agents and mechanism of coagulation of turbid waters using *Moringa oleifera*. Water Research. 1995;**29**(2):703-710. doi:10.1016/0043-1354(94)00161-Y

[29] D. K. Dubey, D. Jyotsna, A. Kumar, R. K. Gulsan. A multipurpose tree: *Moringa oleifera*. International Journal of Pharmaceutical and Chemical Sciences. 2013;**2**(1):415-423.

[30] Bina, B., Mehdinejad, M. H., Dalhammer, G., Rajarao, G., Nikaeen, M., Attar, H. M. Effectiveness of *Moringa oleifera* coagulant protein as natural coagulant aid in removal of turbidity and bacteria from turbid waters. World Academy of Science Engineering and Technology. 2010;**43**:618-620.

[31] G. S. Madrona, G. B. Serpelloni, A. M. Salcedo, L. Nishi, K. Cardoso, R. Bergamasco. Study of the effect of saline solution on the extraction of the *Moringa oleifera* seeds active component for water treatment. Water, Air & Soil Pollution. 2010;**211**(1):409-415. doi:10.1007/s11270-009-0309-0

[32] M. R. Teixeiraa, V. Sousa, M. J. Rosa. Investigating dissolved air flotation performance with cyanobacterial cells and filaments. Water Research. 2010;**44**(11):3337-3344. doi:10.1016/j.watres.2010.03.012

[33] V. Rondeau, D. Commenges, H. Jacqmin-Gadda, J. F. Dartigues. Relation between aluminum concentrations in drinking water and Alzheimer's disease: An 8-year follow-up study. American Journal of Epidemiology. 2000;**152**(1):59-66.

[34] M. C. Bongiovani, F. P. Camacho, L. Nishi, P. F. Coldebella, K. C. Valverde, A. M. Vieira, R. Bergamasco. Improvement of coagulation/flocculation process using a combination of *Moringa oleifera* Lam with anionic polymer in water treatment. Environmental Technology. 2014;**35**(17-20):2227-2236. doi:10.1080/09593330.2014.899398

[35] T. Okuda, A. U. Baes, W. Nishijima, M. Okada. Isolation and characterization of coagulant extracted from *Moringa oleifera* seed by salt solution. Water Research. 2001;**35**(2):405-410. doi:10.1016/S0043-1354(00)00290-6

[36] N. Poumayea, J. Mabinguia, P. Lutgenb, M. Bigan. Contribution to the clarification of surface water from the *Moringa oleifera*: Case M'Poko River to Bangui, Central

African Republic. Chemical Engineering Research and Design. 2012;**90**(12):2346-2352. doi:10.1016/j.cherd.2012.05.017

[37] N. Nwaiwu, B. Lingmu. Studies on the effect of settling time on coliform reduction using *Moringa oleifera* seed powder. Journal of Applied Sciences in Environmental Sanitation. 2011;**6**(3):279-286.

[38] L. Nish, G. S. Madrona, A. L. F. Guilherme, A. M. S. Vieira, A. A. Araújo. Cyanobacteria removal by coagulation/flocculation with seeds of the natural coagulant *Moringa oleifera* Lam. Chemical Engineering Transactions. 2011;**14**:1129-1134.

[39] C. L. Achon, M. M. Barroso, J. S. Cordeiro. Draining beds: Natural system for sludge volume reduction in the water treatment plant. Engenharia Sanitária e Ambiental. 2008;**13**(1):54-62. doi:10.1590/S1413-41522008000100008

[40] B. D. Wahlen, M. W. Robert, C. S. Lance. Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. Bioresource Technology. 2011;**102**(3):2724-2730. doi:10.1016/j.biortech.2010.11.026

[41] F. G. A. Fernandez, J. A. S. Perez, J. M. F. Sevilla, F. G. Camacho, E. M. Grima. Modeling of eicosapentaenoic acid (EPA) production from *Phaeodactylum tricornutum* cultures in tubular photobioreactors: Effects of dilution rate, tube diameter, and solar irradiance. Biotechnology and Bioengineering. 2000;**68**(2):173-183. doi:10.1002/(SICI)1097-0290(20000420)

[42] S. M. Renaud, L. Thinh, G. Lambrinidis, D. L. Parry. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture. 2002;**211**(1-4):195-214. doi:10.1016/S0044-8486(01)00875-4

[43] L. M. Colla, T. E. Bertolin, J. A. V. Costa. Fatty acids profile of Spirulina platensis grown under different temperatures and nitrogen concentrations. Zeitschrift für Naturforschung. 2004;**59**(1-2):55-59. doi:10.1515/znc-2004-1-212

[44] B. W. Nichols, B. J. B. Wood. The occurrence and biosynthesis of gamma-linolenic acid in a blue-green alga, Spirulina platensis. Lipids. 1968;**3**(1):46-50. doi:10.1007/BF02530968

[45] J. P.V. da Silva, T. M. Serra, M. Gossmann, C. R. Wolf, M. R. Meneghetti, S. M.P. Meneghetti. *Moringa oleífera* oil: Studies of characterization and biodiesel production. Biomass and Bioenergy. 2010;**34**(10):1527-1530. doi:10.1016/j.biombioe.2010.04.002

[46] U. Rashida, F. Anwara, B. R. Moserb, G. Knothe. *Moringa oleifera* oil: A possible source of biodiesel. Bioresource Technology. 2008;**99**(17):8175-8179. doi:10.1016/j.biortech.2008.03.066

[47] J. Qu, H. Mao, W. C., S. Gao, Y. Bai, Y. Sun, Y. Geng, J. Ye. Development of marker-free transgenic Jatropha plants with increased levels of seed oleic acid. Biotechnology for Biofuels. 2012;**5**(10). doi:10.1186/1754-6834-5-10

[48] FavARO-Trindade, Carmem Silva; Pinho, SCd; Rocha, G. A. Revisão: Microencapsulação de ingredientes alimentícios. Brazilian Journal of Food Technology. 2008;**11**(2)