

Enhanced water treatment by *Moringa oleifera* seeds extract as the bio-coagulant: role of the extraction method

Nguyen Thanh Hoa and Cao Thi Hue

ABSTRACT

The main objective of this study was to investigate the effectiveness of protein extraction from mature-dried *Moringa oleifera* seeds by three methods: simple pulverization, oil-extraction, and protein-fractionation. Using an electrochemically activated solution as a solvent of the protein extraction process was found to be an extremely effective method due to its significant performance, environmental qualities, chemicals reduction usage and low cost. The chemical composition of protein-fractionation (MO3) indicated that the percent of protein was high at $84\% \pm 0.5\%$ while the percentage of oil was low at $0.5\% \pm 0.1\%$. This leads to increased efficiency in clarifying low turbidity water. In addition, the new bio-coagulant tackled the limitation of previous research, namely the loading of organic compounds into the water after the coagulation process by *Moringa oleifera* seeds. Three substances extracted from *Moringa oleifera* seeds can be used as an alternative to the commercial coagulant polyaluminum chloride (PAC) for water treatment in both lake water and municipal wastewater. The results showed that MO3 showed the highest efficiency in removing turbidity, chemical oxygen demand (COD), and *Escherichia coli* from lake water at 99.3%, 98.46%, and 100%, respectively, compared with results from municipal wastewater at 95.44%, 82.4%, and 99.1%, respectively.

Key words | bio-coagulant, electrochemically activated solution, *Moringa oleifera*, polyaluminum chloride, protein fraction, wastewater

Nguyen Thanh Hoa (corresponding author)
Cao Thi Hue
Thuyloi University,
175 Tay Son, Dong Da district, Ha Noi City,
Vietnam
E-mail: nthoa@tlu.edu.vn

INTRODUCTION

Recently, coagulation and flocculation processes have been widely used for treating water. Common coagulants such as aluminum sulfate and iron salt are usually expensive and affect human health. Many studies have been reported about the relationship between Alzheimer's disease and redundant aluminum (Grabow *et al.* 1985; Ndabigengesere & Narasiah 1998). As recommended by the World Health Organization (Galal-Gorchev *et al.* 1998), the amount of aluminum in drinking water should not exceed 0.2 mg/L, whereas the level of aluminum in standard drinking water in Vietnam is 2 mg/L (QCVN 01:2009/BYT). Moreover, using chemical coagulants leads to a change of pH (alkaline in water after treatment), to the formation of non-

biodegradable sludge and to the incomplete removal of toxic compounds in the water (Ndabigengesere & Narasiah 1998; Valverde *et al.* 2014). Another disadvantage is the high water-treatment cost for poor and developing countries making provision of a clean water supply and water treatment difficult. Therefore, natural coagulants are offering new possibilities in the quest for sustainable water treatment technologies (Jung *et al.* 2018).

Moringa oleifera is a tropical plant belonging to the family of *Moringaceae*. Its seeds have been shown to be one of the most effective main bio-coagulants for water treatment, including the turbidity of surface water, alkalis, organic contaminants through biological oxygen demand

