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Valuation of The Seed Extract Moringa Oleifera for Removal of Cells Microcystis Sp. Enabling The Production of Biodiesel

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The objective of this study was to evaluate the lipid content in the sludge produced by the biomass of Microcystis protocystis, obtained in the process of water treatment coagulation/flocculation/dissolved air flotation (C/F/DAF), using as coagulant Moringa oleifera Lam (MO) seeds. For the tests was used deionized water artificially contaminated with cell cultures M. protocystis, obtaining a cell density of 10^6 cells.mL^{-1}. Cyanobacterium cells were grown in ASM-1 liquid medium sparged with sterile air and maintained under conditions of maximum asepsis, controlled temperature around 24 °C under fluorescent lamps, with a 12h light-12 h dark photo period. The C/F/DAF process was carried out with a solution of 1 % moringa seeds at concentrations varied in the range of 25 - 300 mg.L^{-1}. Biomass lipid was evaluated by lipid composition of esters in transesterification/etherification reaction, adding to the oil, a solution of hexane, chloroform and hydrochloric acid. The esters produced were diluted in a solution of hexane and tricosanoic acid methyl ester as the internal standard in gas chromatographic analysis. Lipid material was characterized as the profile of the major fatty acid. Biomass showed a composition of palmitic acid (23.67 %), oleic acid (24.08 %) and linoleic (12.52 %). These results demostrated that cyanobacterium lipids from M. protocystis have interesting properties for biodiesel production.

1. Introduction

In recent years several studies have been developed towards the use of coagulants natural alternatives to chemical coagulants for obtaining potable water. These initial studies were concerned to assist or replace conventional coagulants in order to improve the stage of coagulation/flocculation, both the quantity of flakes as produced by the absence of metal sludge generated. Subsequently, there has been a concern for the health of the population, especially in relation to Alzheimer's disease, according to studies, can be aggravated by aluminum.

The use of materials of natural origin for clarification of surface water with high turbidity concept is not new. However, there are few studies that evaluate the application of these coagulants in the treatment of eutrophic waters, specifically the Moringa oleifera.

The discovery of the use of the pulp the seeds of Moringa oleifera for water at a lower cost, presents several advantages over chemical coagulants, being biodegradable, non-toxic, not alter the pH of the water, has good color and turbidity removal (Bina et al., 2010) and also promotes removal of bacteria, above 90 %. Further, inhibits the growth of some species of cyanobacteria such as Microcystis aeruginosa (Lürling and Beekman, 2010).

The process of conventional treatment generally has reduced potential for removing cyanobacteria, especially in the sedimentation stage, since cyanobacteria have the characteristic of forming flakes light, tending to float. In this case the step of adding a dissolved air flotation (DAF) is recommended, since in this process the removal occurs through the introduction of air microbubbles, which tend to float to the surface of the flotation tank where they are continuously removed. The continuous removal of this feature can be assigned an

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advantage of flotation in relation to sedimentation, considering the possible damage to the cell wall of the cells by coagulating in a long contact period, which could increase the likelihood of release into the medium cyanotoxins.
In this sense, the present study evaluated the ability of removing cells from the genus Microcystis sp. employing the dissolved air flotation using a flocculating agent like Moringa oleifera. Considering also that there are no papers in the literature describing the use of this cyanobacteria as a source of biomass for lipid synthesis of biodiesel. This work allowed improves this study, including the steps of extraction and characterization of lipids.

2. Materials and Methods

2.1 Samples

For the tests were used a synthetic water obtained from a pure culture of cyanobacterium *Microcystis protocystis* (HBRF01) supplied the laboratory of Hydrobiology of the Sanitation Company of Minas Gerais (COPASA/MG). The culture medium used was ASM-1 (Gorham et al., 1964). Inoculation of cultures was performed fortnightly by following the ratio of inoculum: means (1:9). The pH was adjusted with sodium hydroxide solution (NaOH) 25 to 50%. The cultures were maintained under conditions of maximum asepsis, controlled temperature around 24°C under fluorescent lamps (Philips TLT 20 W/75 s cool), with a 12 h light-12 h dark photo period.

