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Original Research

Analysis of Water Treatment by *Moringa oleifera* Bioflocculant Prepared Via Supercritical Fluid Extraction

Muhammad Azroie Mohamed Yusoff[1,3], Choon Weng Lee[1,2]*, Chui Wei Bong[1,2]

[1]Laboratory of Microbial Ecology, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia
[2]Institute of Ocean and Earth Sciences, University of Malaya, Kuala Lumpur, Malaysia
[3]National Hydraulic Research Institute of Malaysia, Selangor, Malaysia

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Abstract

In this study, we used supercritical fluid extraction to prepare *Moringa oleifera* as a bioflocculant and studied its efficacy in water treatment. Relative to the conventional solvent extraction method, supercritical fluid extraction produced 42% more bioflocculant and was a more efficient method with both time (about seven hours less) and cost savings (about USD30 less). The bioflocculant produced via supercritical fluid extraction was also smaller (18±5 µm) and more similar in size (Coefficient of Variation, CV = 28%) as opposed to conventional solvent extraction (23±8 µm, CV = 35%). It was able to reduce more than 95% of turbidity and up to 60% bacterial population. Its performance in reducing selected heavy metals from water samples was also generally better than aluminium sulfate or alum. Our study showed that with the exception of cost restrictions, *M. oleifera* bioflocculant produced via supercritical fluid extraction has the potential to replace alum in water treatment plants.

Keywords: *Moringa oleifera*; bioflocculant; water treatment; supercritical fluid extraction

Introduction

In a water treatment plant the flocculation step is the main process for turbidity removal. Currently, the flocculation step is carried out with various types of chemical flocculants, e.g., aluminium sulfate or its variants [1]. However, studies have suggested that there may be negative health implications of using alum, such as Alzheimer's disease [2]. Even though it is difficult to show a causal relationship through epidemiological studies, the long-term effects of aluminium cannot be dismissed, and there is a need to control exposure to aluminium in the general population [3]. Other than health implications, another disadvantage to using alum in a water treatment plant is the large sludge volume produced [4] and the high cost of disposing of alum sludge as scheduled waste.

With the growing global population, world water demand has increased seven-fold in the last century and is expected to increase further with the economic expansion of developing countries [1]. Existing freshwater resources need protection and

*e-mail: lee@um.edu.my

new water resources must be developed in order to meet the world's growing demand. This will require better water treatment technology. Therefore, any improvement available for the water treatment process is of importance. Alternative technologies (e.g., membrane) are favored over other technologies for water treatment such as disinfection, distillation, or media filtration because, in principle, they require no chemical additives, thermal inputs, or regeneration of spent media [1]. Another alternative adopts the use of natural flocculants or bioflocculants for turbidity removal in the water treatment process. In this context, *Plantago* spp., common bean seed, chestnut, acorn seed, *Opuntia* spp., chitosan, ipomoea seeds, *Cactus latifaria*, *Cassia obtusifolia*, and *Sterculia lychnophora* have been presented as viable alternatives [5].

A potential bioflocculant investigated in this study is from *Moringa oleifera* seeds [6]. The active component derived from both crushed (powdered) and defatted (oil extracted) seeds of *M. oleifera* is a soluble protein that contains a natural cationic polyelectrolyte that causes flocculation [7]. The conventional method to strip fat from *M. oleifera* seeds involves solvent extraction (SE) using n-hexane [8]. However Ruttarattanamongkol et al. [9] recently proposed using supercritical fluid extraction (SFE) to extract oil from *M. oleifera*. SFE uses high-pressure carbon dioxide (CO_2) as an oil extracting agent and has proven an excellent alternative to chemical solvents [9]. The use of SFE in extraction also eliminates organic solvents and the expensive post-processing step of solvent removal [10].

Although the use of SFE to extract oil from *M. oleifera* has been reported [9], the potential use of *M. oleifera* as bioflocculant after SFE has not been assessed. There is also limited published data to evaluate the effectiveness of *M. oleifera* coagulant versus the conventional alum [11]. Therefore, in this study we investigated the potential of *M. oleifera* bioflocculant in water treatment after SFE extraction. We also reported the optimal water treatment conditions by *M. oleifera* bioflocculant in terms of water turbidity and bacterial population and heavy metal removal.