(BOD₅), chemical oxygen demand (COD) in municipal wastewater, and industrial wastewater including textiles (Vilaseca *et al.* 2014), coffee fermentation (Garde *et al.* 2017), pharmaceutical wastewater (Eri *et al.* 2018), microalgae (Barrado-Moreno *et al.* 2016; Camacho *et al.* 2017), protozoa (Petersen *et al.* 2016), total coliform (Nguyen 2016) and *Escherichia coli* (Dasgupta *et al.* 2016). Many studies have described the predominant mechanisms for toxic reduction utilizing *Moringa oleifera* for adsorption, charge neutralization, and destabilizing particles between the cationic proteins of *Moringa oleifera* and the colloid of water (Jung *et al.* 2018; Villaseñor-Basulto *et al.* 2018). The bioactive agents of *Moringa oleifera* seeds have been determined to be cationic proteins with low molecular weight ranging from 6–16 kDa with isoelectric point above pI 10 (Ndabigengesere *et al.* 1995). Amino acids in *Moringa oleifera* seed include glutamine, arginine and proline, with a total of 60 other residues. The protein peptide assists eight positively charged amino acids (7 arginines and 1 histidine) and 15 glutamine residues. Baptista *et al.* (2017) extracted the *Moringa oleifera* proteins and determined that globulin presented the best performance, promoting high turbidity and color reduction efficiency in low turbidity waters. Also, it is able to remove microorganisms due to the consistency of the antioxidant and antimicrobial. *Moringa oleifera* seed extracts have been observed against *S. aureus*, *E. coli* and *V. cholerae* in the shrimp pond water (Viera *et al.* 2010). In addition, biofilms of microorganisms of clinical interest were removed by *Moringa oleifera* seeds, such as *S. aureus* and *P. aeruginosa* and the yeast *C. albicans*. The bio-compounds most likely involved in this activity are saponins, tannins, isothiocyanates and phenolic compounds, such as alkaloids, particularly flavonoids, which exist in high concentrations in the seeds (Brilhante *et al.* 2017).

In the past, researchers such as Bichi (2013), Petersen *et al.* (2016), and Nguyen (2016) only studied crushed *Moringa oleifera* seeds after removing the seed coat and wings, and grinding to clarify synthetic water from turbidity. However, these substances had effective results in high and medium turbidity (≤ 200 NTU). Thus, development in methods of derivative extraction was investigated. Distilled water, salts and solvents were used to extract the active compound of *Moringa oleifera* before treating the pollutants in wastewater. Sodium chloride solution was used to extract

protein from the *Moringa oleifera* seeds (Okuda *et al.* 1999). This substance was found to have 7.4 times higher coagulation ability than that using distilled water due to the increase in protein dissociations, and protein solubility for coagulation. Madrona *et al.* (2010) described that the reduction in the color and turbidity of aqueous water by *Moringa oleifera* extract prepared by using potassium chloride (KCl) solution was more efficient than with distilled water. Most of the methods of extraction for *Moringa oleifera* seeds were the oil-extract by solvent extraction such as hexane (Muyibi *et al.* 2010; Garcia-Fayos *et al.* 2016), ethanol (Garcia-Fayos *et al.* 2016; Camacho *et al.* 2017) and acetone (Garcia-Fayos *et al.* 2016). On the other hand, the organic, nitrate, and phosphate concentrations in water have been illustrated to grow in the case of powder with either water or salt extracted. Therefore, to solve this limitation of *Moringa oleifera* extract, the purification methods, for instance dialysis, precipitation, delipidation, centrifugation, ion exchange, and lyophilization, have been described. This will improve the processing costs and difficulty for large-scale practical use. Thus, the challenge in the coagulation process is production of new coagulants with high efficiency, eco-friendliness, and biogradated sludge formation to achieve sustainable development.

In the past decade, electrochemically activated solutions (ECAS) have been applied widely in food production industry, biotechnology, healthcare settings and drinking water treatment. Aider *et al.* (2012) concluded that ECAS played a role as self-formation of acidic (anolyte) or base conditions (catholyte), selected protein and fiber extraction, preventing the development of microorganisms on the food or bio-material. According to Cao *et al.* (2017), ECAS including catholyte and anolyte were used for gelatin extraction from fish skins. Using ECAS is an eco-friendly and effective method. As mentioned in this research, the protein extraction of *Moringa oleifera* seeds has been improving this utilization of ECAS as extracted solvent after the oil-extraction step by n-hexane. Protein of *Moringa oleifera* seeds was approximately dissolved into ECAS then precipitated and dried. This extracted product is rarely biodegraded by microorganisms due to the antioxidant from ECAS.

