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Application of defatted *Moringa oleifera* seeds from compressed propane extraction in the removal of emerging microorganisms

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ABSTRACT

The aim of the present study was to investigate the applicability of the defatted residue, generated after oil extraction from *Moringa oleifera* (MO) seeds by pressurised propane, as coagulant in the coagulation/flocculation/dissolved air flotation (C/F/DAF) process for removing emerging microorganisms. MO seeds (with no extraction) were also evaluated for comparison purposes. MO is a natural plant with active bio-coagulating compounds that can be used for water clarification, since it reduces the use of chemical-based coagulants. The experiment consisted of three stages. In the first stage, the species *Cylindrospermopsis raciborskii* was cultivated in the laboratory. The second stage consisted of seed–oil extraction by pressurised propane and application in the C/F/DAF process. The experiments were conducted at temperatures of 30°C–60°C, pressures of 80–120 bar and a constant solvent flow of 1 mL min^{-1}. In the third stage, C/F/DAF assays were performed with the purposes of comparing the efficiencies of the whole powder and defatted powder as coagulants in dosages in the range of 25–500 $mg{\cdot}L^{-1}$. Oil extraction of MO seeds by pressurised propane at a pressure of 80 bar and a temperature of 30°C obtained the highest yield of oil (39%–42%). This indicated that compressed propane is an efficient technique for extracting oil and obtaining a defatted powder with high protein content (41.72%). Between the two preparations of MO seeds as coagulant at their respective optimal dosage (50 $mg{\cdot}L^{-1}$), the defatted powder showed the best efficiency in the removal of chlorophyll-a (97.4%), turbidity (84%), with zeta-potential values close to zero.

Keywords: Moringa oleifera; Cyanobacteria; Dissolved air flotation; Pressurised fluid; Sustainable

1. Introduction

In recent years, several studies have been developed towards the search for sustainable and eco-friendly natural coagulants as an alternative to inorganic and synthetic coagulants to obtain potable water. *Moringa oleifera* (MO) is a tropical plant that belongs to the Moringaceae family. Fourteen species have been so far identified, and all possess coagulant properties in varying degrees [1]. MO is the most widespread species, and grows quickly, even in medium soils having relatively low humidity [2]. It is a fast-growing, drought-resistant tree, native to the Southern foothills of the Himalayas in north-western India, and is widely cultivated in tropical and subtropical areas, where its fresh seed pods and leaves are used as vegetables [1]. MO seed was reported

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to contain an active bio-coagulation compound [3–5], able to reduce high turbidity [6,7] and microorganisms [8–10] from water. Moreover, MO seed extracts produce lower sludge volume when compared with aluminium, are biodegradable and do not affect the pH of the treated water [11,12].

However, the presence of oil and many other organic compounds in the crude extract favours an increase in the amount of organic matter in the treated water [12,13] and prevents its storage and consumption for more than 24 h [14]. This represents a disadvantage for its large-scale application in water treatment, the purification of the crude extract being highly recommended [15]. The extraction of oil from seeds before the crude extract preparation may be an option of suitable purification, allowing oil recovery for food and industrial processes, and the extract for water clarification.

Extensive research has been carried out involving techniques for MO active component extraction and purification to improve its use as coagulant, and to understand the coagulation/flocculation mechanism. The method and the solvents used for extraction can modify the content of the compounds obtained from the MO [16]. Some of those methods are ion-exchange chromatography [15,17], ultrafiltration [18], dialysis [19], oil extraction with organic hexane solvents [20], ethanol [18,21] and extraction of Moringa oil using Soxhlet extraction [22]. However, solvent oil extraction presents some disadvantages: the generally hazardous nature of the chemicals employed, the production of undesirable residues and the necessity of solvent removal at the end of the extraction process [23,24].

Oil extraction using a solvent under supercritical conditions is presented as an alternative to conventional methods, due to the characteristics of the solvents under certain conditions of pressure and temperature, in which they provide greater selectivity in the uses of MO oil [25,26], the high diffusivity of the fluid ensures rapid extraction [27], the degradation of bioactive compounds is eliminated or reduced, resulting in a product without toxic solvent residues [28]. Pressurised propane can be an alternative to supercritical fluid extraction (SFE)-CO_2 in oil extraction of MO seeds, due to the significant reduction of extraction parameters such as time, pressure and temperature, and provides higher extraction yields when compared with SFE-CO_2 [25,29,30].

Despite a series of uses of MO oil, there is little information available about supercritical fluid extraction [26,29,31–35]. There are few studies that report the influence that the oil and its extraction method may have on the proteins of the primary coagulant of the seed extract from MO and on its coagulant activity [11,36,37].

