

Combined natural organic and synthetic inorganic coagulants for surface water treatment

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ABSTRACT

The purified coagulant from *Moringa oleifera* obtained by simple ion exchange purification was used for the removal of turbidity, dissolved organic carbon (DOC) and bacteria from surface water. The natural coagulant was used as a primary coagulant and as a coagulant aid with metal salts. Different water quality and operational parameters such as pH, turbidity and mixing conditions were investigated. Characterization studies using fluorescence excitation emission matrix indicated that the coagulant protein from *M. oleifera* was a tyrosine-like protein. Turbidity and bacteria (*Escherichia coli*) removal efficiency of the purified *M. oleifera* and metal coagulants were similar. While the removal of bacteria by metal salts was mainly due to coagulation/flocculation, the removal by *M. oleifera* coagulant was as a result of both coagulation and growth inhibition, which is expected to improve sludge microbiological quality. Among the operational parameters, slow mixing time significantly influenced performance of natural coagulant. At optimum dosages, the crude extract increased the DOC of the treated water significantly whereas the purified coagulant showed a significant removal of fulvic-like DOC fraction. When used in combination with metal salts, adding of *M. oleifera* before the metal salts showed better turbidity and DOC removal than adding it after the metal salts.

Key words | aluminium sulphate, bacteria, coagulation, DOC, *Moringa oleifera*, turbidity

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INTRODUCTION

There are some constraints to the use of chemical coagulants, such as cost, unavailability in the local area, especially in developing countries, presence of residual metals in the treated water, and large sludge volume. The sludge is often of poor quality as it contains metals which affect the biodegradability and result in poor dewatering characteristics. Studies have linked the presence of residual aluminium in drinking water to the development of Alzheimer's disease (Crapper *et al.* 1973). A number of synthetic organic polymers (polyelectrolytes) are commonly used as primary coagulants (Yeh & Ghosh 1981) or coagulant aids with metal salts (Jarvis *et al.* 2006). Although they are more effective and produce less sludge volume, they are expensive. Organic polymers may contain residual acrylamide or epichlorohydrin monomers which are

reported to have neurotoxicity and strong carcinogenic properties (McCollister *et al.* 1964). There are also naturally occurring polymers that have inherent cationic properties or are modified to yield a cationic charge (Bolto & Gregory 2007). A typical example of this is chitosan (obtained from the shells of crustaceans). Although chitosan is very effective, its cost hinders its use in developing countries.

There is a growing need to develop alternative, cost-effective and environmentally friendly coagulants. *Moringa oleifera* is such an example that has been reported effective in water and wastewater (Bhuptawat *et al.* 2006). It is more effective for high turbidity water compared with low turbidity waters (Muyibi & Evison 1995). The *M. oleifera* seed is also reported to have antibacterial properties (Broin *et al.* 2002; Suarez *et al.* 2003). The studies reported that it was effective

in killing several pathogenic bacterial strains including antibiotic resistant isolates of *Staphylococcus*, *Streptococcus* and *Legionella* species. The coagulant component of the seed is characterized as a water soluble cationic protein with molecular mass ranging from 6 to 14 kDa and isoelectric point of above 10 (Gassenschmidt *et al.* 1995; Ndabigengesere *et al.* 1995; Broin *et al.* 2002; Ghebremichael *et al.* 2005). The main drawback of *M. oleifera* (and also other natural coagulants), when used in the crude form, is the residual dissolved organic carbon (DOC) imparted to the treated water. This drawback has been addressed by purifying the coagulant component from the source (Ndabigengesere & Narasiah 1998). A number of purification protocols are reported in the literature (Gassenschmidt *et al.* 1995; Ndabigengesere *et al.* 1995; Okuda *et al.* 2001). Most of the protocols are cumbersome, expensive and not readily scaleable. A simple ion exchange purification protocol has been developed and tested on clay suspension in the laboratory (Ghebremichael *et al.* 2005).

Although there are many reports on the potential of *M. oleifera* for turbidity removal, there are only a few studies on the effectiveness of the purified coagulant as a primary coagulant or a coagulant aid for natural organic matter (NOM) removal. Previous studies reported the performance of crude *M. oleifera* extract as a coagulant aid with alum for colour and turbidity removal (Muyibi & Alfugara 2003; Kalibbala 2007). Although colour can be used as an indicator of NOM, it does not describe the fractions that are effectively removed.

The main objective of this research was to assess the coagulation effectiveness of the purified coagulant from *M. oleifera* seed for the removal of turbidity, bacteria and DOC from surface (river) water alone or in combination with metal salts.

The specific objectives were to:

1. Investigate the coagulation performance of the purified *M. oleifera* in terms of turbidity, bacteria and NOM removal from surface water and compare its performance with the crude extract, ferric chloride and aluminium sulphate.
2. Study the impact of the purified coagulant on the treated water quality.
3. Study effectiveness of purified *M. oleifera* as a coagulant aid with metal salts for turbidity and NOM removal.

MATERIALS AND METHOD

Water and coagulant sources

Dried *M. oleifera* seeds were purchased from Herbal Point Services in Zaria, Nigeria, and stored at room temperature. The coagulant from the seed was extracted and purified according to Ghebremichael *et al.* (2006). Alum and ferric chloride suspensions were prepared as a 1% stock solution in distilled water. As much as possible the coagulant solutions were prepared fresh to avoid ageing phenomena and improve reproducibility.

Water samples were collected from the River Mues in Rotterdam and canal water in Delft, The Netherlands. The average raw water qualities from the two sources are summarized in Table 1. In order to vary the initial turbidity, the water samples were spiked with a clay suspension. The clay suspension was prepared as 0.5% w/v solution by adding kaolin to tap water. The suspension was stirred for about 1 hour and allowed to settle for up to 24 hours for complete hydration. The supernatant was used for the experiment and dilutions were prepared to obtain the desired initial turbidity.