Polyaluminium chloride (PAC) has become popular in the coagulation–flocculation process due to its many

advantages over metallic salts (aluminum and iron salts). Many researchers found that PAC had better efficiency at low temperature (Yu *et al.* 2007), fewer aluminum residuals, less sludge formation, a wide pH range (5.5–8) of raw water for activity, and more rapid flocculation (Wei *et al.* 2015). Recently, most studies on the coagulation mechanisms of PAC conclude towards the combination of the adsorption–charge neutralization process, such as electrostatic patch, sweep coagulation and bridge aggregation, due to the high positive charges of Al_{13} [$AlO_4Al_{12}(OH)_{24}(H_2O)_{72}^{7+}$] and Al_{30} [$Al_{30}O_8(OH)_{56}(H_2O)_{24}^{18+}$] (Popa *et al.* 2010; Lin *et al.* 2014). Gregory & Duan (2001) have investigated the effectiveness of different pre-hydrolysing coagulants for wastewater treatment and concluded that PAC products give more rapid flocculation and stronger flocs than alum at optimal dosage. Yarahmadi *et al.* (2009) reported the comparison of the effect of the *Moringa oleifera* seeds extraction by different concentrations of sodium chloride with the traditional coagulant PAC in the purification of turbid water from 10 to 1,000 NTU. These authors showed that *Moringa oleifera* seeds were more efficient than PAC in treating high turbidity water. In contrast, PAC had a better result for purification in low turbidity, whereas the defatted presscake from *Moringa oleifera* seeds was found to be more effective than the commercial coagulation agents PAC to treat black dye 19 in pH 5–8 at any temperature (Tie *et al.* 2015). Thus, it is necessary to investigate the use of *Moringa oleifera* seeds extract and the difference between *Moringa oleifera* seed and the commercial coagulant PAC to treat surface waters and wastewaters.

The use of a bio-coagulant based on plants such as *Moringa oleifera* seeds would assist sustainable, economic environmental practices by presenting great benefits such as biodegradability, low index of residual sludge formation and non-toxicity, and a non-corrosive nature, and harnessing them for water and wastewater treatment would help reduce chemical dependency (Oladoja 2014). The objectives of this study were: (1) to analyse the influence of extracted forms *Moringa oleifera* as a coagulant, and to characterize the *Moringa oleifera* extract powder by its chemical constituents; (2) to compare the effect on turbidity, COD and *E. coli* removal in lake water and municipal wastewater between the *Moringa oleifera* extract and the

conventional coagulant PAC; and (3) to suggest the optimum dosage of coagulants for treatment of water.

MATERIAL AND METHODS

Chemicals

The following chemicals were used in, whereas this study. n-hexane, potassium dichromate (K_2CrO_7), silver sulfate (Ag_2SO_4), sulfuric acid (H_2SO_4), ammonium iron(II) sulfate ($(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$), 1,10-phenanthroline and mTEC agar were purchased from Merck, Germany with a purity of 99%. Polyaluminium chloride (PAC), named PAC-HB or $Al_z(OH)_x(Cl)_y(SO_4)_z$, was a light-yellow powder with the following characteristics: 30% Al_2O_3 , basicity at 71.3%, pH 4.14 (10 g/solution) and water insoluble at 0.2%. PAC-HB was obtained from the Viet Tri Chemical Joint-Stock Company, Vietnam.

Extraction of *Moringa oleifera* seeds

Moringa oleifera seeds were harvested from Binh Thuan province, Vietnam. Seeds were chosen that were not decayed, old, or infected with seed pests. These seeds were dried in the oven (Blinder, FD 115, USA) for 24 h at 40 °C. After that, the seeds were crushed into powder using a blender (Sunhouse, SHD5580, Vietnam). This powder was known as MO1.

Continuously, the MO1 was defatted by extracting with n-hexane. The extraction process was carried out 3 times with an n-hexane/powder ratio of 3:1 (v/w) at 45 °C in 15 minutes under ultrasound by the ultrasonic cleaner (Sharpertek, SH80-2 L, USA). The solid was collected by filter papers (Whatman, 4, UK) and then dried at 50 °C (Blinder, FD 115, USA) (MO2).