There are few studies about the influence of the oil and its extraction method on the coagulant activity of MO in the removal of toxic cyanobacteria. The presence of cyanobacteria in drinking water reservoirs represents a high risk to human health, for many cyanobacterial blooms are comprised of toxin-producing species [38]. Therefore, toxic cyanobacteria contribute to the reduction of water quality and are responsible for producing cyanotoxins. These toxins are very dangerous to human and animal health, and are responsible for the death of wild and domestic animals, and human health issues ranging from contact irritations and gastrointestinal distress to acute, chronic and lethal poisonings [38]. Cases have been reported worldwide of lethal poisoning in animals by cyanotoxins. They are also known cases of human death attributed to exposure to these toxins; therefore, it is necessary to develop and implement treatment technologies for the removal of cyanobacteria from drinking water reservoirs.

Previous studies have shown that MO seed extracts are quite efficient as bio-flocculants and coagulants in microalgae biomass separation processes and harvesting [3,39] and can further inhibit the growth until their removal of some cyanobacteria species, such as *Microcystis*, *Chlorella* and *Scenedesmus* [1,39,40], thus contributing to the control of toxins from cyanobacterial blooms.

Cylindrospermopsis raciborskii is a bloom-forming cyanobacterium with complex population dynamics and toxicity [41]. It is of concern because it is capable of producing toxins with implications for human and animal health, has a high level of flexibility with respect to light and nutrients, with higher temperatures and levels of carbon dioxide also promoting growth [42].

Therefore, in this work, the applicability of defatted residues, generated after oil extraction from MO seeds by pressurised propane, were evaluated as coagulants in the C/F/DAF process for removal of *C. raciborskii*. Thus, this research pretends to contribute to finding a viable alternative to improve the quality of water produced in water treatment plants using natural coagulants.

2. Experimental section

2.1. Materials

MO seeds were provided by the Federal University of Sergipe, Brazil. Propane (99.9 wt% in the liquid phase) for the SFE method was provided by Sigma-Aldrich (São Paulo, SP, Brazil). For coagulant preparation, MO seeds were dried and ground to a powder in a Wiley mill (model Te-633 TEC Mill) purchased from Tec-Nal (Piracicaba, SP, Brazil).

2.2. Cyanobacterial cells

Cylindrospermopsis raciborskii was kindly provided by Prof. Dr. Maria do Carmo Bittencourt, from the Brazilian Cyanobacterial Collection of the University of São Paulo (BCCUSP), which allowed for the completion of this study. *C. raciborskii* used in the experiments were grown in laboratory in ASM-1 medium, composed of inorganic substances. These cultures were maintained under conditions of maximum asepsis, at a controlled temperature around 24°C, with a 12 h light/12 h dark photo period.

The study water was prepared from *C. raciborskii* culture and showed the following characteristics (Table 1):

2.3. Extraction method

2.3.1. Compressed propane extraction apparatus and experimental procedure

The experiments were conducted at a bench extraction unit consisting of a propane cylinder, two thermostatically controlled baths, two syringe pumps (ISCO, model 500D, Lincoln, USA), a jacketed extraction vessel with capacity of 78.6 mL, an absolute pressure transducer (Smar, LD301, Smar, Sertãozinho, SP, Brazil) equipped with a portable

programmer (Smar, model HT 201, Sertãozinho, SP, Brazil) at 0.12 bar precision, and a glass vessel to receive the extract removed from the extraction chamber. Additional details on the experimental apparatus and procedure have been described in our previous studies [26,29,31,32].

The extractor was fed with 0.015 kg of seeds (drying of seeds was performed according to the AOAC method) and the remaining space of the extraction cell was filled with glass beads (inert bed). Thus, the propane was added to the extractor and passed initially through the inert bed and then through the powdered seeds. After reaching the desired temperature for extraction, the pump and extractor were simultaneously pressurised. After the operating pressure was reached, the system was left at rest to reach equilibrium (30 min). The propane was then pumped into the extractor at a flow rate of 1 mL min^{-1} for 60–90 min until the extracted oil weight was constant. The experiments were performed in pressure and temperature ranges of 80–120 bar and 30°C–60°C, respectively. The extraction yield was calculated by the ratio of the extracted oil mass to the initial dried sample mass

2.4. Coagulation/flocculation/dissolved air flotation (C/F/DAF) tests

The C/F/DAF assays were conducted using 'Flotest' equipment (Nova Ética, Model 218/3) with MO as coagulant in two different preparations: defatted powder (PO_{def}) by compressed propane extraction and integral powder (PO_{int}).