Experimental methods

Standard jar test experiments were carried out to assess the effects of pH and mixing conditions. The pH of the sample water was adjusted using 0.1M HCl and 0.1M sodium hydroxide solutions. Ranges of pH from 4 to 9 were studied. In the case of combined coagulants, the first coagulant was added during the first minute of rapid mixing and the second coagulant was added during the second minute of rapid mixing (120 rpm); the slow mixing was performed at 45 rpm for 20 minutes. The order of chemical addition was varied with three options: 1) addition of *M. oleifera* before the metal coagulant; 2) addition of *M. oleifera* after the metal coagulant; and 3) prior mixing of *M. oleifera* and metal coagulant in the stock solution.

Bacteria removal experiments were carried out by inoculating cultured *Escherichia coli* (in a chromocult agar medium) into jar test beakers filled with Delft canal water. The cultured *E. coli* bacteria were concentrated to about 10^7 CFU ml⁻¹ and 1 ml was spiked and the standard

Table 1 | Characteristics of source water samples

| Water quality parameter | River Maas | Range | Delft Canal | Range |
|---|------------|------------|-------------|-----------|
| | Average | | Average | |
| Turbidity, NTU | 26 | 20–45 | 35 | 24–60 |
| pH | 8 | | 7.8 | |
| Electrical conductivity ($\mu\text{s cm}^{-1}$) | 635 | 540–700 | 829 | 750–900 |
| DOC, mg l^{-1} | 4.63 | 3–8 | 15.8 | 14.7–17.4 |
| UV ₂₅₄ , cm^{-1} | 0.098 | 0.08–0.122 | 0.51 | 0.4–0.5 |
| SUVA, $\text{L mg}^{-1} \text{m}^{-1}$ | 2.25 | | 3.2 | |

jar test procedure was followed. After 1 h settling samples were taken from the supernatant, diluted 10- 100- and 1,000-fold, inoculated on chromocult plates and incubated at 35–37°C for 22–24 hours. Colonies were counted to estimate bacteria numbers. Bacteria counts were also made from the settled sludge. About 15 ml of settled sludge was removed and placed in a beaker. To each sludge sample, 2.1 ml of 0.1 M citrate buffer (pH 5.5) was added and mixed vigorously using a vortex for several minutes. These samples were also diluted and plated in a similar way to the supernatant water.

In addition to settled water quality, sludge volume was also estimated. Once the flocs were settled for about 1 h, the supernatant was decanted and the settled flocs were poured into an Imhoff cone and allowed to settle for 15 minutes and the reading on the volume scale was recorded.

Analytical methods

Turbidity was measured using a turbidity meter (HACH 2100N). The EC and pH were measured using a WTW and 300i conductivity meter and METROHM-691 pH meter, respectively. Organic content was measured in terms of DOC, UV₂₅₄ and fluorescence excitation-emission matrix (EEM). DOC for filtered water samples were measured using a Siever model 700 portable total organic carbon analyser (O&I Corporation). UV₂₅₄ was measured using a Perkin Elmer (Lambda 20 1.11) spectrophotometer.

The EEM spectra of the different samples were measured using a Horiba Jobin Yvon FluoroMax-3 spectrofluorometer with a xenon lamp as the excitation source. The samples were diluted to approximately 1.0 mg l^{-1} of DOC with 0.01 M KCl solution and the pH was adjusted to 2.8

(± 0.1) using concentrated HCl to correct the inner filter effect and to minimize possible metal complexation of DOC. Three-dimensional spectra were obtained by measuring the emission spectra in the range 290–500 nm repeatedly, at the excitation wavelengths from 240 to 450 nm, spaced by 10 nm. Using Matlab, program spectra were converted into an excitation-emission matrix and three-dimensional plots and contour maps were produced.

The interpretations of the EEM data obtained in this study were based on the characterisation of NOM into humic-like (Ex/Em 250–260/380–420 nm), fulvic-like (Ex/Em 330–350/420–480) and protein-like (Ex/Em 270–280/300–350) fractions (Coble 1996). Fluorescence index (FI) values defined as the ratio of fluorescence emission intensity at wavelength 450 nm to that at 500 nm (E₄₅₀/E₅₀₀) were also estimated to distinguish microbially derived fulvic acids from terrestrially derived fulvic acids (McKnight *et al.* 2001).

RESULTS AND DISCUSSION

Purification and characterization of *M. oleifera* coagulant protein

The DOC values of the crude and purified coagulants were found to be 2.3 and $1.48 \text{ mg DOC ml}^{-1}$, respectively. Previous studies reported that the purified coagulant is composed solely of protein whereas the crude extract contains proteins and other organic compounds such as carbohydrates and lipids (Gassenschmidt *et al.* 1995; Ghebremichael *et al.* 2006). Hence the use of the crude extract results in residual DOC because of the non-active organic compounds.

Excitation-emission matrix (EEM) of the purified coagulant and crude extract showed a maximum peak at excitation 270 nm and emission 306 nm, which corresponds to tyrosine-like organic matter (Figure 1). This differs from research by Kwaambwa & Maikokera (2007) which stated the fluorescence emission to be dominated by tryptophan emission. However previous research by Gassenschmidt *et al.* (1995) indicated the absence of tryptophan residues through the reaction of one fraction of *M. oleifera* with Ehrlich's reagent.

Turbidity removal

Figure 2(a) shows that significantly lower dosage of the purified coagulant was required to achieve the same coagulation effect as the crude extract. At the optimum dose of 7 mg DOC l⁻¹ (purified) and 58 mg DOC l⁻¹ (crude), about 97% turbidity removal was achieved. The significant difference is explained by the fact that the purified coagulant comprises pure proteins that are active in coagulation whereas the crude extract DOC consists of different active and non-active organic compounds.