The defatted flour was suspended in an electrochemically activated solution which was produced using an electroactivator (Aquapribor, AP1, Russia) with pH 9.0 in a relation 1/10 (w/v). The suspension was stirred by magnetic stirrer (IKA, RT5P, China) for 1 hour at room temperature and then centrifuged (Hettich, Eba 200, Germany) at 9,000 rpm for 20 min at 10 °C. The precipitate was freeze-dried at room temperature about 20–25 °C

(MO3). All three materials were stored in a desiccator (Boeco, GLA 415262302, Germany).

The coagulant stock

Moringa oleifera extract powder stock

One gram of *Moringa oleifera* powder extract was dissolved in 1,000 mL of distilled water to obtain the stock coagulant. The mixture was blended using a magnetic stirrer (IKA, RT5P, China) for 30 min at high speed to extract the active proteins of *Moringa oleifera*. The suspension was then filtered through a filter paper (Whatman, 41, UK) with a pore size 20–25 μm in a beaker to get a stock solution of 1 g/L.

Preparation of the PAC stock

The PAC powder (PAC-HB, Viet Tri, Vietnam) was dissolved into deionized water to prepare a concentrated stock solution (2 mol Al/L). To enhance reproducibility and avoid degradation of the solution, a fresh coagulant solution was prepared from the stock solution the day before each set of experiments.

Water samples

The lake water samples were taken at Van Quan Lake, Ha Dong district, Ha Noi, and the municipal wastewater was collected from To Lich River which is the main artificial drain of domestic wastewater in Ha Noi. Tables 1 and 2 show the quality of lake water and river water, respectively. All samples were stored in the pharmaceutical refrigerator (Panasonic MPR-S313, Japan) at 4 °C within 24 hours.

Experimental design

The design of the Jar Test experiment was based on Nguyen (2016) with a coagulant dosage of 0–60 mg/L for the lake water. The municipal wastewater used 0–120 mg/L of coagulants. The coagulation process was carried out using the Jar Test (VELP, JLT6, Italia) and involved a rapid mixing of the mixture coagulants into the water, slow mixing, and a sedimentation stage in a batch process. In

Table 1 | Characteristic of the water samples

Parameter	Unit	Lake water samples	Municipal wastewater samples	QCVN 08 – 2015/ BTNMT B ₁
t°	°C	28.5 ± 0.02	29 ± 0.02	–
pH		7.64 ± 0.06	7.1 ± 0.05	5.5–9
DO	mg/L	2.2 ± 0.1	1.2 ± 0.1	≥4
Turbidity	NTU	44 ± 1	81.2 ± 2.5	–
TSS	mg/L	53.24 ± 0.5	103.24 ± 0.5	100
BOD ₅	mg/L	60.55 ± 0.3	111.5 ± 0.2	15
COD	mg/L	93.77 ± 0.25	159.3 ± 0.7	30
NH ₄ ⁺	mg/L	1.3 ± 0.05	19.3 ± 0.05	0.9
NO ₂ ⁻	mg/L	0.37 ± 0.3	9.37 ± 0.2	0.05
NO ₃ ⁻	mg/L	1.2 ± 0.1	27.2 ± 0.1	10
<i>E. coli</i>	CFU/mL	118 ± 1.5	535 ± 0.6	50
Total coliform	MPN/mL	9,700	<10,000	7,500

Sample location and description: Lake water – Van Quan Lake, Ha Dong province, Ha Noi city, 19–23 February 2018, green water and smell; Municipal water – To Lich River, Dong Da province, Ha Noi city, 19–23 February 2018, high turbidity water and foul smell.