Experimental

Preparation of *M. oleifera* Seeds as Bioflocculant

M. oleifera was obtained from the state of Sabah in Malaysia. We used only good-quality seeds from dry pods. The seeds were removed from their shells and the kernels were blended into medium fine powder using a domestic blender. The powder was then oven dried at 50ºC overnight (Memmert, Germany) to decrease its moisture content (Fig. 1). We then extracted the oil inside the *M. oleifera* powder via the SFE method [12] using a TST Oven Extraction system (OV-SCF-1000, Taiwan). Briefly, the extraction was carried out at 250 Bar and 50 to 60ºC with 10 min of

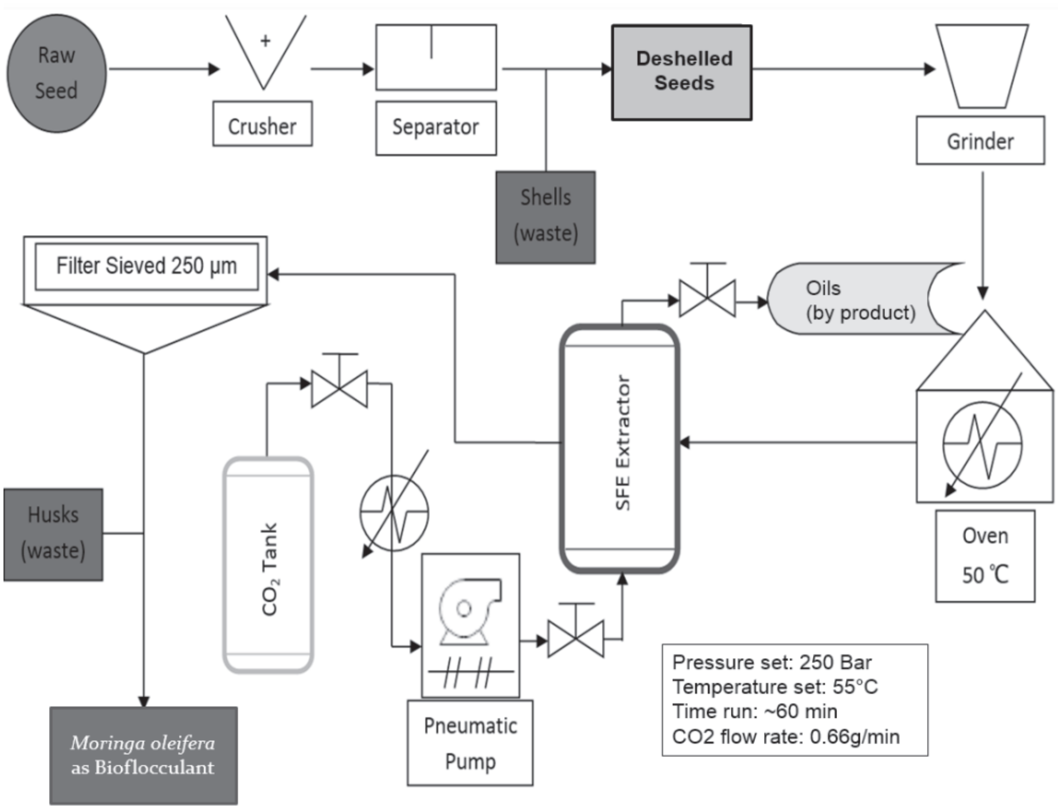

Fig. 1. Flow diagram showing the steps in the preparation of *M. oleifera* bioflocculant.

Table 1. Comparison of reagents and utility costs incurred in producing oil-extracted *M. oleifera* bioflocculant via solvent extraction (SE) and supercritical fluid extraction (SFE). The Malaysian Electricity Tariff is MYR 0.218 (~USD 0.05) for the first 200 kWh. Estimated price in USD based on a MYR4-to-USD1 conversion rate.