The purified *M. oleifera*, alum and ferric chloride had similar performance for turbidity removal with optimum dosages of 7 mg DOC l⁻¹, 2.4 mg Al l⁻¹ and 10.3 mg Fe l⁻¹ and corresponding residual turbidity values of 1.8, 0.63 and 0.43 NTU, respectively (Figure 2(b)). Visual observation indicated that the flocs had significant differences in terms of size and rate of formation. The *M. oleifera* coagulants produced small flocs with lower settling velocity after about

5 minutes whereas the metal coagulants produced large flocs almost instantaneously, attributed to the formation of hydroxide precipitates.

The treated water quality results indicate that *M. oleifera* did not affect the pH while increasing the TDS (total dissolved solids) concentration due to the use of NaCl during purification. On the other hand the metal salts depressed the pH (Figure 2(c)) but did not increase the TDS level. At the optimum *M. oleifera* dosage of 7 mg DOC l⁻¹, the TDS value increased by 13% and the final TDS value was below the WHO guideline value (WHO 1996). Ndabigengesere & Narasiah (1998) reported similar effects on the pH and TDS of water treated by *M. oleifera*.

There was a significant difference in the sludge volume produced by the different coagulants (Figure 2(d)). At the optimal dose of 7 mg DOC l⁻¹, the *M. oleifera* coagulant produced 1.2 ml l⁻¹ of sludge, whereas alum (at a dosage of 4.0 mg Al l⁻¹) produced 6.0 ml l⁻¹ of sludge and ferric chloride (at a dosage of 6.2 mg Fe l⁻¹) produced 5.0 ml l⁻¹ of sludge. The sludge volume for the inorganic coagulants is almost four times higher. Similar findings were reported by Ndabigengesere *et al.* (1995). The larger sludge volume produced by the inorganic coagulants compared with *M. oleifera* is explained by the addition of hydroxide precipitates from the metal coagulants. The biodegradable nature and small sludge volume from *M. oleifera* makes it very attractive from a sludge management point of view. On the other hand the dewatering characteristics of the sludge are expected to improve when natural coagulants are used.

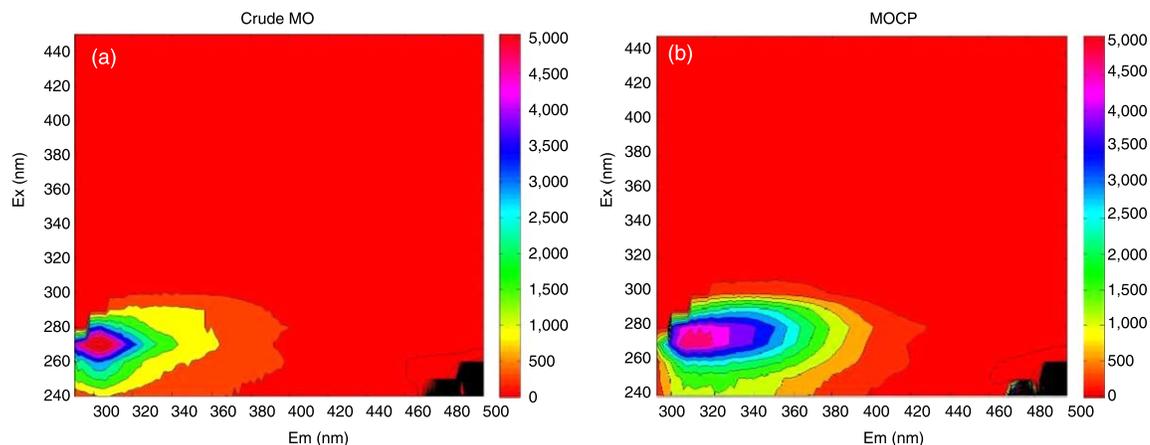


Figure 1 | EEM spectra for *M. oleifera* extracts: (a) crude extract; (b) purified coagulant.