The optimised operating conditions for C/F/DAF were chosen based on literature and preliminary assays. For the C/F step, the operating conditions used were a G_{mr} of 810 s^{-1}, t_{mr} of 20 s, G_f of 30 s^{-1} and T_f of 20 min. For the DAF step, the best results were obtained at a P_{sat} of 4 bar, T_{sat} of 8 min, R of 15% and V_f of 5 cm·min^{-1}.

2.5. Analytical methods

All samples were analysed for DOC (TOC-5000, Shimadzu, Japan), turbidity (HACH 2100N) and pH (Crison Basic 20+). For chlorophyll a (chl_a) analysis, the samples were filtered through Whatman GF/F filter paper and the chlorophylls were extracted using 10 mL of acetone (90%) using Standard Methods [43] and the chl_a concentration was computed from Lorenzen [44] equations. The zeta potential of the samples was also measured. Analysis was performed in a Zetasizer Nano ZS90 (Malvern Inc., United Kingdom) by electrophoresis mobility measurements at 25°C using a disposable polycarbonate capillary cell (DTS1061, Malvern Inc.).

Table 1
Characteristics of study water

Parameters	Values
Turbidity (NTU)	30.0 ± 0.35
Apparent colour (uH)	124 ± 2.12
pH	7.5 ± 0.21
DOC (mg·L^{-1})	4.075 ± 0.77
Chlorophyll-a (μg·L^{-1})	150 ± 1.62
N (×10^6 cell·mL^{-1})	1 ± 0.49

Note: Results given in mean concentrations ± standard deviation.

The morphological characteristics of MO seeds before and after oil extraction were evaluated using a scanning electron microscope (SS 550 Shimadzu SuperScan SS-550, Shimadzu Corporation, Japan). Samples were manually dispersed on double-face conducting bands on aluminium sample frames and coated with a thin gold layer lining at 20 KV for 20 min in a metallised Shimadzu ion IC 50 prior to analysis.

MO integral powder supercritical were also characterised for protein content, moisture, ash and fats/oils. Protein content was determined by Kjeldahl nitrogen analysis (protein = N(%) × 6.25), following the methodology described in Standard Methods. Moisture content was determined by weighing in a crucible and drying in an oven at 105°C, until a constant weight was obtained. Ash content was measured at 550°C for 3 h. Fats/oils were determined by the Bligh and Dyer method.

2.6. Statistical procedures

STATISTICA 8.0 software was used for all statistical analysis. Statistical significance was considered when $p < 0.05$. Analysis of variance (ANOVA) with Tukey's test was carried out to verify the significance of differences between the means.

3. Results and discussions

3.1. Extraction of MO seed oil by supercritical fluid extraction

The extraction of MO seed oil occurred in periods up to 90 min with a yield of 39%–42% for temperatures and pressures ranging from 30°C to 60°C and 80 to 120 bar, respectively (Table 2).

The experimental condition which presented the highest oil yield was at the lower pressure of 80 bar and temperature of 30°C. At higher temperatures and pressures, the yield tended to decrease, as observed in the conditions 3 and 5. It can be observed that the profile of extractions was similar under all conditions studied; however, an increase in pressure did not influence the oil yield. This shows that condition 1 (30°C/80 bar) is sufficient to obtain good extractions of MO oil (42%) in a shorter time. Table 3 shows other studies in the literature that used CO_2 and propane for the extraction of oil from seeds.

Based on the aforementioned studies, it is observed that to obtain a satisfactory yield of MO oil using supercritical CO_2, it is necessary to use high pressures (400–500 bar) during extraction periods of 2–3 h, or extraction periods of 5–7 h at

Table 2
Experimental conditions and yield of MO seed oil obtained by supercritical fluid extraction

Operational Conditions	Temperature (°C)	Pressure (bar)	Time (min)	Density (g·cm^{-3})	Yield (m/m)
1	30	80	65	0.513	42.02
2	30	120	70	0.504	41.35
3	45	100	60	0.459	39.60
4	60	80	75	0.473	41.56
5	60	120	90	0.488	39.89

pressures of 200–300 bar. Thus, it emphasises the use of propane solvent in the extraction of MO oil due to lower time, pressure and temperature required, as obtained in the study.

3.2. Chemical characterisation of MO seeds

The integral powder (PO_{int}) of MO seeds presented high oil content, protein and carbohydrates and, after SFE method, it was noted that the seed oil had been substantially removed and the protein was not entrained with the oil remaining in the defatted powder (PO_{def}) (Table 4).

