Moringa oleifera-derived coagulants for water treatment: Floc structure, residual organics, and performance tradeoffs

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Abstract

The study explored the suitability of unfractionated extracts from the seeds of the *Moringa oleifera* tree as a coagulant for water treatment. The coagulant was obtained by soaking crushed and sieved seeds in a low salinity aqueous solution: a simple and inexpensive alternative to conventional coagulants in settings where specialized expertise and equipment are lacking. The performance of *M. oleifera*-derived coagulants was quantified in terms of turbidity removal, bacteriophage clearance, concentration of residual organics, as well as meta-parameters such as floc size and fractal dimension. Treating high turbidity clay suspensions at the optimal coagulant dosage (14.7 mg(DOC)/L) and flocculation mixing conditions ($\bar{G}$ = 22.4 s^{-1}) removed > 94% of turbidity, similar to that recorded in reference tests with alum. Floc size distribution shifted to larger sizes during the first 10 min of flocculation with no change afterwards, while the floc fractal dimension, D_f, continued to increase, pointing to the gradual formation of denser (D_f = 2.1 to 2.2), more settleable flocs. Preliminary tests with MS2 bacteriophage showed that coagulation with *M. oleifera* decreased the viable MS2 titer by ~ 1.3 log, which was significantly above the turbidity removal (~ 1 log). The extraction process, however, allowed a large amount of residual organics (> 78% of extracted DOC) into the treated water. Combining the coagulants with downstream filtration and adsorption, employing UV or solar disinfection, or limiting applications to non-potable reuse are suggested for mitigating the concerns related to residual DOC.

Keywords

natural coagulants; coagulation; flocculation; fractal dimension; turbidity; virus removal; MS2 bacteriophage; residual organics

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1. Introduction

Coagulation and flocculation are commonly used in water and wastewater treatment to remove colloidal materials. Compared to inorganic and synthetic organic coagulants, natural organic coagulants produce less sludge and their performance is less affected by pH (Oladoja 2015). They are biodegradable and they tend to leave the pH of the treated water unchanged. They can often be sourced locally and at a low expense. These properties make natural coagulants more accessible, easier to use, and more sustainable than the other types of coagulants (Kansal and Kumari 2014). The use of natural organic substances to treat water has a long history with applications including adsorption, coagulation and disinfection. Treating water using various parts of plants such as *Strychnos potatorum* was prescribed in the ancient Indian text, Sushruta Samhita (Baker 1949). Water treatment using seeds of *Moringa oleifera* (*M. oleifera*) has received much attention recently (Kansal and Kumari 2014; Villaseñor-Basulto et al. 2018). Native to the Indian subcontinent, *M. oleifera* grows in South and Southeast Asia as well as in Africa, Central America and the Caribbean, northern parts of South America, Middle East, and Oceania. *M. oleifera* is grown commercially on a large scale; in India alone, the annual production of immature pods is at ~ 1.2×10^9 kg (Paliwal and Sharma 2011). The plant grows well even on infertile soil, it is a legume, it is nutritious, and it has medical uses: hence, there are several advantages to growing *M. oleifera*, especially in regions with economic water scarcity.

Shelled seeds of *M. oleifera* contain ~ 36.7% protein, 34.6% lipids, and 5.0% carbohydrate by mass (Ndabigengesere et al. 1995). To extract the coagulant, the wings and seed shell are typically removed from dried seeds, which are then crushed into powder. Coagulants can be extracted by soaking in water (Jahn and Dirar 1979) or in solutions of salt – commonly NaCl, but also $NaNO_3$ and KCl (Okuda et al. 1999). Although simple and inexpensive, this extraction process increases water's organic carbon content, a major concern that limits the range of possible uses of the treated water. To reduce organic carbon in treated water, the seed powder can be defatted using organic solvents prior to extraction. More selective extraction methods (e. g. ion exchange, chemical precipitation) can also be used to minimize residual organics. These approaches, however, add costs and require access to specialized equipment, chemicals, and expertise, defeating the key advantages of natural coagulants – their accessibility and ease of use.

A number of studies have attempted to isolate coagulating components with the goal of characterizing them or reducing residual organic carbon in the treated water. Purification methods included precipitation, dialysis, ion-exchange chromatography, and gel electrophoresis (Gassenschmidt et al. 1995; Ndabigengesere et al. 1995). Different results were obtained based on the extraction and purification methods used. This suggests that the seeds of *M. oleifera* contain a mixture of coagulants whose relative abundance in an extract is extraction- and purification process-specific. Notably, water and salt extraction seem to extract the same proteins, but with different abundances

(Ghebremichael et al. 2005). Fractionation of a water extract resulted in eight cationic protein fractions; nearly 95% of these proteins were part of four fractions that were effective as coagulants (Nordmark et al. 2016).