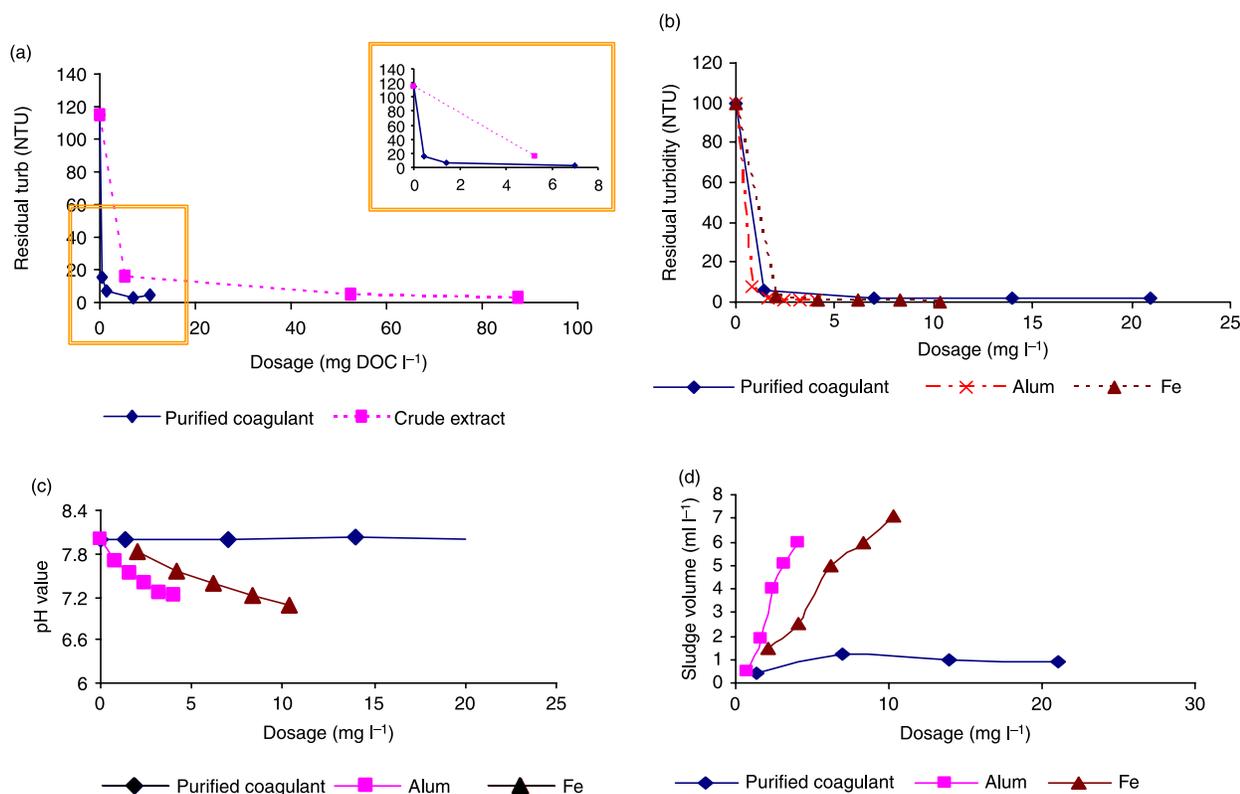


Figure 2 | Comparisons of turbidity removal, final pH and sludge production: (a) purified *M. oleifera* and crude extract with initial turbidity of 120 NTU; (b) purified extract and metal salts with initial turbidity of 100 NTU; (c) pH of settled water; (d) sludge volume (dosages of *M. oleifera* are expressed in mg DOC l⁻¹).

Ozcar & Sengil (2000) reported that sludge formed from the usage of tannin as a coagulant aid had better de-waterability than the sludge formed by Al₂(SO₄)₃.

NOM removal

The source water from the River Maas had humic- and fulvic-like peaks at Ex/Em = 250/412 and 300/408, respectively. The source was found to have an FI of 1.0 and the source of NOM can be described as allochthonous, which is expected since the river drains a vegetated watershed in contact with plants and soils.

The effect of coagulant dose on the settled water DOC and UV₂₅₄ are shown in Figure 3(a) and (b). The metal coagulants resulted in better removal compared with the *M. oleifera* coagulant. The DOC removals at 4.0 mg Al l⁻¹ and 10.3 mg Fe l⁻¹ were between 15 and 20%. These removals are low compared with reports from the literature (about 35%, Qin *et al.* 2006). The low DOC removal may be attributed to the low SUVA values of the raw water,

which indicates that the water was mostly dominated by non-humic and low molecular weight material (see Table 1). DOC removal for this type of water has been found to be difficult by conventional treatment. The maximum removal of UV₂₅₄ by *M. oleifera*, alum and ferric chloride were 18%, 43% and 41%, respectively. The high UV₂₅₄ removal compared with DOC is consistent with other studies which suggested that coagulation is effective for the removal of aromatic/hydrophobic fractions of NOM.

Figure 3(a) shows that, with an overdose of *M. oleifera* (above 7 mg l⁻¹), the DOC started to increase sharply; however, overdosing did not increase the UV₂₅₄ (Figure 3(b)). Since the purified coagulant is composed of protein, overdosing is expected to increase the protein fraction only.

EEM results of *M. oleifera* treated water (Figure 4) indicate that the coagulant reduced the fulvic-like and humic-like fractions. At excessively high coagulant dosage, high fulvic-humic reductions were followed by the appearance of a protein-like peak. This is obviously due to the composition of the coagulant. This is consistent with

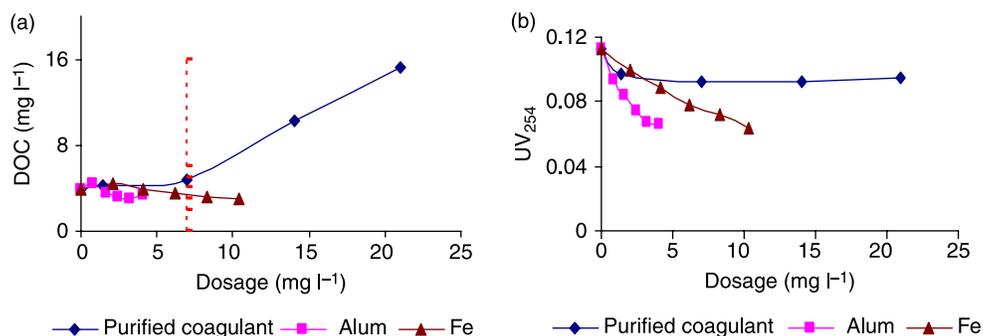


Figure 3 | Quality of water treated by coagulation with *M. oleifera*, alum and ferric chloride: (a) DOC, (b) UV₂₅₄ (doses of purified coagulant are expressed in terms of mg DOC l⁻¹).

the results of DOC analysis which showed that beyond the optimum dosage (7 mg DOC l⁻¹, Figure 3(a)) the residual DOC started to increase rapidly. To avoid excessive dosage it is recommended to use the *M. oleifera* in combination with metal coagulants (as discussed later).

In order to quantify the reduction of the humic-like and fulvic-like fraction, differential fluorescence EEMs were obtained by subtracting peak-specific fluorescence values of the treated water from those of the raw water as shown in

Table 2. Specific fluorescence values (fluorescence/DOC) were estimated based on a 1 mg l⁻¹ DOC value of the samples. At the optimum dosage of 7 mg DOC l⁻¹ 75% and 10% removal of the fulvic-like and humic-like fractions were observed, respectively.