It can be observed that the percentage of protein obtained for PO_{def} ranged from 37.03% to 41.72%. The conditions 1, 3 and 4 had the highest levels of protein and were statistically different from the conditions 2 and 5. For the oil content, the condition 1 was evaluated to be statistically different from the other conditions. Regarding moisture values, ash and carbohydrates there was no statistical difference between the experimental conditions. In addition, according to the ANOVA test, there were statistical differences between PO_{def} and PO_{int}; however, the Tukey test showed that PO_{def} was the best coagulant preparation, due to the fact that it contained a high level of carbohydrates and a low level of moisture, which decreases the possibility of degradation. The values of oil, protein and carbohydrates for PO_{int} were similar to those reported by Gidde et al. [45], about 37%, 37% and 16%, respectively. Similar composition values for protein and lipids were also found by Ndabigengesere et al. [46] and Gallão et al. [47], who in turn, verified that protein is the compound found in greater amounts in MO seeds – about 40% – followed by a lipid content of approximately 20%. Silva et al. [48], obtained proteins and lipids values near 40%.

Based on the outcomes, PO_{def} was chosen, using the condition 1 of DAF process for the next C/F/DAF tests, due to the fact that lower temperature and pressure values are directly related to the reduction of energy costs.

Fig. 1 shows the microstructures of the defatted powder using supercritical and integral powder, showing that the number of pores in the seeds increases after oil extraction.

According to Araújo et al. [49], the MO seeds have adsorbent properties, that is, deformations on the surface are visible, containing spaces available for conditions which favour adsorption of metal or organic chemical species in their interstices. By comparing visually the electronic micrographs of Figs. 1(a) and (b), it is possible to infer information about the efficiency of the supercritical extraction process. Practically all seeds cells are broken by the effect of pressure, due probably to the rapid solubilisation of the oil by propane. The great advantage of oil extraction is to reduce the organic load from the seeds and to produce edible oil as a by-product.

3.3. Coagulation/flocculation/dissolved air flotation (C/F/DAF) tests with PO_{int} and PO_{def}

The residual averages of the turbidity, colour, and chlorophyll-a parameters of the water treated by C/F/DAF with PO_{int} and PO_{def} are presented in Table 5. It is worth remembering that the optimal operating conditions were G_{mr} = 810 s^{-1}, t_{mr} = 20 s, G_f = 30 s^{-1}, t_f = 20 min, P_{sat} = 4 bar, t_{sat} = 8 min, R = 15% and V_f = 5 $cm \cdot min^{-1}$. The dosages in the trials varied from 25 to 1,000 $mg \cdot L^{-1}$.

In general, analysing each parameter, lower residuals of turbidity (<9.0 NTU), colour (<40 uH) and chlorophyll-a (<40 $\mu g \cdot L^{-1}$) were observed with dosages of 200–300 $mg \cdot L^{-1}$ for the PO_{int} coagulant and lower residuals of turbidity (<5.0 NTU), colour (<30 uH) and chlorophyll-a (<30 $\mu g \cdot L^{-1}$) with dosage from 50 to 100 $mg \cdot L^{-1}$ for the PO_{def} coagulant, this being in accordance with current legislation.

Table 3
Studies with different conditions in the extraction of oil of seeds

Material	Conditions (temperature/pressure/solvent)	Extraction time (h)	Oil yield (%)	References
MO	30°C, 80 bar, propane	1.08	42.00	This work
MO	47.35°C, 235 bar, CO_2	7	28.00	[21]
MO	102°C, 500 bar, CO_2	2	37.00	[23]
Sesame	60°C, 120 bar, propane	0.91	34.10	[29]
Canola	60°C, 120 bar, propane	1.42	23.83	[31]

Table 4
Average values in the centesimal analysis of MO integral powder and defatted powder

	Operational conditions for supercritical fluid extraction (PO_{def})					
Dry base composition	PO_{int}	1	2	3	4	5
Moisture	10.9 ± 0.09[a]	14.5 ± 0.40[b]	14.9 ± 0.22[b]	14.7 ± 0.09[b]	14.9 ± 0.02[b]	14.59 ± 0.08[b]
Ash	5.23 ± 0.06[a]	5.96 ± 0.10[a]	5.85 ± 0.30[a]	5.61 ± 0.21[a]	5.65 ± 0.08[a]	5.79 ± 0.03[a]
Protein	40.2 ± 0.32[a]	41.7 ± 0.05[a]	37.0 ± 0.08[b]	41.4 ± 0.05[a]	41.3 ± 0.06[a]	38.85 ± 0.20[b]
Oils	29.5 ± 0.18[a]	1.87 ± 0.35[b]	2.96 ± 0.03[c]	2.80 ± 0.29[c]	2.74 ± 0.40[c]	2.78 ± 0.32[c]
Carbohydrates	14.0 ± 0.22[a]	35.9 ± 0.02[b]	39.1 ± 0.01[b]	35.4 ±0.08[b]	35.3 ± 0.010[b]	38.08 ± 0.02[b]

Note: The letters (a,b,c) represent the Tukey's representation statistical analysis – "Results are usually drawn in a box plot. Levels that are not significantly different one each other are represented with the same letter." Within the same line, the averages followed by the same upper-case letter are not statistically different from each other, by Tukey test at 5% significance level. The interpretation of results should be carried out in relation to the values arranged horizontally.