The mechanism of coagulation is believed to be adsorption and charge neutralization (Ndabigengesere et al. 1995; Nordmark et al. 2018). The proteins involved in coagulation are strongly cationic with high pI values (Nordmark et al. 2016). While the active agents in *M. oleifera* seeds have been studied and the coagulation mechanisms are partly understood, the formation and structure of flocs was, to our knowledge, explored only in one study. Flocculation of monodisperse latex nanoparticles using purified *M. oleifera* derived proteins led to flocs with a high fractal dimension (close to 3) suggesting restructuring that followed reaction-limited aggregation (Hellsing et al. 2014). In water treatment practice, however, a broad range of suspended materials need to be removed, often in the clay size range. The flocs formed from clays and other morphologically complex materials can be very different from those resulting from monodisperse spherical beads. The reduction in turbidity caused by the removal of clays is indeed a common test in studies assessing the performance of the coagulants in *M. oleifera* seeds (Jahn and Dirar 1979), with removals over 90% being typically noted (Kansal and Kumari 2014; Villaseñor-Basulto et al. 2018). The efficiency of *M. oleifera* in removing microorganisms in coagulation-flocculation processes has also been studied, with coliforms as the most commonly used target (Salles et al. 2014; Shebek et al. 2015).

The present work focuses on the non-ideal, yet, in many settings, relevant, case of unfractionated *M. oleifera* extracts, obtained by leaching and filtering under conditions practicable in a non-laboratory environment. The primary goal of the study was to explore the suitability of unfractionated *M. oleifera*-derived extract as a coagulant for turbidity removal. We hypothesized that the flocculation time needed to maximize floc fractal dimension, D_f, would lead to improved turbidity removal. In addition to the data on floc structure, residual organics, and turbidity removal, the study presents preliminary results on the reduction in the titer of infective MS2 bacteriophage, a commonly used surrogate for human viruses. Based on the removal data and residual dissolved organic carbon (DOC) values, we discuss process design constraints and tradeoffs, as well as implications of the results for downstream treatment processes such as filtration and disinfection.

2. Materials and Methods

2.1 Reagents and synthetic feed water

Moringa oleifera seeds were obtained from Paisley Farm and Crafts (Willoughby, OH). FCC-grade kaolin powder was purchased from Spectrum Chemical (Brunswick, NJ).

Reagent-grade NaCl, ReagentPlus-grade $Na_2S_2O_8$, and ACS reagent-grade H_3PO_4 were procured from Sigma-Aldrich. All aqueous solutions were prepared using deionized (DI) water (resistivity of ~ 0.077 MΩ·cm, pH ~5.9). Stock aqueous suspension of kaolin (15 g/L) was stirred for at least 24 h to hydrate clay particles. In each coagulation-flocculation jar, 1980 mL of DI water and 20 mL of stock kaolin suspension were mixed at 100 rpm for at least 30 min to prepare a suspension with ~ 225 NTU turbidity (Hach 2100N turbidimeter). Procedures for MS2 Propagation and quantification are described in the Supplementary Information (SI) file.

2.2 Preparation of *Moringa oleifera*-derived coagulant

Seeds of *M. oleifera* were shelled and kernels were powdered using mortar and pestle. Crushed seed fragments were separated using a stack of sieves (Gilson). Landázuri et al. showed that particle size of crushed *M. oleifera*'s seeds affects the coagulant's efficiency (Landázuri et al. 2018). In our tests, the 300 to 600 µm size range fraction consistently proved to be easy to make while also being effective. In field applications and non-laboratory settings, pilot testing would be used to determine optimum dosage. To prepare the coagulant extract, 2.5 g of this size fraction were mixed with 250 mL of 10 mM NaCl aqueous solution for 10 min in a blender (Osterizer 6630) and vacuum-filtered through a 0.45 µm mixed cellulose ester membrane (Membrane Solutions) to remove seeds fragments. This achieves an extraction ratio of 10 g of seeds per litre, which was chosen because the resultant coagulant dosage volumes would be of the order of 30 mL - an easy volume to treat 1 L of water in the field while still using a relatively small quantity of water to prepare coagulant solution. We chose to work with 10 mM of NaCl to avail the benefits of extraction with the help of salt while also avoiding excessive reliance on purified salt and keeping salinity of the treated water low. Our extraction method is based on the procedures and observations of previous studies (Ghebremichael et al. 2005) (Jahn and Dirar 1979) (Ndabigengesere et al. 1995) (Okuda et al. 1999). The filtrate was analysed for dissolved organic carbon (DOC) by heated persulphate wet oxidation (Aurora 1030W total organic carbon analyser, OI Analytical) and for particle size distribution using dynamic light scattering (Malvern Zetasizer). To provide a comparative basis, a commercial coagulant, $Al_2(SO_4)_3 \cdot 18H_2O$ (alum) was also tested by preparing a stock solution of 0.4 g/L to administer required dosages against the same feeds and under the same test conditions. Alum is one of the two most commonly used commercial coagulants (the other is $FeCl_3$) due to its treatment efficiency, availability, and low cost.