	Solvent Extraction (SE)	Supercritical Fluid Extraction (SFE)
Extraction agent	n-Hexane 99%	Carbon Dioxide (CO_2) 99%
Average local price L^{-1}	USD 12.50 L^{-1}	USD 0.66 L^{-1} + USD 0.01 L^{-1} (N_2 gas for flushing)
Usage to produce for 1 kg bioflocculant	6 L n-Hexane	68.29 L CO_2
Reagent total cost usage for 1 kg	USD 75.00	USD 45.75
Extracting instrument	Hanon SOX406 Fat Analyzer	TST OV-SCF-10000 Extraction Oven
Electricity power usage	1 Kilowatt (kW)	1.76 kW
Extraction time for 1 kg bioflocculant	9 hours	2 hours
Electricity Cost usage	USD 0.05 h^{-1}	USD 0.09 h^{-1}
Total Electricity Cost usage	USD 0.45	USD 0.18
Reagent and Utilities Cost for 1 kg bioflocculant produced	USD 75.45	USD 45.93
M. oleifera cost for 1 kg produced (local price)	USD 11.00	USD 11.00
Grand total for 1 kg bioflocculant produced	**USD 86.45**	**USD 56.93**

static extraction and 30 min of dynamic extraction with an industrial-grade CO_2 flow rate of 0.66 g min^{-1}. The final product was sieved through a 250 µm mesh to remove any remaining husk.

Jar Test

Jar testing simulates the flocculation process with treatment chemicals in a water treatment plant and helps determine the right amount of treatment chemicals to improve the plant's performance. We collected river water using a sterile 50-liter high-density poly-ethylene plastic container at the Labu River water intake point at Sungai Labu Water Treatment Plant, Salak Tinggi (2°N 47'21", 101°E 44'15"), and kept it cold at 4ºC until analysis. Turbidity, pH, total suspended solids (TSS) and heavy metals – aluminium (Al), barium (Ba), chromium (Cr), cuprum (Cu), ferum (Fe), manganese (Mn), nickel (Ni), plumbum (Pb), and zinc (Zn) – were analyzed according to APHA guidelines [13]. Turbidity was measured using a turbidimeter (Thermo, USA), whereas pH and TSS were measured by a handheld pH meter (Mettler Toledo, Spain) and a UV-visible spectrophotometer (DR 5000 Hach, USA), respectively. Heavy metal analyses were by inductive coupled plasma or ICP-OES (Perkin Elmer, USA), with detection limits within the SEMI C10-1109 guidelines. We also enumerated culturable heterotrophic microflora on four different types of microbiological media (Reasoner's 2 agar (R2A), Tryptone Glucose Yeast agar (TGYA), Casitone Glycerol Yeast Autolysate agar (CGYA) and MacConkey Agar) via the spread plate method. MacConkey agar was used for the enumeration of fecal pollution indicators such as coliform. All plates were incubated aerobically at 35ºC overnight. Microbiological counts were also carried out according to standard APHA guidelines.

Table 2. Before and after comparison of (A) turbidity in NTU and (B) total suspended solids (TSS, mg L^{-1}) in jar test analyses at various dosages (10-1000 mg L^{-1}) of *M. oleifera* bioflocculant versus the control alum (25 mg L^{-1}).

Dosage (mg L^{-1}) *M. oleifera*	A. Turbidity (NTU)		% Reduction
	Before	After	
1000	853±2	43±1	95.0
500	834±5	31±3	96.2
100	821±4	24±1	97.0
50	835±2	21±2	97.5
10	712±4	10±1	98.6
Alum			
25	919±3	6±1	99.3
Dosage (mg L^{-1}) *M. oleifera*	B. TSS (mg L^{-1})		% Reduction
	Before	After	
1000	2907±4	147±3	94.9
500	2846±5	87±1	96.9
100	2811±2	82±4	97.1
50	2863±7	61±5	97.9
10	2452±3	33±2	98.7
Alum			
25	3153±7	20±1	99.4

Table 3. Comparison of heavy metal removal by alum and *M. oleifera* bioflocculant from supercritical fluid extraction (SFE). Instrument detection limits (µg L^{-1}): Al (1.86), Ba (0. 529), Cr (0. 838), Cu (0. 953), Fe (0. 736), Mn (0. 176), Ni (1.22), Pb (8.56), and Zn (0.527).