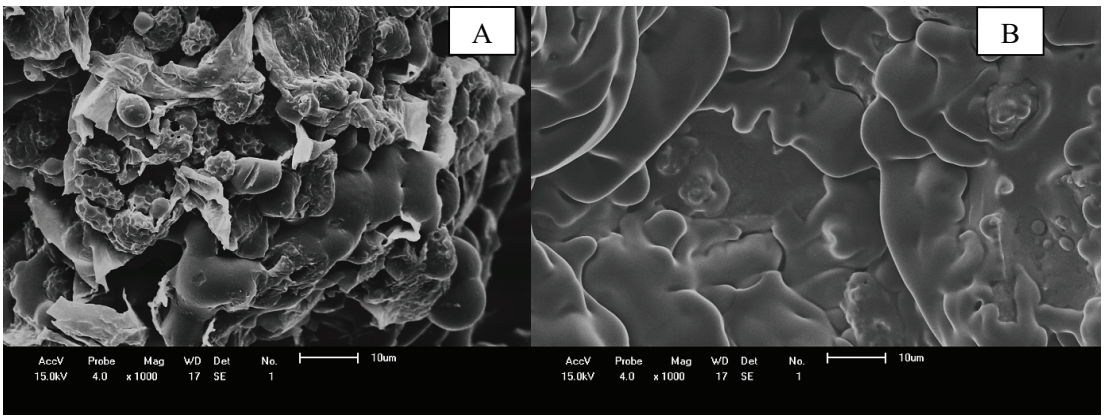

Fig. 1. Microstructures of seeds: (A) defatted powder using supercritical extraction; (B) integral powder (magnified 2000x).

Table 5
Residual average of turbidity, colour and chlorophyll-a to determine the optimal dosage of PO_{int} and PO_{def}

Dosage (mg L^{-1})	Residual turbidity (NTU)[a]		Residual colour (uH)[a]		Residual chlorophyll-a (μg·L^{-1})[a]	
	PO_{int}	PO_{def}	$PO_{(int)}$	PO_{def}	$PO_{(int)}$	PO_{def}
25	11.52 ± 2.97	5.52 ± 0.01	28 ± 0.29	32 ± 3.14	38.20 ± 0.81	9.24 ± 0.67
50	10.86 ± 0.85	4.74 ± 0.01	25 ± 0.24	22 ± 1.43	40.70 ± 1.78	6.25 ± 0.10
100	9.54 ± 0.57	4.26 ± 1.13	28 ± 2.00	21 ± 0.57	31.68 ± 0.48	6.96 ± 0.65
200	8.04 ± 1.41	6.60 ± 0.71	19 ± 0.71	55 ± 0.29	26.40 ± 1.41	15.84 ± 1.12
300	8.88 ± 0.28	10.26 ± 0.99	25 ± 1.27	50 ± 0.29	27.72 ± 1.08	32.92 ± 0.75
400	12.66 ± 1.41	12.36 ± 0.71	21 ± 1.16	75 ± 0.29	33.05 ± 0.99	36.55 ± 1.07
500	12.40 ± 0.94	14.28 ± 2.69	19 ± 1.13	84 ± 2.57	44.90 ± 1.49	38.50 ± 0.50
600	14.28 ± 2.12	17.58 ± 1.13	53 ± 0.29	119 ± 2.57	64.60 ± 0.52	51.46 ± 1.41
700	15.06 ± 0.57	19.32 ± 10.1	34 ± 2.57	117 ± 0.57	71.20 ± 0.62	56.60 ± 0.37
800	18.48 ± 0.85	19.80 ± 1.41	117 ± 0.57	119 ± 0.29	92.48 ± 0.64	67.16 ± 0.37
900	20.94 ± 3.82	20.28 ± 0.14	119 ± 1.14	119 ± 0.57	116.25 ± 0.74	83.00 ± 0.37
1,000	22.68 ± 1.70	22.69 ± 1.10	119 ± 0.86	121 ± 0.29	142.60 ± 1.63	86.96 ± 0.37

[a]Residual results are represented as the average ± standard deviation.

In Fig. 2, the graph of the removal of turbidity, colour and chlorophyll-a parameters is shown. Assays of each preparation of the MO coagulant are represented by different rows, and dosages are arranged along the x-axis.