2.3 Jar test procedure

The jar tests were conducted using a programmable jar tester (model 7790-960, Phipps & Bird) and six 2 L square jars by following the typical three-step procedure of coagulation, flocculation, and settling: i) coagulation for 1 min at 100 rpm, ii) flocculation for 40 min at 20, 30, or 40 rpm, and iii) settling for 120 min. Based on preliminary tests,

we chose settings that were optimal, based on final water quality and the amount of time and effort required. For example, flocculation times of 20, 40, and 60 min were evaluated in preliminary tests using a feed volume of 1 L, coagulant extraction for 2 min into 0.2 M NaCl and un-sieved seed fragments. The results obtained for 40 min flocculation time were better than those of 20 min at all doses except one, by a margin greater than that of the error. Performance for 60 min flocculation was either close to or worse than for 40 min. Thus 40 min flocculation was adopted for all future tests. After settling, ~ 30 mL samples of the treated water were withdrawn from sampling ports in each jar for turbidity measurement. Mixing parameters used in flocculation tests are summarized in Table S1. These parameters remained the same for jar tests with *Moringa oleifera*-based coagulants, alum, and NaCl: the only significant difference between these tests was the coagulant used. While coagulant dosage volumes changed in case of tests using alum, the resultant difference in test volumes was only ~1%, which would not have had a significant effect. In a separate set of jar tests, 20 µL of MS2 stock (~ 10^{11} PFU/mL) were pipetted into 2000 mL of the kaolin suspension in foil-wrapped jars to yield total MS2 concentration of ~ 10^5 PFU/mL. At the end of each of these tests, 50 mL samples were collected from the sampling ports of the jars and stored in the dark at 4 °C prior to conducting a double-plaque layer assay the following day.

2.4 Floc characterization

Laser diffraction analysis (Mastersizer 2000, Malvern) was used to measure the particle size distribution and fractal dimension of flocs before coagulation, after 10, 20, 30, and 40 min of flocculation time, and at the end of settling. The refractive index of primary scatterers (kaolin) was taken to be 1.57 (an average value for the 1.47 – 1.68 range considered typical for clay minerals (Mukherjee 2013)), which is close to that of a typical protein (Fischer et al. 2004). Details of the sampling protocol are provided in the SI file. The fractal dimension of flocs, D_f, was obtained from light diffraction data as described in an earlier study (Amjad et al. 2015). Briefly, D_f was calculated from the double logarithmic plot of the dependence of the intensity of scattered light, I, on the scattering vector, Q: $I(Q) = Q^{D_f}$, where $Q = \frac{4\pi n}{\lambda} sin\left(\frac{\varphi}{2}\right)$, n is the refractive index of water, φ is the scattering angle, and λ is the wavelength of the incident light (632.8 nm). Flocs were also characterized using confocal laser scanning microscopy (CLSM, Olympus Fluoview FV1000 Inverted microscope). Samples were drawn from the test jar using a dropper and placed in a chambered cover-glass slide for brightfield imaging.

3. Results and Discussion

Dynamic light scattering tests with *M. oleifera* seed extract yielded a size distribution with a single peak centered at ~ 12 nm. Assuming spherical scatterers with the density of 1.35 g/cm^3 (typical for proteins) this hydrodynamic diameter translates to a molar mass

of ~ 680 kDa (Fig. S1, see SI), which is much higher than values reported earlier for the proteins in the native state (< 50 kDa (Gassenschmidt et al. 1995; Ndabigengesere et al. 1995; Nordmark et al. 2016)). This discrepancy can be explained by the following considerations. First, the assumption that the coagulants are spherical in shape contradicts literature (Gassenschmidt et al. 1995; Kwaambwa and Maikokera 2008; Suarez et al. 2005). Second, since no purification processes were employed, the extract would have had several components with no coagulant role; they likely accounted for the vast majority of particles in the mixture. Indeed, the assumption that density of the particles would equal that of a typical protein (i. e. 1.35 g/cm^3) may not be justified. The second possible explanation is supported by the high concentration of residual DOC (section 3.1, Fig. 1). Size measurements must be attempted through more suitable methods such as HPLC, after sufficient purification.