2.2 *Moringa oleifera* Lam coagulant preparation- crude extract (MO)

For MO coagulant preparation, mature seeds of *M. oleifera* were used, from the Federal University of Sergipe (UFS, Brazil). One gram of peeled seeds were weighed and crushed with 100 mL of distilled water in a blender. Subsequently, the solution was stirred for 30 min and filtered under vacuum on membranes of 0.45 µm, obtaining a solution 1.0 % m/v of MO seeds (Cardoso et al., 2008).

2.3 Coagulation/flocculation/DAF tests

Experimental tests were conducted in flotest equipment Nova Ética - Model 218/3 with three acrylic vessels which work in parallel with the saturation chamber, at an intermittent flow (batch). The bases of each vessel comprise two acrylic plates spaced 5 cm from one another, with channels at the bottom plates for quicker transport and distribution of previously saturated water. The pressure chamber has a 2L useful capability of water. Its saturation results from air inclusion by an air outside the laboratory. The upper part of the chamber is equipped with a pressure regulator valve with filter, needle valves for fine adjustment of pressure in the chamber, manometer and other items. The base of the chamber has three sphere valves with specific functions: to regulate the inlet of clarified water into the chamber, the air inlet and water saturated outlet for vessels. Three replicates were performed for each assay. Figure 1. shows a schematic of the equipment floteste: (1) air compressor, (2) deionized water tank, (3) camera saturation and (4) jar specifically for flotation.

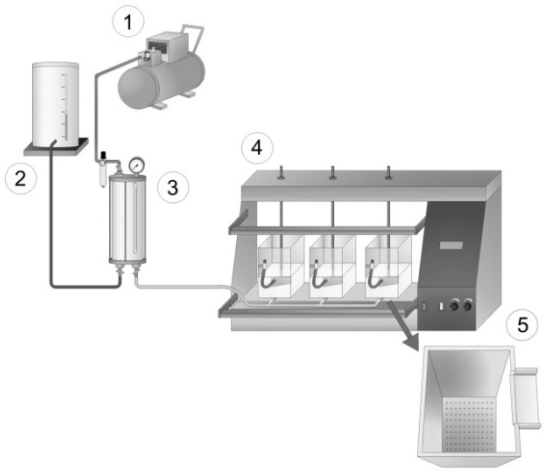

Figure 1: Floteste representation of the equipment used in the tests of C/F/DAF.

For flotation tests, the operational conditions used were: (a) coagulation at G_C 380 s^{-1} for 2 min, using 25 – 300 $mg.L^{-1}$ MO seeds; (b) flocculation at G_F 70 s^{-1} for 8 min; (c) DAF for 8 min, a relative pressure of 5 bar and an applied recycle ratio (*R/Q*) of 8% (Ribau Teixeira and Rosa, 2006).

2.4 Extraction by washing successively

For testing, weighed 1 g of biomass dry type Falcon tube, add 10 mL of hexane and placed in a bath, with stirring, at 60°C for 30 min. Then, the sample was centrifuged for 5 minutes at 4,000 rpm and the supernatant solvent was transferred to a preweighed flask (P1) being repeated for six times. At the end, the solvent was evaporated on a rotary evaporator. The flask containing the fatty matter was moved to oven (60 °C) until a constant weight (P2). And by the mass difference between P1 and P2 was determined total lipid content.

2.5 Transesterification/esterification in situ

To assess the production of esters made to transesterification/esterification in situ according to Lewis et al. (2000). For the test, weighed 20 mg dry weight in test tubes of 10 mL. Adding 3 mL of hexane, chloroform, hydrochloric acid (10:1:1). Then, stirring was performed manually and taken into bath at 60 ° C for 120 min. After the test, was added 1 mL of deionized water and then added 2 mL (triplicate) of a solution of hexane and chloroform (4:1). After phase separation, the supernatant was collected in other tube, for solvent evaporation. The esters produced were diluted in a solution of hexane and tricosanoic acid methyl ester as internal standard in chromatographic analysis.