From the results in Fig. 2, it was noted that there was an increase in turbidity removal efficiency when the oil from MO seeds was extracted. A hypothesis is that the oil content in the seeds forms an emulsion or pellicle coating that can inhibit contact with the surface and thus reduce the formation of flocs. Fig. 2 shows graphics of apparent colour and chlorophyll-a removal; in general, analysing each preparation of MO, high removals of apparent colour (>70%) and chlorophyll-a (>80%) were observed at the lower doses of the PO_{def} relative to the PO_{int}, requiring higher doses (>100 mg·L^{-1}).

According to the statistical analysis, the interaction between variables is clear; not only from the graphics analysis, but also because of the ANOVA report, which provides a *p*-value less than 0.05 in each case. The Tukey test for multiple comparisons was used to determine the optimal dosage of the coagulant and which coagulant is the best to be used.

The summary of the average values of turbidity, apparent colour and chlorophyll-a removals for the two lower (PO_{int} and PO_{def}) coagulants are shown in Table 6.

As for the removal of parameters, statistical analysis indicates that different preparations of coagulants are statistically different between individual dosages, with the exception of apparent colour parameter at lower dosages (25–100 mg·L^{-1}). There was no statistical difference between the preparations of MO in dosages ranging from 25 to 100 mg·L^{-1} for PO_{def}, and from 200 to 300 mg·L^{-1} for PO_{int}. According to Rolim et al. [50], a concentration of 200 mg·L^{-1} of MO seed extract does not represent any health risk.

Similar behaviour was found by Carvalho Bongiovani et al. [51], used the crude extract (aqueous solution) and the defatted extract (oil was extracted with hexane and ethanol). The authors observed higher removal efficiency in all evaluated parameters (turbidity, apparent colour, absorption in UV_{254nm}) when the oil was extracted with hexane using lower dosages (30 mg·L^{-1}) compared with aqueous extract (50 mg·L^{1}).

The summary of the results is presented in Table 7 and reveals that the optimal dosage of MO powder that displayed the lowest turbidity, colour and chlorophyll-a residuals ($p < 0.05$) was dependent on coagulant preparation.

The average floc sizes of the synthetic water and after C/F/DAF process with different preparations of MO as coagulant in their optimal dosages are shown in Table 8.

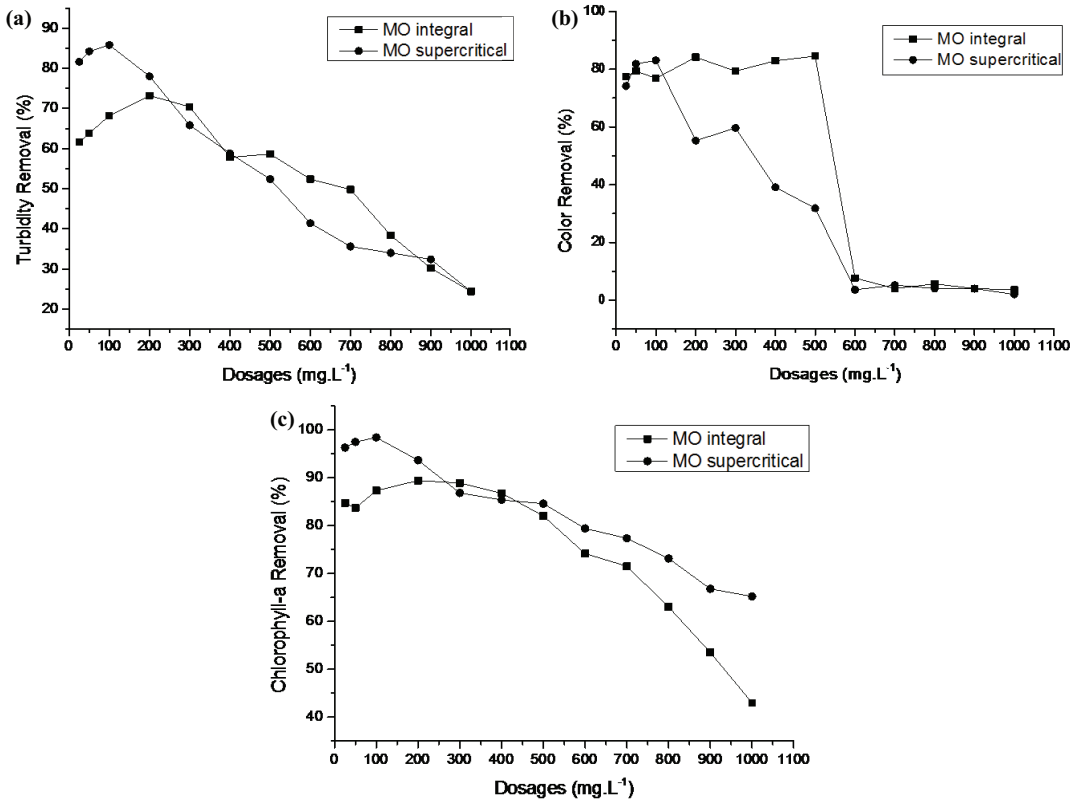

Fig. 2. (a) Turbidity, (b) colour and (c) chlorophyll-a removals in the optimised process of the C/F/DAF using different dosages of MO coagulant preparations.