Metal	Type of coagulant and dosage (mg L^{-1})	Initial metal concentration at 0 min (mg L^{-1})	Final metal concentration at 15 min (mg L^{-1})	p-value (t-test)
Al	SFE (10)	19.62±1.74	0.11±0.02	1.6×10^{-54}
	Alum (25)		0.15±0.02	1.8×10^{-54}
Ba	SFE (10)	0.16±0.02	0.01±0.00	1.2×10^{-49}
	Alum (25)		0.02±0.00	1.9×10^{-48}
Cr	SFE (10)	0.02±0.00	ND	9.4×10^{-41}
	Alum (25)		ND	4.6×10^{-40}
Cu	SFE (10)	0.05±0.04	ND	1.1×10^{-7}
	Alum (25)		ND	7.5×10^{-8}
Fe	SFE (10)	47.73±6.91	0.19±0.1	1.8×10^{-42}
	Alum (25)		0.1±0.02	1.6×10^{-42}
Mn	SFE (10)	0.73±0.05	0.02±0.01	2.4×10^{-57}
	Alum (25)		0.04±0.01	1.4×10^{-56}
Ni	SFE (10)	0.02±0.04	ND	6.4×10^{-6}
	Alum (25)		ND	2.6×10^{-6}
Pb	SFE (10)	0.08±0.01	ND	4.4×10^{-29}
	Alum (25)		ND	3.2×10^{-56}
Zn	SFE (10)	0.39±0.1	ND	1.2×10^{-31}
	Alum (25)		ND	7.2×10^{-25}

We carried out the jar test analysis using a jar tester (Lovibond Flocculator, Germany) according to standard sedimentation jar test analysis, where for each test run a one-liter test solution was mixed at 200 rpm for 5 min and sedimentation was for 15 min. For each jar test assay, *M. oleifera* bioflocculant at different treatment dosages were carried out against the control alum, and selected parameters (turbidity, TSS, culturable bacteria, and heavy metal concentration) before and after each jar test analysis were recorded. All values were reported as mean ± standard deviation (S.D.) unless otherwise stated. Count data were log-transformed before statistical analyses to meet parametric assumptions of equality of variance and normal distribution. Analysis of variance and Tukey's test were used to test the significance of the reduction of selected parameters after flocculation.

Results and Discussion

Preparation of *M. oleifera* as Bioflocculant

In this study, the yield of *M. oleifera* bioflocculant extracted via SFE technique was 42.1±0.1% of the initial weight and is comparable to the yield reported by Palafox et al. [14]. The yield from SFE was higher than the conventional SE method using n-hexane as a solvent ($t = 9.79$, $p<0.001$). The amount of oil extracted using the SFE method was also higher than the SE method ($t = 4.56$, $p<0.05$). Concurrently, less husk was produced from the SFE method, i.e., about 28.3±0.2% of initial weight as compared to 33.0±0.3% from the SE method ($t = 10.58$, $p<0.001$). Using the SFE method, we produced about 420 g kg^{-1} (initial weight) of pure *M. oleifera* bioflocculant, 300 g kg^{-1} of extracted oil, and 280 g kg^{-1} husk waste. The diameter of particles via SFE method ranged from 10 to 35 µm (18±5 µm, n = 110) and were smaller than via the SE method (11-39 µm, 23±8 µm, n = 110) ($t = 5.185$, $p = 1 \times 10^{-6}$). This was probably due to the high pressure (250 bar) applied during the SFE extraction process, which is known to result in smaller-sized particles [15]. In contrast, the SE method was significantly less efficient in extracting oil from *M. oleifera*, and the bioflocculant particles formed were larger and visibly not uniform. The more efficient oil extraction via SFE method is of a great advantage as it also reduced the organic load from the seeds. The reduced organic load can help overcome the problem of increased organic load, water colour, taste, and odour in water treatment when using *M. oleifera* bioflocculant [16].