Bacteria removal

Studies on the removal of bacteria from surface water spiked with *E. coli* indicated that *M. oleifera* was as effective

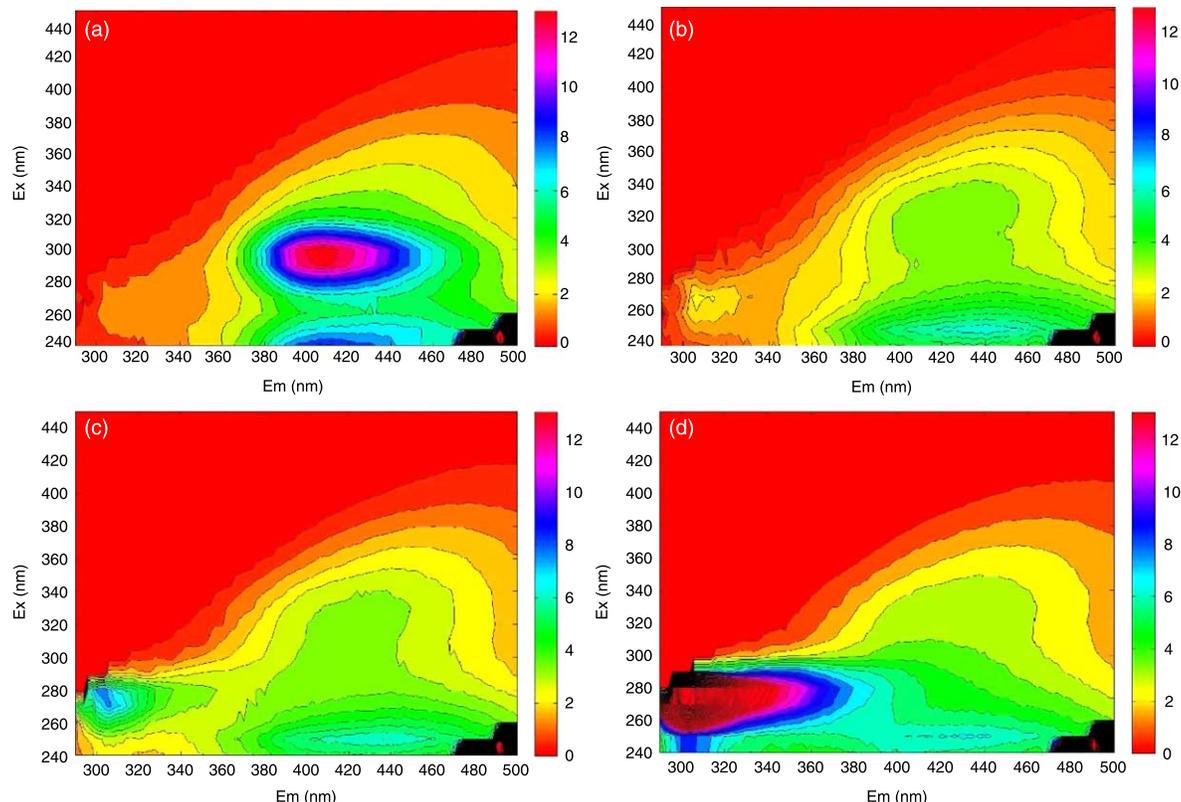


Figure 4 | EEM of (a) raw water (River Meuse); (b) 1.4 mg DOC l⁻¹ dosage; (c) 7 mg DOC l⁻¹ dosage; (d) 9.8 mg DOC l⁻¹ dosage.

Table 2 | Estimation of DOC fractions removal of water treated with *M. oleifera*

| <i>M. oleifera</i> dosage | Humic-like Specific fluorescence | % removal | Fulvic-like Specific fluorescence | % removal |
|----------------------------|----------------------------------|-----------|-----------------------------------|-----------|
| 0 mg DOC l ⁻¹ | 7.17 | | 13.4 | |
| 1.4 mg DOC l ⁻¹ | 6.50 | 9 | 3.36 | 75 |
| 7 mg DOC l ⁻¹ | 6.43 | 10 | 3.38 | 75 |
| 9.8 mg DOC l ⁻¹ | 6.98 | 3 | 4.19 | 69 |

as alum. In order to assess whether the removal was only by coagulation/flocculation or also by other mechanisms, microbial counts were made on both the supernatant water and sludge.

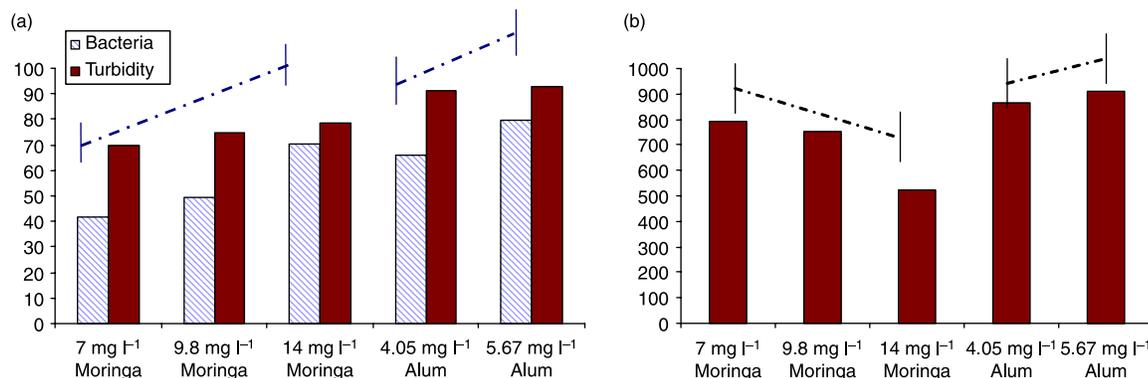
Figure 5(a) shows that removal of bacteria increased with increase in dosage for both coagulants. It is assumed that the main mechanism for bacteria removal is similar to that of turbidity removal (coagulation/flocculation). Chow *et al.* (1999) reported that when alum was used to treat water spiked with cyanobacteria, the cells in the supernatant and sludge were not damaged and remained intact. In this study bacteria removal by *M. oleifera* was observed to be not only by coagulation/flocculation but also by other means (growth inhibition). Comparison of alum and *M. oleifera* indicated that for similar turbidity removal, the microbial reduction by *M. oleifera* was higher. This indicates that although both coagulants have removed turbidity equally, the viable bacteria count in the supernatant treated by *M. oleifera* sample was lower, indicating growth inhibition. This observation is also supported by the bacterial count in the sludge. The bacterial count in the *M. oleifera* treated sludge was observed to decrease with an increase in the *M. oleifera* dosage (Figure 5(b)). In the case