Table 2 | Chemical composition of protein *Moringa oleifera* seed extract

	Moisture (%)	Protein (%)	Lipid (%)	Mineral (%)	Carbohydrate (%)
MO1	7.2 ± 0.3	33.4 ± 0.2	34.5 ± 0.4	6.4 ± 0.7	18.5 ± 0.3
MO2	6.8 ± 0.3	46.2 ± 0.3	1.5 ± 0.1	16.4 ± 0.4	29.1 ± 0.2
MO3	8.8 ± 0.2	84.0 ± 0.5	0.5 ± 0.1	1.5 ± 0.3	5.2 ± 0.1

Notes: MO1 - simple powder of *Moringa oleifera* seeds; MO2 - fat-extracted *Moringa oleifera* seeds; MO3 - fractionation protein of *Moringa oleifera* seeds.

order to reproduce the coagulation and flocculation stage in the water treatment, in each round of the experiment, 1 L water sample (lake water or municipal wastewater) was poured into the beaker and stirred rapidly for 4 minutes with *Moringa oleifera* seed extracts predetermined concentration then stirred for 20 minutes (the rotational speed of the stirring paddle for fast and slow stirring was 100 and 40 rpm, respectively). Next, the suspensions were left to stand for 30 minutes. After the coagulation process, pH, turbidity, COD, and *E. coli* were identified in the lake water and the municipal wastewater. These parameters represent pollution of particulates, organic matter, and microorganisms. All experiments took place at room temperature (25 °C ± 0.5). In Vietnam, a tropical country, the temperature of surface waters and domestic wastewaters is

relatively stable. Thus, temperature is not a significant factor in the optimal dosage of coagulants. The initial pH of the sample did not change during the coagulation process because the pH of both samples was neutral at around 7. These parameters were in the optimal range for coagulation process by *Moringa oleifera* extract powders and PAC.

Analytical methods

Turbidity was measured by a portable turbidimeter (Hach 2100Q, USA), pH values of samples were tested by a pH meter (SI Analytics, Lab 850, Germany).

E. coli were analyzed by the membrane filtration method following method 1603 of United States Environmental Protection Agency (USEPA 2014). The result provides a direct count of *E. coli* in water or wastewater based on the growth of colonies on the surface of a membrane filter. A sample is filtered through the membrane, so the bacteria is retained on the surface membrane. After that, the membrane is placed on a selective and differential culture, modified mTEC agar, then incubated at 44.5 ± 0.2 °C for 22 ± 2 hours (Incucell 111, MMM Medcenter Einrichtungen GmbH, Germany). The results are that the number of colonies on the modified mTEC agar is red or magenta in color after the incubation period.

COD was calculated by the open reflux method SMEWW 5220.B:2012 using a COD reactor, (Hach, DRB 200, USA). Potassium dichromate ($K_2Cr_2O_7$) is used as a strong oxidant to degraded matters. After digestion, the remaining unreduced dichromate $K_2Cr_2O_7$ is titrated with ferrous ammonium sulfate ($(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$) to find the amount of $K_2Cr_2O_7$ consumed and the oxidizable compounds are calculated in terms of oxygen equivalent. Some samples with very low COD or with highly miscellaneous solids content may need to be analyzed in replicate to yield the most positive data. Results are further enhanced by reacting a maximum quantity of dichromate, showing that some residual dichromate remains.

The turbidity, COD, and *E. coli* removal efficiency (H%) were calculated using Equation (1) as follows:

$$H = \frac{C_0 - C}{C_0} \times 100\% \quad (1)$$

where C_0 and C are the initial and final concentration of turbidity (NTU), COD (mg/L) and *E. coli* (CFU/mL) in water, respectively.

The chemical composition of *Moringa oleifera* extract seed powders (moisture, ash, fat and protein contents) was determined according to standard methods 934.01, 942.05, 991.36 and 954.01, respectively (AOAC 2000).