2.6 Analytical methods

Whereas color and turbidity were measured in a HACH DR/2010 spectrophotometer, according to the procedure recommended by Standard Methods (APHA, 2005), pH was measured by a Digimed DM-2 pH meter according to the manufacturer's methodology. Removal degree of M. protocystis cells was monitored by the Utermöhl method (Lund et al., 1958), which involves the counting of sediment organisms in a special chamber using an inverted microscope.

2.7 Characterization of the surface of the sludge

A Scanning Electron Microscope (SEM) SS 550 Superscan Shimadzu SuperScan SS-550. Shimadzu Corporation, Kyoto, Japan) was employed so that the morphological characteristics of sludge before extraction oil. Samples were manually dispersed on double-face conducting bands on aluminum sample frames and covered by a gold thin layer lining at 20 kV for 20 min in a metalized Shimadzu ion IC 50 (Japan) prior to analysis.

3. Results

3.1 Determination of optimal dosage of MO coagulant dosage in C/F/DAF tests

The mean of the residual and removal efficiencies for parameters color, turbidity and *M. protocystis* cells with MO coagulant tests are shown in Table 1.

In general, analyzing each parameter, high removals of turbidez (> 30 %), color (> 70 %) and *M. protocystis cells* (> 80 %) were observed using lower dosages. The decrease in efficiency of removal of turbidity in water after adding the MO coagulant can be explained by the increase in organic load. This is justifiable given that *Moringa oleifera* Lam is an oilseed rich in organic substances, such as oil, protein, fat, vitamins, etc. This increase of the parameters in waters treated with MO is observed in other studies applied to the treatment of water for drinking.

During the tests, it was observed that the addition of the coagulant does not influence the pH of the medium after the process of C/F/DAF and remained stable in all concentrations, with neutral values. Thus, regardless of dosage Moringa, the pH of the water does not influence, and is considered as one of the advantages of coagulating agent like Moringa oleifera Lam (Nkurunziza et al., 2009), unlike treatment with chemical coagulants in which it is necessary to adjust pH to improve its water coagulant action, increasing the cost of water treatment.

Table 1 - Means of residual and removal efficiencies to determine the optimal dosage of MO coagulant.

Dosage ($mg.L^{-1}$)	Residual Turbidity (NTU)	Turbidity Removal (%)	Residual Color (uH)	Color Removal (%)	Residual *M. protocystis* ($10^6 cell \cdot mL^{-1}$)	*M. protocystis* Removal (%)
25	30.50 ± 0.23	39.00	190.0 ± 0.38	74.80	$17x10^4$ ± 0.04	83.00
50	29.50 ± 0.94	41.00	201.5 ± 0.83	73.28	$25x10^4$ ± 0.36	75.00
75	36.00 ± 0.34	28.00	214.0 ± 0.58	71.62	$30x10^4$ ± 0.18	70.00
100	43.50 ± 0.96	13.00	206.0 ± 0.06	71.68	$69x10^4$ ± 0.75	31.00
125	42.00 ± 0.90	16.00	209.5 ± 0.59	72.21	$43x10^4$ ± 0.37	57.00
150	47.50 ± 0.79	5.00	211.0 ± 0.46	72.02	$24x10^4$ ± 0.07	76.00
175	45.00 ± 0.32	10.00	219.0 ± 0.13	70.95	$23x10^4$ ± 0.77	77.00
200	35.50 ± 0.70	29.00	220.0 ± 0.19	70.76	$23x10^4$ ± 0.74	77.00
225	42.50 ± 0.25	15.00	234.5 ± 0.09	68.90	$15x10^4$ ± 0.43	85.00
250	48.00 ± 0.14	4.00	199.0 ± 0.01	73.61	$50x10^4$ ± 0.32	50.00
275	48.00 ± 0.20	4.00	209.5 ± 0.59	72.21	$12x10^4$ ± 0.55	88.00
300	48.00 ± 0.22	4.00	209.5 ± 0.50	72.21	$26x10^4$ ± 0.65	74.00

Cyanobacteria present some advantages in view of the microalgae as a source of raw materials in the synthesis of biodiesel such as lipids produced by cyanobacteria have fatty acid composition simpler than other microalgae, which facilitates the transesterification reaction. Their cellular shell is simpler, because it is prokaryotic micro-organisms, facilitating the extraction step of lipids, in addition to having high photosynthetic capacity, cyanobacteria are excellent fixing atmospheric N_2 exhibit higher growth rate compared to other microalgae and can be easily modified by genetic manipulation (Liu et al., 2010).