of alum, however, the bacterial count in the sludge increased as coagulant dosage increased suggesting that the viability of the cells was not affected by the alum coagulant. These findings imply that *M. oleifera* actually inhibited the growth of the bacteria. Suarez *et al.* (2003) demonstrated the ability of a recombinant *M. oleifera* protein to decrease the viability of gram-negative and gram-positive bacterial cells and to mediate the aggregation of negatively charged particles in suspension.

Effects of raw water quality and operational parameters

The main water quality and operational parameters that were investigated were raw water turbidity, pH, rapid and slow mixing conditions.

Studies have reported that the crude extract from *M. oleifera* is effective for high turbidity water while its effectiveness is reduced for low turbidity (Muyibi & Okuofu 1995). Results from this study (Figure 6) indicated that the residual turbidity in both the high and low turbidity waters was similar with higher (%) removal (90–100%) for high turbidity water (above 100 NTU).

**Figure 5** | *E. coli* removals by *M. oleifera* and alum in jar test experiments: (a) turbidity and bacteria removal (%) in the supernatant; (b) bacterial count in the sludge ($\times 10^7 \text{ ml}^{-1}$).

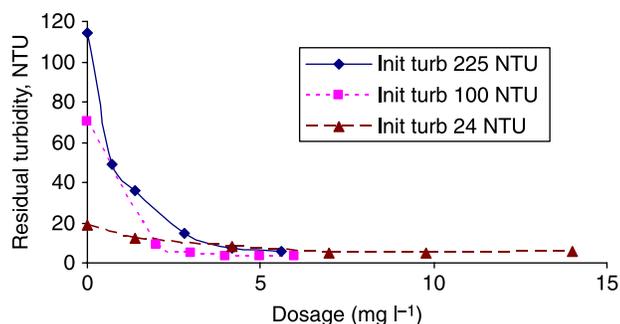


Figure 6 | Effect of initial turbidity on performance of *M. oleifera*.

For the low turbidity water the removal was only 70–80%. The optimum dosage for the low initial turbidity was lower than for the high turbidity waters. This may be explained by the increased colloidal particles as seeds for adsorption sites and higher rate of collision between particles for better agglomeration.

The coagulation activity of *M. oleifera* was determined at various pH levels ranging from 4 to 9 and the results indicated that better coagulation performance was observed

at lower pH with lowest residual DOC at pH 5. The difference in turbidity removal for the various pH levels was not significant. Better performance at lower pH levels can be attributed to the high isoelectric point (pI above 10) of the coagulant protein from *M. oleifera*. At low pH the charge density of the protein increases and hence better coagulation. Metal coagulants are also known to achieve better NOM removal at low pH by enhanced coagulation.

Experiments on varying the mixing intensity and mixing time with *M. oleifera* dosing indicated that slow mixing time had significant impact whereas rapid mixing intensity and time did not (Figure 7). When using *M. oleifera*, stable cationic proteins are applied and the speed with which they are dispersed (during rapid mixing) may not be critical for their effectiveness as opposed to metal coagulants where instant and vigorous mixing is critical. In the case of slow mixing, increasing mixing time from 10 to 40 minutes reduced the residual turbidity and DOC. This can be attributed to the fact that increased contact time allowed for more collisions and formation of bigger flocs. The final