3.1. Jar tests with *Moringa oleifera*-derived coagulants: Turbidity removal

For flat paddles and square jars (Cornwell and Bishop 1983), 20 rpm, 30 rpm, and 40 rpm mixing rates correspond to mean velocity gradients, $\bar{G}$, of 13.0, 22.4, and 35.0 s^{-1} (Table 1). Several coagulant dosages in the 6 to 25 mg(DOC)/L were evaluated with each volume-based dosage being tested in triplicates. Coagulant dosages in units of mg(DOC)/L were calculated based on the volume of the added extract and its DOC content. From the process control perspective, measuring the dosage in terms of DOC helps factor out the variability of the coagulant concentration among different extractions. The uncertainty has various sources including the size distribution of *M. oleifera* seed fragments after grinding and sieving, the variability in seed composition, and the efficiency of extraction.

Optimum dosage decreased with increasing flocculation mixing rates for the tested range of flocculation mixing rates (Table 1). The lowest value of the residual turbidity was 12.8 NTU, above the 5 NTU limit recommended by the World Health Organization for drinking water. While the residual turbidity was high, it was reduced by well over 90% (Fig. 1), which would lighten the load on any downstream processes such as sand filters (Samineni et al. 2019), and decrease the interference of turbidity with disinfection. The maximum turbidity removals for flocculation mixing rates of 20 rpm, 30 rpm, and 40 rpm were (93.0 ± 0.2)%, (94.2 ± 0.2)%, and (92.8 ± 0.7)%, respectively (Table 1). The optimal flocculation mixing rate of 30 rpm is likely a compromise between the increased frequency of particle-particle collisions and increased floc breakup at higher mixing rate. In comparison, turbidity removals using alum for these flocculation mixing rates were (94.1 ± 0.2)%, (94.8 ± 0.2)%, and (92.7 ± 0.6)%. While statistically significant, the difference in performance among the three mixing rates was small.

To factor out the coagulating effect of NaCl (a component of the extraction solution) and the dilution caused by adding coagulant, 0.01 M NaCl was also tested as a coagulant against kaolin suspensions. The coagulating activity of NaCl in these baseline tests

resulted in < 5% decrease in turbidity (Fig. S4). Further, to compare the performance of *M. oleifera*-derived coagulants with a commercial coagulant, jar tests with alum were performed as a reference. At the optimum dosage values, the performance of alum and the coagulants in *M. oleifera* seeds were very similar (Fig. S5). While turbidity removal by alum is highest at pH 7.5 to 8.5, this would require a large dosage of alum, which would result in large residuals of Al^{3+}, which is undesirable (Faust and Aly 1998). The other peak for turbidity removal by alum occurs between pH 3.5 and 5.5, which is outside the range of the vast majority of natural waters. Our study used raw water with pH of ~ 7, and treatment using alum at the optimum dose caused pH to decrease to ~ 6.5, except in the case of flocculation at 40 rpm, where pH dropped to ~6.75. In contrast, the performance of *M. oleifera*-based coagulants is not significantly affected by pH, and their use does not significantly affect pH (Ndabigengesere and Narasiah 1996). This is a clear advantage that *M. oleifera*-based coagulants have over alum.

Table 1. Jar test data for different flocculation mixing rates. Experimental errors correspond to the standard deviation of the lowest three values of residual turbidity, with no two values coming from the same test. Details on the calculation of the average velocity gradient, $\bar{G}$, are given in Table S1 and Fig. S2.

[a] The value was selected based on testing of 10 different fixed volume-based dosages and choosing the volume that gave the best average turbidity removal.
[b] Values correspond to the optimal coagulant dose.

Flocculation mixing rate, rpm	Average velocity gradient, s^{-1}	Optimal coagulant dose[a], mg(DOC)/L	Turbidity removal[b], %	Residual DOC[a], mg(DOC)/L
20	13.0	19.6 ± 0.6	93.0 ± 0.2	15.8 ± 0.1
30	22.4	14.7 ± 1.8	94.2 ± 0.2	13.1 ± 1.4
40	35.0	12.2 ± 1.1	92.8 ± 0.7	11.1 ± 0.8

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Turbidity is a curve with a minimum for both