From the estimated cost to produce one kg of bioflocculant, we showed that the SFE method was about USD30 cheaper than the SE method (Table 1). Extraction via SE method also took up more time (nine hours) than via SFE method (two hours). Moreover, the *M. oleifera* bioflocculant after solvent extraction is readily contaminated with solvent [8], which is toxic and harmful to the environment [17]. Consequently, additional processing steps in the SE extraction system had to be implemented to eliminate chemical residue [14]. Although this is usually done via a series of distillation units under vacuum and other ancillary apparatus (such as a deodorizer and degumming [18]), these additional processing steps would have incurred extra cost and time. We showed that extraction via SFE was a viable and better method with both time and cost savings relative to the SE method.

Jar Test Analysis

In the jar test analyses, the effectiveness of *M. oleifera* bioflocculant from the SFE method as a flocculating agent for water treatment was compared with alum, which is the common coagulant used in conventional water treatment. The average turbidity of the raw water sample for each assay ranged from 712 to 919 NTU (nephelometric turbidity unit) and was highly turbid. The amount of *M. oleifera* bioflocculant used in the jar test analysis ranged from 10 mg L^{-1} to 1000 mg L^{-1}, and the turbidity of the raw water was reduced by 95.0% to 98.6% ($F = 168.7$, $df = 5$, $p = 1.096 \times 10^{-10}$) (Table 2). This level of reduction, however, was still lower than alum, which reduced water turbidity by 99.3% ($q>6.53$, $p<0.0061$). Similarly, *M. oleifera* bioflocculant reduced TSS by 94.9% to 98.7% ($F = 661.1$, $df = 5$, $p = 3.27 \times 10^{-14}$), which was less than the reduction achieved by alum (99.4%) ($q>11.50$, $p<0.0002$).

With the *M. oleifera* bioflocculant we were able to achieve at least 95% reduction in turbidity and TSS. The highest reduction was obtained with 10 mg L^{-1} dosage, at which turbidity and TSS were reduced by 98%. The SFE extracted *M. oleifera* bioflocculant performed strikingly better than when using filtered ground seed suspension, i.e., about 94% turbidity reduction at 400 mg L^{-1} dosage [19]. Our results were also clearly better than when using *M. oleifera* bioflocculant extracted with ether, which reduced turbidity by 90% at 500 mg L^{-1} [16]. Our results showed vast improvement in the reduction of turbidity and TSS relative to previous extraction methods [20, 21], and suggested the viability of SFE-extracted *M. oleifera* bioflocculant as a replacement for alum in water treatment.

Table 3 shows the heavy metal removal by *M. oleifera* bioflocculant from SFE and alum in a jar test analysis at the optimum dosage of 10 mg L^{-1} for *M. oleifera* bioflocculant and 25 mg L^{-1} for alum. Both bioflocculant and alum significantly reduced the concentration of the tested heavy metal. Tests showed that both bioflocculant and alum removed Cr, Cu, Ni, Pb, and Zn to non-detectable or below the detection limit of the analytical instrument. For Al, Ba, and Mn, *M. oleifera* bioflocculant also reduced the heavy metal concentration, but at a higher degree than alum. The only exception was Fe, where alum reduced Fe concentration at a higher degree than *M. oleifera* bioflocculant. The bioflocculant generally performed better than alum in removing heavy metals, and could

Table 4. Culturable bacterial count on R2A, CGYA, TGYA, and MacConkey Agar in jar test analyses before and after treatment with varying dosages of *M. oleifera* bioflocculant and alum.