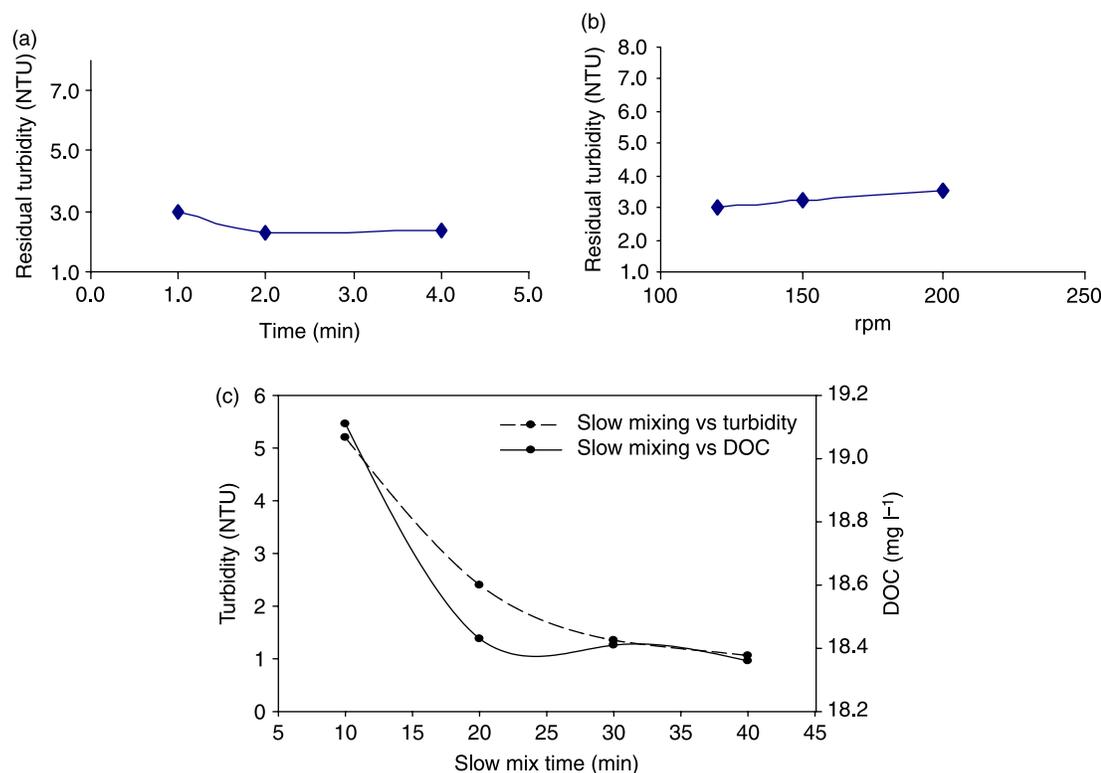


Figure 7 | The effects of rapid mixing and slow mixing conditions: (a) effect of rapid mixing time; (b) effect of rapid mixing speed; (c) effect of slow mixing time for turbidity and DOC removal.

turbidity values of the settled water (raw water 100 NTU) with slow mixing times of 10, 20 and 40 min were 5.2, 2.4 and 1.1 NTU, respectively. The corresponding final DOC values were 19.4, 18.4 and 18.3, respectively.

M. oleifera as a coagulant aid with metal salts

In this study *M. oleifera* has been used as a coagulant aid by varying the dosage and the order of application. Three combinations were investigated: i) each coagulant alone; ii) alum followed by *M. oleifera* and vice versa; and iii) pre-mixing of the coagulants in a stock solution. The order of dosing of the coagulants showed significant difference particularly in terms of DOC removal. When alum dosage was followed by *M. oleifera* the residual turbidity and DOC were significantly higher than when *M. oleifera* was followed by alum. When alum is first added, most of the colloids would be destabilized and the *M. oleifera* would be exposed to low turbidity water, hence its effectiveness decreases. As a result, the *M. oleifera* coagulant remains in solution leading to increased residual DOC. On the other hand when *M. oleifera* is added first it would be exposed to high turbidity water, forming micro-flocs that would be entrapped and adsorbed to the hydroxide precipitates. This results in lower turbidity and DOC residuals. In the case of polyelectrolytes as coagulant aids, their addition after the main coagulants is effective owing to inter-particle bridging, which works well even for low turbidity water (Aguilar *et al.* 2003).

Pre-mixing of the *M. oleifera* and metal salts did not result in effective coagulation performance. Although there may not be a clear explanation for this, it may be due to a complexation reaction between the metal salts and the protein.

Use of a coagulant aid resulted in better turbidity removal compared with the use of alum alone. On average the (%) turbidity removal increased by over 20% when *M. oleifera* was used as a coagulant aid (Table 3). The use of a coagulant aid reduced alum requirements by about 50–75%. Similar observations were made by Lilliehöök (2005) who reported that the use of aluminium could be reduced by 60%.

In terms of DOC removal, the addition of *M. oleifera* prior to alum dosage gave the highest removal of between 23 and 40%. This is comparable to DOC removals of 5 to 40% by alum and cationic polymer (Lee & Westerhoff 2006).

Table 3 | Performance of *M. oleifera* as a coagulant aid with alum for turbidity and DOC removal

| Coagulant dose (mg l ⁻¹) | Turbidity | DOC |
|--------------------------------------|-----------|-------|
| <i>Coagulant1/coagulant2</i> | | |
| Raw water | 116 | 15.59 |
| 1.4 <i>M. oleifera</i> /0.4 Al | 22.0 | 15.52 |
| 1.4 <i>M. oleifera</i> /0.8 Al | 6.8 | 15.29 |
| 1.4 <i>M. oleifera</i> /1.6 Al | 3.4 | 14.95 |
| 2.8 <i>M. oleifera</i> /0.4 Al | 8.8 | 15.99 |
| 2.8 <i>M. oleifera</i> /0.8 Al | 4.5 | 15.71 |
| 2.8 <i>M. oleifera</i> /1.6 Al | 2.8 | 15.18 |
| 0.4 Al | 40.0 | 16.04 |
| 0.8 Al | 13.0 | 15.39 |
| 1.6 Al | 4.5 | 15.15 |

CONCLUSIONS

The purpose of this study was to purify *M. Oleifera* seed coagulant and use it for surface water treatment. The main conclusions of this study are:

1. Characterization by EEM analysis confirmed that the active component of the *M. oleifera* seed coagulant was mainly tyrosine.
2. The purified coagulant showed better coagulation activity in terms of turbidity removal, with dosages five times lower than the crude extract. The purified coagulant effectively removed more than 95% of turbidity for highly turbid waters. The *M. oleifera* was found to be as effective as the metal coagulants (alum and ferric chloride) and produced about 25% of the sludge volume compared with the metal salts. The study also showed that *M. oleifera* was effective as a coagulant aid with metal salts and its use could reduce the use of metal coagulants by about 60%.
3. *M. oleifera* was effective at high initial turbidity compared with low initial turbidity.
4. At the optimum dosage, the purified *M. oleifera* could remove up to 75% of the humic-like fractions of DOC.
5. The bacteria removal mechanism of *M. oleifera* was not only by coagulation/flocculation but also by growth inhibition.

The use of biocoagulants such as *M. oleifera* has a number of advantages over inorganic salts in terms of cost, availability, low sludge volume and effective sludge disposal. This study demonstrates that biomaterials can be effectively

used to complement and/or substitute industrial inorganic coagulants. The environmental and public health benefits can give them a competitive advantage both in developing and developed countries.