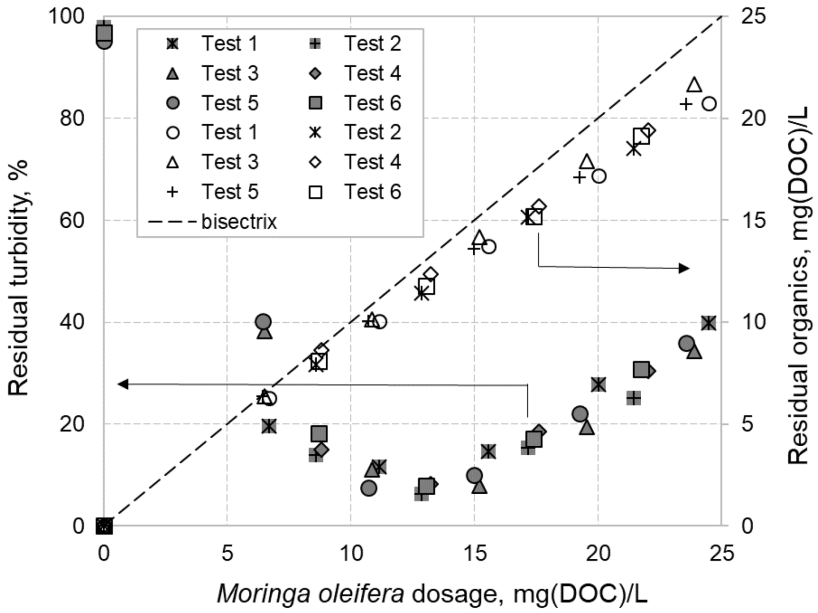

Figure 1. Residual turbidity (filled symbols) and residual DOC (empty symbols) as functions of *M. oleifera* coagulant dosage in jar tests with the flocculation mixing rate of 30 rpm. Initial turbidity was 228 ± 10 NTU. Different symbols correspond to different jar tests. The dashed line corresponds to the condition of no removal of coagulant-associated DOC. Results from jar tests with flocculation mixing rates of 20 rpm and 40 rpm are given in Fig. S3. Instrumental error in turbidity measurements was 2% + 0.01 NTU (HACH 2013).

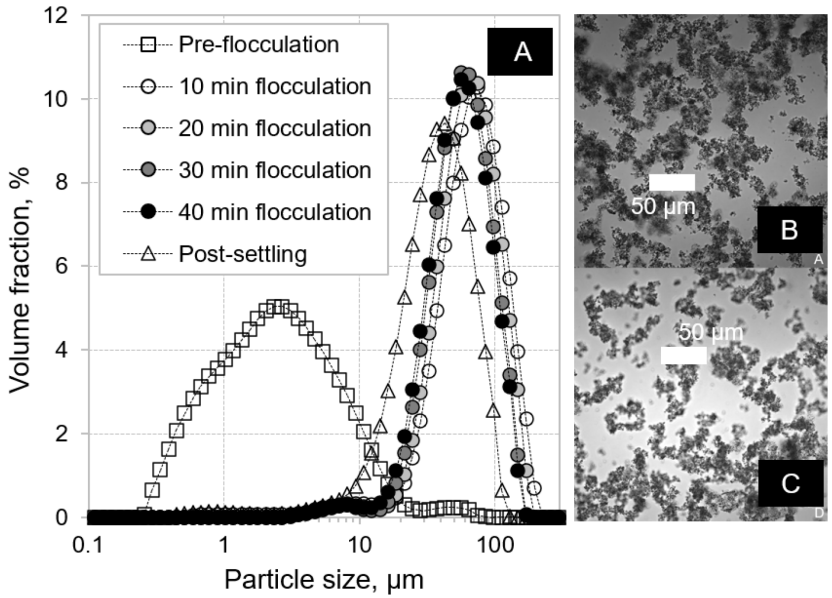

Figure 2. Evolution of floc size distribution (A) in a test with a flocculation mixing rate of 30 rpm and *M. oleifera* dosage of 14.2 mg(DOC)/L. Lines are added to guide the eye. Graphs for all replicate tests at 20 rpm, 30 rpm and 40 rpm are given in the SI file. Results for post-settling size distribution should be treated with caution as the laser obscuration ratio was < 10% (Fig. S9). Laser scanning microscopy images (B, C) of flocs at two different locations at the end of 40 min of flocculation at 30 rpm.

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3.2 Jar tests with *Moringa oleifera*-derived coagulants: Residual organics in the treated water

Measured DOC includes both coagulation-active and coagulation-inactive components whose relative abundance can vary. At least 78% of the DOC in the *M. oleifera* extract remained in the treated water. This can be explained by the fact that the seeds of *M. oleifera* contain a variety of organic compounds, which do not play a role in coagulation and flocculation. The residual DOC data (Fig. 1; empty symbols vs dashed line) shows that the inactive species is a much more abundant fraction. Notably, higher dosages of the coagulant led to residual DOC values that were a lower percentage of the DOC added. This appears counterintuitive, since an excess dosage would mean that a greater percentage of added coagulant would be unused, and therefore remain in the treated water. The observation can be explained under the hypothesis that a significant fraction of coagulation-inactive components are uncharged or negatively charged species so as to be removable by adsorption to settleable charge-compensated solids. At higher coagulant dosages, kaolin's negative surface charge is more fully neutralized, facilitating removal of coagulation-inactive components as well. The effect should be more pronounced when the coagulant dosage is excessive, causing kaolin to become positively charged (Black et al. 1966). This hypothesis was tested by adding kaolin in various dosages to the treated water. Consistent with the hypothesis, kaolin addition only resulted in an increase in turbidity. The tests showed that residual organics in treated water are coagulation-inactive.