Statistical analysis

All the experiments were conducted in triplicate to ensure reproducibility of results. The final results were the average of the three. All statistical analyses of the data were performed using a Student's paired *t*-test. Statistical significance was defined as $p < 0.05$, analysed using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Chemical composition of protein *Moringa oleifera* extract

Table 2 displays the chemical composition of three materials from *Moringa oleifera* extract. The results illustrated that the extraction methods of *Moringa oleifera* seeds was as expected: *Moringa oleifera* integral powder (MO1) shows a higher oil content than *Moringa oleifera* seed after fatty extractions (MO2) and protein fractions (MO3). They were reduced by 95.65% and 98.55% for MO2 and MO3, respectively. The oil-free component was the important step in the *Moringa oleifera* extraction because it increases organic pollutants by the addition of fatty acid into water. Moreover, it is a valuable product that can be collected and reused (Nordmark *et al.* 2016). Table 2 also demonstrates that the protein fractionation MO3 had the best protein value, around 84%. The percentage of extracted protein was much higher than that extracted by salt, or ethanol (Madrona *et al.* 2012; Camacho *et al.* 2017). The values were 32% and 42.9%, respectively. The MO3 samples were mostly protein (on dry weight) and the low lipid, ash and carbohydrate content showed the effect of extraction by electrochemical activated water (catholyte with pH 9.0). Generally, alkaline or acid solution were used in protein

extraction. Proteins of *Moringa oleifera* seeds were observed as a natural cationic polyelectrolyte and display the main characteristics for coagulation capability. They connected with suspended particles in the water by charge neutralization to form the floc, which was removed easily from water in the sedimentation process (Baptista *et al.* 2017; Villaseñor-Basulto *et al.* 2018).

Quality of sample water

Quality of the lake water (Van Quan) and municipal wastewater (To Lich River) were determined and are shown in Table 1. Comparing Vietnam's standard value for surface water quality class B1 for irrigation (QCVN 08: 2015/ BTNMT, 2015), the pH value of both water samples standardized at neutral pH. In general, wastewater from To Lich River is neutral because a major source is wastewater from the daily activities of the city's residential area, and industrial wastewater or production wastewater only account for a small part. The pH of Van Quan lake water is slightly basic because of development of algae. DO values for the water samples were low at about 2.2 and 1 mg/L for the lake water and municipal wastewater, respectively. They did not meet the conditions of the habitat for aquatic plants and animals. Both water samples were seriously polluted with high BOD₅ and COD levels. BOD₅ levels were as high as 65.55 to 111.5 mg/L for the lake water and municipal wastewater, respectively. The COD value for the To Lich River exceeds 5 times that for class B1 of Vietnam's standard of surface water quality, whereas the COD of the lake was, at 93.77 mg/L, more than three times the COD value in QCVN 08:2015/BTNMT (column B1: 30 mg/L). The main component of To Lich River and Van Quan lake is wastewater, so the concentration of nutrients in the river water is often very high. The results of analysis for NH₄⁺, NO₂⁻, NO₃⁻ of To Lich River were approximately 19.3, 9.37, 27.2 mg/L, many times higher than the prescribed standard in QCVN 08:2015/BTNMT column B1 (0.9, 0.05, 10 mg/L, respectively). For the Van Quan lake, these results were lower than those for To Lich river, however, they were still higher than the standard of Vietnam's surface water quality. Microbial pathogens in both water samples were determined by *E. coli* and total coliform parameters. Both water samples had values higher than the value defined in

the Standard and exceeded it by many times. Although the turbidity of water samples was not modified in Standard QCVN 08:2015/BTNMT, we can conclude that the two water samples were polluted with particles using the total suspended solids (TSS) parameter. TSS parameter in both water samples exceeded the Standard (class B1 at 50 mg/L). In summary, both water samples were polluted with regard to turbidity, organic contaminants, and microorganisms.

Coagulation is utilized as a primary process of surface water and wastewater treatment in the control of particulates, microorganisms, and natural and synthetic organic matter. Thus, in this research, we chose three parameters: turbidity, COD, and *E. coli*, to investigate the efficiency of the proposed coagulation process. They not only represent types of pollution for surface water and municipal wastewater but also were easily analyzed.