The results showed the species M. protocystis presented the lipid fraction of approximately 29.6 % compared to the dry biomass. The Resulting lipid materials were characterized the fatty acid profile having the composition shown in Table 2.

It can be seen in Table 2 that the biomass present in the chemical composition of approximately 47.58 % saturated fatty acids, among which stand out in greater quantity palmitic acid (23.67 %) and lauric acid (11.20 %). The other 50 % are made up of 42.49 % unsaturated fatty acids, especially oleic acid (24.08 %) and linoleic (12.52 %), and 9.93 % unidentified. Depending on the results of the total lipid fraction in the dry biomass and the fatty acid profile, it appears that this species is presented as a potential source of raw material for biodiesel synthesis. Figure 2 show the microstructures of the sludge before extraction of the oil.

Table 2: Chromatographic profile of the sludge generated by the C/F/DAF process.

	% of average fatty acids present in *M. propoc*
Fatty acids	With 275 $mg.L^{-1}$ *Moringa oleife*
C6:0	0.04
C8:0	0.62
C10:0	0.86
C12:0	11.20
C14:0	5.33
C15:0	0.19
C16:0	23.67
C17:0	0.17
C17:1	0.02
C18:0	4.92
C20:0	0.24
C22:0	0.23

Table 2: Chromatographic profile of the sludge generated by the C/F/DAF process (cont.d)

	C24:0	0.09
Subtotal		**47.58**
Monounsaturated	C16:1	1.21
	C17:1	0.05
	C18:1	24.08
	C20:1	0.38
	C22:1	2.05
	C24:1	0.06
Subtotal		**27.83**
polyunsaturated	C18:2	12.52
	C18:3	0.72
	C18:3	1.42
Subtotal		**14.66**
Not identified		9.93

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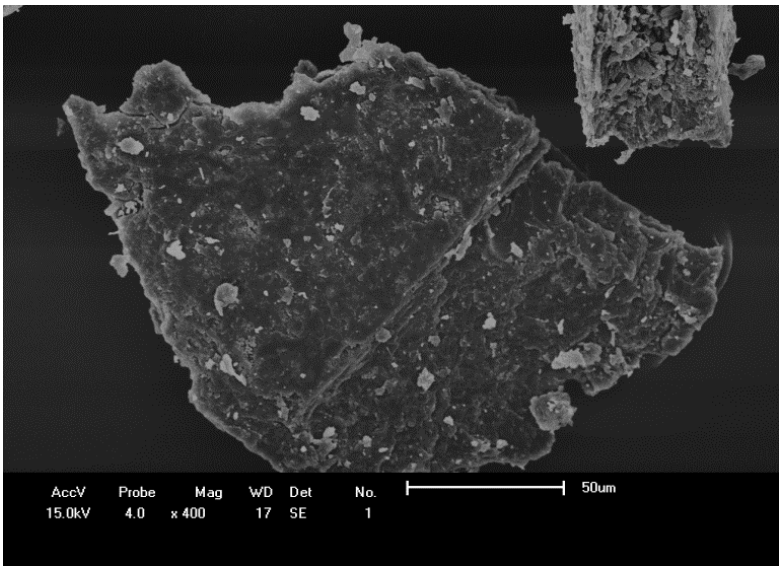

Figure 2: Microstructures of sludge, magnified 400X.

4. Conclusions

Depending on the results obtained, it can be considered that the process combined C/F/DAF with Moringa oleifera, satisfactory results and may be a suitable alternative for treating water with blooms, not only to reduce the color and turbidity but mainly as a raw material for biodiesel synthesis.

The FAD with optimum conditions of employment of aqueous extraction can achieve removal efficiency Microcytis propocystis cells above 80% .

The efficiency of the removal process will depend on factors such as the quality of the water used, the species of cyanobacteria and their morphological and physiological characteristics, the type and dosage of coagulant used in coagulation pH, time and velocity gradient flocculation adopted, among others.

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