3.3 Particle size distribution and fractal dimension of flocs

The peak of the size distribution shifted towards larger sizes during first 10 min of flocculation. Some of the flocs were large enough to be visible to the naked eye. Consistent with this observation, the size data (Fig. 2) indicated presence of flocs > 100 μm, which is close to the smallest size resolvable by a naked eye. For the remaining 30 min, the distribution remained almost the same, pointing to a dynamic equilibrium between aggregation of particles and breakage due to mixing. The lack of floc growth would occur if the particle size approached the Kolmogorov length scale (Kolmogorov 1941c, b, a). Under our experimental conditions, mixing at 20, 30, and 40 rpm translates to Kolmogorov length scales of ~ 266, 202, 165 μm (see SI, section S1). By the end of the settling stage, particle size distribution shifted slightly towards lower sizes, which can be explained by settling of larger flocs. The flocs left behind after settling were still much larger than the initial particles. Thus, the difference between settleable and non-settleable fractions appears to be in the structure of the flocs.

Floc structure, quantified in terms of D_f, was considered as a meta-parameter connecting process design with treatment efficiency. The fractal dimension of flocs was close to 2.1 (Fig. 3), pointing to reaction-limited aggregation as the flocculation mechanism (Lin et al. 1990). The final D_f values were much lower than those reported earlier for monodisperse polystyrene nanoparticles coagulated by isolated *M. oleifera*

proteins (Hellsing et al. 2014). A direct comparison is difficult given that both coagulants and suspension were different; however, our results show that for practical feeds (e. g. containing clays in the suspended fraction) and more feasible coagulant extraction processes, more porous flocs are likely to form, calling for adjustments in the flocculation process (e. g. longer flocculation times). Indeed, the observed increase in D_f during flocculation indicates densification of flocs. Thus, despite little growth in floc size after the first 10 min (Fig. 2), continued flocculation helped make the flocs more settleable. This is corroborated by preliminary tests on the optimization of flocculation time where 40 min yielded better results than shorter tests (see SI). Fractal dimension of non-settleable flocs was significantly ($p < 0.005$) smaller than that of the ensemble of flocs prior to settling (Fig. 3). This result, together with the observation of a minimal change in floc size distribution after settling, gives further credence to the conclusion that the difference between the settled and unsettled particles is in the internal structure of flocs.

3.4 MS2 bacteriophage removal

In a set of preliminary tests, coagulation performance was evaluated with respect to virus removal. Bacteriophage MS2 was selected as a model microorganism; small and hydrophilic (Dang and Tarabara 2019), MS2 is more difficult to remove than larger and hydrophobic viruses, and for this reason, MS2 is commonly used as a conservative surrogate to estimate virus removal by unit processes (EPA 1991). Both *M. oleifera* and alum helped removed turbidity and viable MS2, with removal values significantly above those recorded in control tests (Fig. 4). Turbidity removals with alum and with *M. oleifera* were 1.07 log and 0.99 log with no statistically significant difference between the two coagulants. (These removal values were slightly lower than in tests with clay only (see section 3.1), where application of alum and *M. oleifera* led to turbidity reductions of 1.28 log and 1.24 log, respectively). *Moringa oleifera* showed only 0.3. log additional removal of MS2 over that of turbidity. The minimal, if any, virucidal activity of *M. oleifera* is in contrast to its antibacterial properties attributable to membrane cell damage (Shebek et al. 2015), a mechanism that should not be relevant for non-enveloped viruses such as MS2. Whether the lipid layer of enveloped viruses can be damaged by *M. oleifera*-derived cationic polypeptides is an open question. Removal of viable MS2 was much higher in tests with alum (Fig. 4). The difference can be attributed to a higher inactivation of MS2 by alum. Indeed, virus inactivation by aluminum and iron salts has been reported before, although values for MS2 inactivation by alum were 1.5 and lower (Heffron and Mayer 2016).

To our knowledge, our study is the first report on virus removal by *M. oleifera*-derived coagulants in a batch reactor. The interaction of MS2 with a *M. oleifera*-coated surface, however, has been explored in two previous studies. In a recent study, MS2 adsorption onto rice husk ash functionalized by *M. oleifera* seed protein could be adequately described by Freundlich isotherm but showed relatively low adsorption capacity and was affected by solution pH (Pisharody et al. 2021). In an investigation of MS2 removal by

sand filters modified by *M. oleifera* extract, the very high (7 logs) removal of the bacteriophage was attributed to specific molecular interactions between a chitin-binding protein and MS2 capsid proteins (Samineni et al. 2019).