Table 6
Average values of turbidity, apparent colour and chlorophyll-a removals in the lower dosages of coagulants PO_{int} and PO_{def}

Dosage ($mg \cdot L^{-1}$)	Turbidity removal (%)		Colour removal (%)		Chlorophyll-a removal (%)	
	PO_{int}	PO_{def}	PO_{int}	PO_{def}	PO_{int}	PO_{def}
25	$61.60 \pm 0.85^{A/a}$	$81.60 \pm 0.49^{B/a}$	$77 \pm 2.12^{A/a}$	$74 \pm 2.83^{A/a}$	$84.72 \pm 0.81^{A/a,b}$	$96.30 \pm 0.00^{A/a,b}$
50	$63.80 \pm 0.49^{A/a,b}$	$84.20 \pm 0.13^{B/a,b}$	$79 \pm 3.54^{A/a}$	$81 \pm 1.41^{A/a}$	$83.72 \pm 0.21^{A/b}$	$97.50 \pm 0.14^{B/a}$
100	$68.20 \pm 0.41^{A/b}$	$85.80 \pm 0.43^{B/b}$	$76 \pm 2.83^{A/a}$	$83 \pm 0.71^{A/a}$	$87.33 \pm 0.07^{A/a,b}$	$98.42 \pm 0.07^{B/a}$
200	$73.20 \pm 0.18^{A/c}$	$78.00 \pm 0.34^{B/c}$	$84 \pm 0.00^{A/b}$	$55 \pm 1.41^{B/b}$	$89.44 \pm 0.71^{A/b}$	$93.66 \pm 0.49^{B/b}$
300	$70.40 \pm 0.28^{A/d}$	$65.80 \pm 0.35^{B/b,c}$	$79 \pm 1.41^{A/b}$	$50 \pm 0.71^{B/a}$	$88.91 \pm 0.07^{A/b}$	$86.83 \pm 0.28^{B/c}$

Note: The letters in the superscript represent theTukey's representation statistical analysis – "Results are usually drawn in a box plot. Levels that are not significantly different one each other are represented with the same letter." Within the same row, averages followed by the same capital letter are not statistically different from each other, by Tukey test at 5% significance level. The interpretation of results should be carried out in relation to values arranged horizontally (row). Within the same column, the averages followed by the same lower-case letter do not differ statistically among themselves by Tukey test at 5% significance level. The interpretation of results should be carried out in relation to values arranged vertically (column).

According to the statistical analysis, there was a significant increase in the floc size after C/F/DAF process, particularly when the PO_{def} was used at lower dosages.

Cyanobacterial cell removal by coagulation/flocculation is governed by the same principles as those applied to the removal of colloidal or suspended particles. Cyanobacteria with essentially spherical structures and smooth surfaces may be destabilised by a charge neutralisation mechanism and may aggregate by the principle of adsorption coagulation [52]. It has been suggested that after reducing the magnitude of the negative zeta potential, charge neutralisation and destabilisation occur, and optimal removal conditions could be observed [53–55]. In addition, the zeta potentials of coagulants PO_{int} and PO_{def} were determined, to evaluate the charge. The values obtained were +10.38 mV for PO_{int} coagulant and +17.35 mV for PO_{def} coagulant, which may mean, as mentioned in the literature, that the protein of MO is cationic. The averages of zeta potential values for both coagulants are shown in Fig. 3.

It is observed in Fig. 3 that the zeta potential varied between –3.54 mV and +23.31 mV for PO_{int} coagulant, and between –0.10 mV and +20.46 mV for PO_{def}, with the average value of the raw water of –9.15 mV. This value is in agreement with previous studies, which claim that practically all aqueous colloids are electronegative (negative charge), including algae.