Type of Dosage (*M. oleifera* or alum) in mg L^{-1}	Media		% Reduction
	R2A (cfu ml^{-1})		
	Before	After	
M. oleifera 1000	$7.7±0.6 \times 10^4$	$2.9±0.9 \times 10^4$	61.6
M. oleifera 500	$7.4±0.5 \times 10^4$	$3.2±0.8 \times 10^4$	57.1
M. oleifera 100	$7.4±0.6 \times 10^4$	$3.9±1.7 \times 10^4$	47.2
M. oleifera 50	$7.3±0.6 \times 10^4$	$3.3±0.7 \times 10^4$	55.7
M. oleifera 10	$7.6±0.9 \times 10^4$	$3.4±0.9 \times 10^4$	54.9
Alum 25	$7.6±0.5 \times 10^4$	$5.9±1.8 \times 10^2$	99.2
	CGYA (cfu ml^{-1})		
	Before	After	
M. oleifera 1000	$2.4±0.5 \times 10^4$	$1.0±0.4 \times 10^4$	59.4
M. oleifera 500	$2.5±0.4 \times 10^4$	$1.0±0.5 \times 10^4$	59.7
M. oleifera 100	$2.6±0.5 \times 10^4$	$1.0±0.4 \times 10^4$	62.2
M. oleifera 50	$2.5±0.2 \times 10^4$	$1.1±0.6 \times 10^4$	57.6
M. oleifera 10	$2.5±0.3 \times 10^4$	$1.1±0.5 \times 10^4$	56.3
Alum 25	$2.3±0.5 \times 10^4$	$9.7±1.4 \times 10^1$	99.6
	TGYA (cfu ml^{-1})		
	Before	After	
M. oleifera 1000	$6.5±0.3 \times 10^4$	$3.0±0.9 \times 10^4$	53.4
M. oleifera 500	$6.6±0.5 \times 10^4$	$2.9±0.8 \times 10^4$	55.3
M. oleifera 100	$6.8±0.4 \times 10^4$	$3.0±0.9 \times 10^4$	56.0
M. oleifera 50	$6.4±0.3 \times 10^4$	$3.0±0.9 \times 10^4$	52.1
M. oleifera 10	$6.3±0.3 \times 10^4$	$3.3±0.9 \times 10^4$	47.4
Alum 25	$6.4±0.2 \times 10^4$	$3.3±1.4 \times 10^2$	99.5
	MacConkey Agar (cfu ml^{-1})		
	Before	After	
M. oleifera 1000	$6.4±0.3 \times 10^4$	$2.9±0.7 \times 10^4$	53.4
M. oleifera 500	$6.1±0.4 \times 10^4$	$3.4±0.7 \times 10^4$	43.8
M. oleifera 100	$6.5±0.3 \times 10^4$	$3.3±0.7 \times 10^4$	49.2
M. oleifera 50	$6.4±0.3 \times 10^4$	$3.1±0.8 \times 10^4$	51.6
M. oleifera 10	$6.2±0.2 \times 10^4$	$2.9±0.6 \times 10^4$	53.3
Alum 25	$6.6±0.3 \times 10^4$	$5.7±1.6 \times 10^2$	99.1

Table 5. Estimated cost of conventional water treatment system using alum as coagulant versus *M. oleifera* bioflocculant. *Estimated price in USD based on Kualiti Alam Ltd (a local scheduled waste handling company). MLD = million liter day^{-1}, MT = metric ton, WTP = water treatment plant.

	Alum coagulant	*M. oleifera* bioflocculant
Origin	Aluminium sulfate	*M. oleifera*
Average local price kg^{-1}	< USD 0.08 kg^{-1}	USD 11.00 kg^{-1}
Routine treatment dosage	25 mg L^{-1} For a 100 MLD WTP: 2500 kg day^{-1}	10 mg L^{-1} For a 100 MLD WTP: 1000 kg day^{-1}
Estimated cost of coagulants in WTP	USD 0.002 m^{-3} USD 200.00 day^{-1} USD 6,000.00 $month^{-1}$	USD 0.11 m^{-3} USD 11,000.00 day^{-1} USD 330,000.00 $month^{-1}$
Sludge	Categorized as scheduled waste and has to be treated before disposal	Is not scheduled waste and considered as environmentally harmless
Cost of sludge disposal and treatment*	USD 684.00 MT^{-1}	None
Estimated sludge produced	10 MT $month^{-1}$	5 MT $month^{-1}$
Estimated costs of sludge disposal	USD 6,835.00 $month^{-1}$	None. Sludge could instead be sold as organic fertilizer for USD 455 $month^{-1}$ at a market value of USD 91.00 MT^{-1}
Estimated monthly operating cost of coagulant purchase and disposal	USD 13,000	USD 330,000
Side effects	Diseases related to excessive Al^{+} residue in treated water (Suarez-Fernandez et al.1999)	None

be used to treat raw water with heavy metal elements, thus providing an alternative to alum.