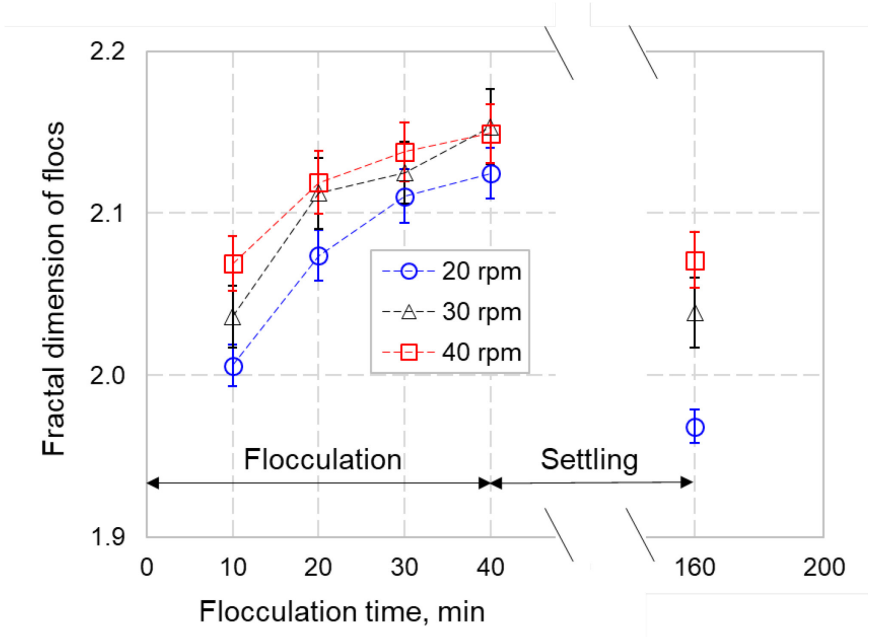

Figure 3. Fractal dimension of flocs, D_f, as a function of flocculation time. Also shown is D_f for residual flocs that remain suspended after 120 min of settling. Lines are added to guide the eye. Error bars represent standard deviations. Tables S3 and S4 summarize data on the statistical significance of differences.

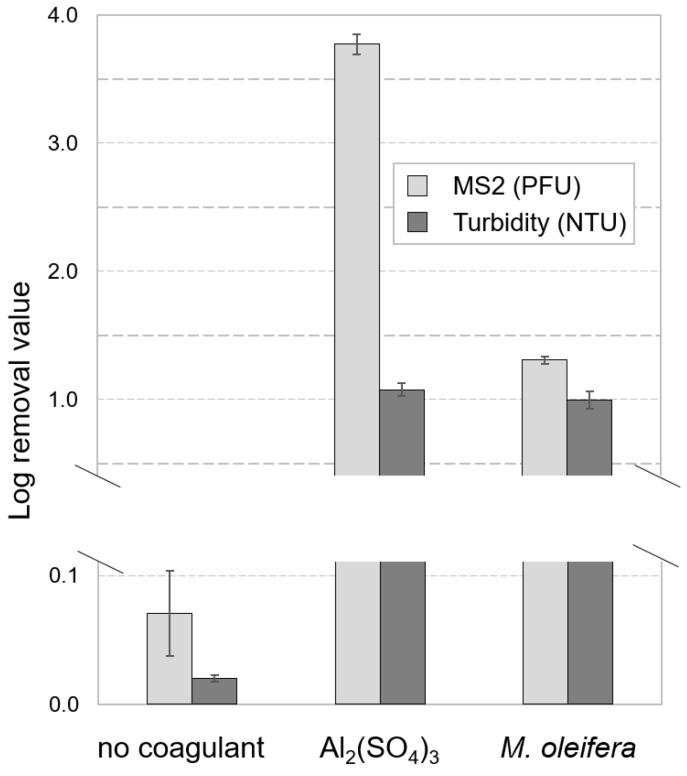

Figure 4. MS2 and turbidity removal in tests with alum and *M. oleifera* as coagulants. Tests with no coagulant were used to establish baselines. Flocculation mixing rate: 30 rpm.