REFERENCES

- Aguilar, M. I., Sáez, J., Lloréns, M., Soler, A. & Ortuño, J. F. 2003 Microscopic observation of particle reduction in slaughterhouse wastewater by coagulation-flocculation using ferric sulphate as coagulant and different coagulant aids. *Water Res.* **37**(9), 2233–2241.
- Bolto, B. & Gregory, J. 2007 Organic polyelectrolytes in water treatment. *Water Res.* **41**(11), 2301–2324.
- Bhuptawat, H., Folkard, G. K. & Chaudhari, S. 2006 Innovative physico-chemical treatment of wastewater incorporating *Moringa oleifera* seed coagulant. *J. Hazard. Mater.* **142**(1–2), 477–482.
- Broin, M., Santaella, C., Cuine, S., Kokou, K., Peltier, G. & Joët, T. 2002 Flocculent activity of a recombinant protein from *Moringa oleifera* Lam. seeds. *Appl. Microbiol. Biotechnol.* **60**(1), 114–119.
- Chow, C. W. K., Drikas, M., House, J., Burch, M. D. & Velzeboer, R. M. A. 1999 The impact of conventional water treatment processes on cells of the cyanobacterium *Microcystis aeruginosa*. *Water Res.* **33**(15), 3253–3262.
- Coble, P. G. 1996 Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Mar. Chem.* **51**(4), 325–346.
- Crapper, D. R., Krishnan, S. S. & Dalton, A. J. 1973 Brain aluminum distribution in Alzheimer's disease and experimental neurofibrillary degeneration. *Sci. Tech. Froid* **180**(4085), 511–513.
- Gassenschmidt, U., Jany, K. D., Bernhard, T. & Niebergall, H. 1995 Isolation and characterization of a flocculating protein from *Moringa oleifera* Lam. *BBA- Gen. Subjects* **1243**(3), 477–481.
- Ghebremichael, K. A., Gunaratna, K. R., Henriksson, H., Brumer, H. & Dallhammar, G. 2005 A simple purification and activity assay of the coagulant protein from *Moringa oleifera* seed. *Water Res.* **39**(11), 2338–2344.
- Ghebremichael, K., Gunaratna, K. R. & Dalhammar, G. 2006 Single step ion exchange purification of the coagulant protein from *Moringa oleifera* seed. *Appl. Microbiol. Biotechnol.* **70**(5), 526–532.
- Jarvis, P., Jefferson, B. & Parsons, S. A. 2006 Floc structural characteristics using conventional coagulation for a high doc, low alkalinity surface water source. *Water Res.* **40**(14), 2727–2737.
- Kalibbala, H.M. 2007 Application of indigenous materials in drinking water treatment, www.lwr.kth.se/Publikationer/PDF_Files/LWR_LIC_2036.pdf (accessed 22 January 2009).
- Kwaambwa, H. M. & Maikokera, R. 2007 A fluorescence spectroscopic study of a coagulating protein extracted from *Moringa oleifera* seeds. *Colloid. Surface. B* **60**, 213–220.
- Lee, W. & Westerhoff, P. 2006 Dissolved organic nitrogen removal during water treatment by aluminum sulphate and cationic polymer coagulation. *Water Res.* **40**(20), 3767–3774.
- Lilliehöök, H. 2005 Use of sand filtration on river water flocculated with *Moringa oleifera*. MSc thesis, Lulea University of Technology, Sweden.
- McCullister, D. D., Oyen, F. & Rowe, V. K. 1964 Toxicology of acrylamide. *Toxicol. Appl. Pharm.* **6**(2), 172–181.
- McKnight, D. M., Boyer, E. W., Westerhoff, P. K., Doran, P. T., Kulbe, T. & Andersen, D. T. 2001 Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnol. Oceanogr.* **46**(1), 38–48.
- Muyibi, S. & Alfugara, A. 2003 Treatment of surface water with *Moringa oleifera* seed extract and alum: a comparative study using a pilot scale water treatment plant. *Int. J. Environ. Stud.* **60**(6), 617–626.
- Muyibi, S. A. & Evison, L. M. 1995 Optimizing physical parameters affecting coagulation of turbid water with *Moringa oleifera* seeds. *Wat. Res.* **29**(12), 2689–2695.
- Muyibi, S. A. & Okuofu, C. A. 1995 Coagulation of low turbidity surface waters with *Moringa oleifera* seeds. *Int. J. Environ. Stud.* **48**(3), 263–273.
- Ndabigengesere, A. & Narasiah, K. S. 1998 Quality of water treated by coagulation using *Moringa oleifera* seeds. *Water Res.* **32**(3), 781–791.
- Ndabigengesere, A., Narasiah, K. S. & Talbot, B. G. 1995 Active agents and mechanism of coagulation of turbid waters using *Moringa oleifera*. *Water Res.* **29**(2), 703–710.
- Okuda, T., Baes, A. U., Nishijima, W. & Okada, M. 2001 Isolation and characterization of coagulant extracted from *Moringa oleifera* seed by salt solution. *Water Res.* **35**(2), 405–410.
- Ozacar, M. & Sengil, I. A. 2000 Effectiveness of tannins obtained from valonia as a coagulant aid for dewatering of sludge. *Water Res.* **34**(4), 1407–1412.
- Qin, J.-J., O, M. H., Kekre, K. A., Knops, F. & Miller, P. 2006 Impact of coagulation pH on enhanced removal of natural organic matter in treatment of reservoir water. *Sep. Purif. Technol.* **49**(3), 295–298.
- Suarez, M., Entenza, J. M., Doerries, C., Meyer, E., Bourquin, L., Sutherland, J., Marison, I., Moreillon, P. & Mermod, N. 2003 Expression of a plant-derived peptide harbouring water-cleaning and antimicrobial activities. *Biotechnol. Bioeng.* **81**(1), 13–20.
- Sutherland, J. P. 2001 Process for preparing coagulants for water treatment. Patent cooperation treaty application.
- WHO 1996 *Guidelines for Drinking Water Quality. Standards and Health*, (Vol. 1), p. 540.
- Yeh, H. H. & Ghosh, M. M. 1981 Selecting polymers for direct filtration. *J. Am. Water Wks Assoc.* **73**(4), 211–218.

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