In this study, we also assessed the effect of *M. oleifera* bioflocculant toward bacterial abundance based on culture-dependent isolation on R2A, CGYA, TGYA, and MacConkey agars. The abundance of culturable bacteria decreased in every jar test analysis, and the percentage of reduction when bioflocculant was used ranged from 44% to 62% (Table 4). The percentage of reduction was strikingly lower than with alum, which reduced bacteria by >99% ($t = 41.15$, $p<0.001$). There was also no discernible pattern in the percentage of reduction with increasing bioflocculant dosage. The average bacterial reduction on R2A, CGYA, TGYA, and MacConkey agars were 55±5%, 59±2%, 53±3%, and 50±4%, respectively. Although *M. oleifera* bioflocculant possesses bactericidal compounds that inhibit bacterial growth [22] – for example 4(α-L-rhamnosyloxy)-benzyl isothiocynate [23] – the bacterial reduction was more likely due to the polypeptides in the bioflocculant as increasing dosage of the bioflocculant did not increase bacterial reduction. These polypeptides are known to coagulate particles and bacteria in the suspension [5], and might have already reached saturating conditions at a low bioflocculant dosage.

Although others have reported higher bacterial reduction (66% to 93%) with *M. oleifera* bioflocculant [11], the water sample used had low initial counts (6 cfu ml^{-1} to 100 cfu ml^{-1}), which was about two orders lower than in this study. The water sample used in this study represented typical raw water used in drinking water treatment systems in Malaysia. Therefore, our results showed that one of the challenges for using *M. oleifera* bioflocculant was the residual bacterial counts, which were relatively higher than when using alum. Although the addition of second-stage sand filtration [11] or chlorination [24] can remove bacteria satisfactorily, further studies have to be carried out to confirm this.

Economic Feasibility of *M. oleifera* Bioflocculant in Water Treatment System

Although we had shown earlier that the production cost of the *M. oleifera* bioflocculant via SFE was lower than SE, we examined the economic feasibility on the use of *M. oleifera* bioflocculant versus alum in a conventional water treatment system (Table 5). In this study, the optimum dosage for water treatment using *M. oleifera* bioflocculant was 10 mg L^{-1}, which was less than the amount of alum required (25 mg L^{-1}). Similarly, the amount of sludge produced when using the bioflocculant was smaller and considered to be environmentally safe. In contrast, sludge produced from alum flocculation is categorized as scheduled waste, and disposal of this sludge incurs cost [25]. From Table 5, the estimated monthly operating cost of bioflocculant purchase and disposal for the *M. oleifera* bioflocculant was about 30 times higher than alum. Unlike alum that is widely available for water treatment plant operators worldwide, *M. oleifera* bioflocculant is currently only processed by vitamin and supplement manufacturers. Therefore, the current market price for *M. oleifera* bioflocculant is strikingly higher than alum. At current prices, the use of *M. oleifera* bioflocculant is untenable even though there is also a financially hidden cost in managing diseases related to excessive aluminium-ion (Al^{3+}) residue in treated water [3]. If the production cost of *M. oleifera* bioflocculant can be reduced by an order, its estimated water treatment cost would only be about 2.5 times more, and this is probably more acceptable to the public since *M. oleifera* bioflocculant has shown itself to be capable of treating raw water and is environmentally friendly and safe toward human health.

Conclusions

In this study, the SFE method was a more efficient method in the production of *M. oleifera* bioflocculant than the SE method. More bioflocculant was extracted per weight of raw seeds, and the bioflocculant produced was smaller and more similar in size. Bioflocculant via SFE could reduce >95% of turbidity and suspended solids, up to 60% bacterial abundance, and heavy metals. Apart from the higher residual bacterial abundance, bioflocculant performed as well as alum. Therefore, SFE showed promise in eliminating residual chemicals in bioflocculant preparation. At present, although *M. oleifera* bioflocculant has the potential to replace alum in water treatment plants, current prices must be reduced further to make it tenable.

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Conflict of Interest

The authors declare no conflict of interest.

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