Furthermore, the zeta potential values were observed near zero at a dosage of 100 mg·L^{-1} for PO_{int} and 50 mg·L^{-1} for PO_{def}. Therefore, these dosages are enough for an efficient coagulation/flocculation, as previously determined by statistical analysis. There are few studies of the zeta potential measurement of water treated with MO as coagulant, and they suggest that the main coagulation mechanism is charge neutralisation, because there is an increase in zeta potential with increasing MO dosage. In this study, dosages higher than 500 mg·L^{-1} for both MO coagulant preparations, PO_{int} and PO_{def}, resulted in an overdosage, which resulted in a zeta potential increase to positive values of +23.31 mV and +20.46 mV, respectively. It was also observed that the isoelectric point of MO is around pH 6, and at neutral pH values varies between –5 and –11 mV, before and after oil extraction. Thus, oil extraction by pressurised propane does not change the charge of the MO seeds.

This finding is one of the principal concerns in applying natural coagulants to drinking water treatment processes. Future experiments include the continuous monitoring of DOC and the study of removal options. One option includes the use of powder activated carbon, which is an adsorbent already used in water treatment plants. Finally, pH and conductivity do not present significant differences between the two coagulants used.

Table 7
Optimal dosages of MO for different coagulant preparations

Coagulant	Dosage (mg L^{-1})	Average removal (%)[a]		
		Turbidity	Colour	Chlorophyll-a
PO_{int}	200–300	72.0 ± 1.98	82.0 ± 3.43	89.1 ± 0.37
PO_{def}	25–100	84.0 ± 1.13	81.0 ± 0.86	97.4 ± 0.65

[a]Results of removals are represented by average ± standard deviation.

Table 8
Floc size of synthetic water (control) and after C/F/DAF process with different preparations of MO as coagulants in optimal dosages

Sample	Floc size (nm)
Control	594.5 ± 2.043[a]
PO_{int}	2,351.8 ± 0.808[a]
PO_{def}	4,496.9 ± 0.584[a]

[a]Different statistical groups (Tukey test, $p < 0.05$). The results being represented by average ± standard deviation.

4. Conclusions

This work demonstrates that it is possible to remove *C. raciborskii* from surface water, using MO defatted powder (PO_{def}) by compressed propane extraction, and MO integral powder, as coagulants in the coagulation/flocculation/DAF process. Results showed that using a dosage of 50 mg·L^{-1} of MO defatted powder, removals as high as 97.4% for chlorophyll-a and 84% for turbidity were obtained from medium-turbidity waters (30 NTU). The mechanism proposed for cyanobacteria and turbidity removal was charge neutralisation based on zeta potential values and coagulant doses used. MO is a sustainable alternative water treatment that should be considered together or not with other treatment technologies. Finally, these results demonstrate that MO is a viable alternative to diminish the use of inorganic coagulants in the clarification of surface waters, and represents a more sustainable option to water treatment managers. One of the major concerns in using natural coagulants identified in preliminary results is the increase of DOC in treated water, which represents a challenge for future experiments.

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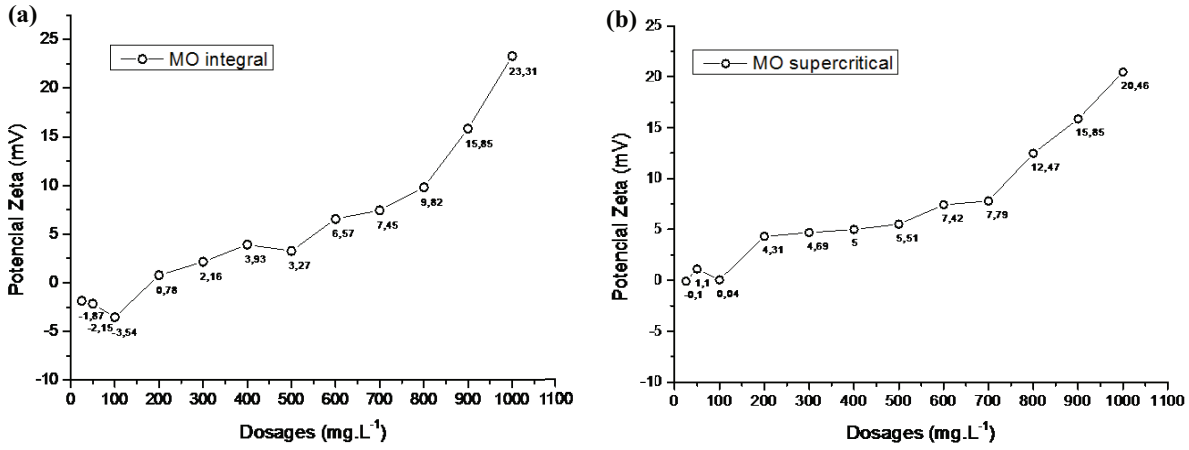

Fig. 3. Zeta potential analysis in the optimised process of C/F/DAF using different dosages of (a) PO_{int} and (b) PO_{def}.

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