3.5 Implications for water treatment practice

The ability to extract coagulants using dilute electrolytes makes the process inexpensive, simple, and, therefore, of possible value in settings where chemical coagulants, and specialized knowledge are not available. Neither vacuum filtration (used in this study to speed up filtration) nor the use of a 0.45 μm membrane (can be replaced by a cloth to remove suspended seed-fragments) is of critical importance in real world applications. However, batch-to-batch changes in the makeup, extraction efficiency and coagulating ability of extractants mean that volume-based dosages are not reliable: optimum dosages must be determined through more complex and expensive methods such as DOC measurements, which are not practicable in cases of low-income regions with a dearth of skilled labour. To provide an estimate of this variation, the DOC data of the extracts used in this work have an average value of 892.3 mg(DOC)/L, standard deviation of 52.4 mg(DOC)/L, and a range of 795.8 ± 35.9 mg(DOC)/L to 972.9 ± 7.5 mg(DOC)/L. This concern is mitigated by the fact that unlike alum, *M. oleifera*-based coagulants are effective over broad ranges of dosage. For example, in our jar tests with flocculation at 30 rpm, the range of dosages producing at least 90% turbidity reduction was 11.45 to 17.56 mg(DOC)/L for *M. oleifera* coagulants, and 4.63 to 6.33 mg/L for alum. The higher value of this range for *M. oleifera* coagulants was 53.4% greater than the lower value of the range, compared to 36.5% in case of alum. A more important challenge is that without additional processing steps (e. g. defatting, fractionation), the extract of *M. oleifera* seeds contributes organics to the treated water, which may facilitate regrowth of microbes, interfere with disinfection, and in case of chemical disinfection, lead to the formation of disinfection by-products (e. g. in the case of chlorination – trihalomethanes). When residual turbidity is sufficiently low, UV or solar may be the best disinfection options. Of these, solar disinfection would be the most practical option in regions with economic water scarcity. The data on floc size and structure (Fig. 3) point to the possibility of using *M. oleifera* coagulation as a pre-treatment for downstream surface filtration (e. g. ceramic filter (Abebe et al. 2016)): given the large size of non-settleable flocs, low head-loss separation by a microfilter may be feasible as long as excessive floc breakup is avoided. Slow sand filtration may be preferable as a simple and inexpensive process with the ability to partially remove colloidal and even dissolved organics (Collins et al. 1992). Other treatment processes that rely on *M. oleifera*-derived materials (e. g. adsorption to leaves and bark (George et al. 2016), filtration through *M oleifera*-modified granular media (Samineni et al. 2019)) may be of special interest.

4. Conclusion

The study evaluated coagulants in crude (i. e. unfractionated) extracts from *M. oleifera* seeds against high turbidity model feed waters. Extraction into a low salinity aqueous solution offers an affordable alternative to conventional coagulants, which is feasible in

settings where specialized expertise and equipment are lacking. Coagulation-flocculation performance was quantified in terms of turbidity and bacteriophage removal, concentration of residual organics, as well as floc size and fractal dimension. Turbidity removal at the optimal coagulant dosage (14.7 mg(DOC)/L) was > 94%, similar to that recorded in reference tests with alum. The best performance was obtained at an intermediate flocculation mixing rate ($\bar{G}$ = 22.4 s^{-1}; 30 rpm). Floc size distribution shifted to larger sizes only during the first 10 min of flocculation, while the fractal dimension of flocs continued to increase up to the 2.1 - 2.2 range, suggesting that the mechanism of flocculation was reaction-limited aggregation. Flocculation for a longer period (> 10 min) was useful as it led to flocs with better settling characteristics. Large, lower fractal dimension flocs that did not settle should be easily removed by downstream filtration. In virus removal tests, coagulation with *M. oleifera* decreased the viable MS2 titer by ~ 1.3 log, higher than the turbidity log removal value in these experiments (~ 1 log). Importantly, the extraction procedure allowed > 78% of extracted DOC into the treated water, with higher dosages of the coagulant leading to lower DOC residuals as a percentage of the DOC added. Consistent with this observation, residual organics were shown to be coagulation-inactive. Residual DOC is a major concern as it may facilitate microbial regrowth, interfere with disinfection, and lead to the formation of disinfection by-products. Combining the coagulants with other treatment process such as downstream filtration and adsorption, employing physical disinfection (e. g. UV or solar), or limiting applications to non-potable use can mitigate these concerns. Slow-sand filtration and adsorption by charcoal, wood, or *M. oleifera* bark and leaves may be especially worth exploring.

Declarations

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

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Authors' contributions

AM, BSW, and VVT conceptualized the study. AM participated in all aspects of the experimental work. KDH performed tests on MS2 removal and quantification. BSW, DJT, and RG contributed to the optimization of coagulant extraction and coagulation-flocculation research. AM and VVT participated in results validation. All authors contributed to the development of the methodology and the formal analysis of data; AM, KDH, and VVT worked on data visualization and curated the data. VVT supervised and administered the project. AM and VVT developed the original draft of the manuscript. All authors reviewed and edited subsequent drafts and approved the final manuscript.

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Supplementary Information

The SI file contains data on particle size measurements of coagulants; particle size distribution vs time for two additional flocculation mixing rates; residual turbidity and DOC as a function of coagulant dosage for two additional flocculation mixing rates; calculation details for and summary of mixing parameters; data from baseline tests with 0.01 M NaCl and alum as coagulants; details of MS2 propagation and quantification procedures; description of light diffraction measurements; and summary of p-values for statistical differences for fractal dimension data.