Treatment of lake water with different *Moringa oleifera* extract powders

Figures 1 and 2 show different parameters (pH, the turbidity, COD and *E. coli*) analyzed from the lake water after the coagulation process by simply crushed *Moringa oleifera* seeds (MO1), oil-extraction *Moringa oleifera* seeds (MO2), fraction protein of *Moringa oleifera* seeds (MO3) and PAC.

It can be seen from Figure 1(a) that three substances did not significantly affect the pH value, which remained almost constant at around 7.64 for all dosages tested. In contrast, the pH value decreased from 7.64 to 5.51 for PAC. Coagulant PAC contains aluminum in the form of a polymer, and also forms aluminum hydroxide in contact with water, becoming cationic through the reaction with the alkalinity of water. Increased acidity may happen due to the aluminum trivalent cation that acts as a Lewis acid and accepts ion-electron pairs. Formation of the H⁺ ions to reduce the pH of the water followed Equation (2). This was similar to previous research on the coagulation process using PAC (Yan *et al.* 2008; Hendrawati *et al.* 2016),



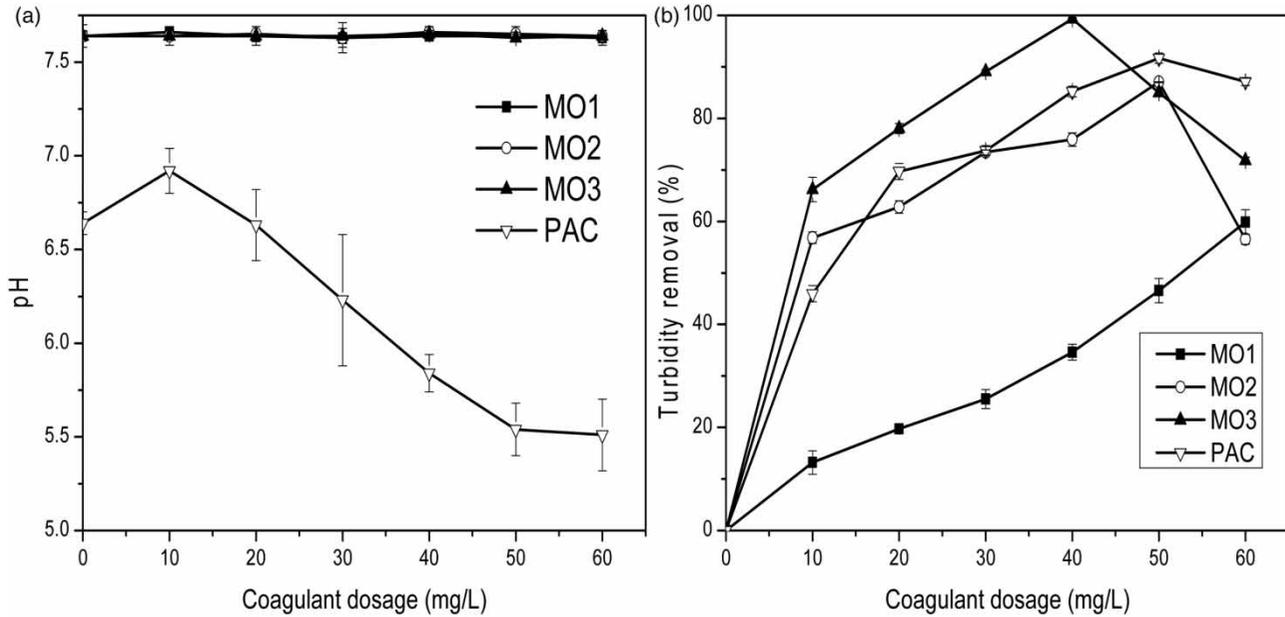


Figure 1 | (a) pH residual and (b) turbidity removal (%) in lake water with *Moringa oleifera* seed extract flours and PAC and optimum coagulant dosages.

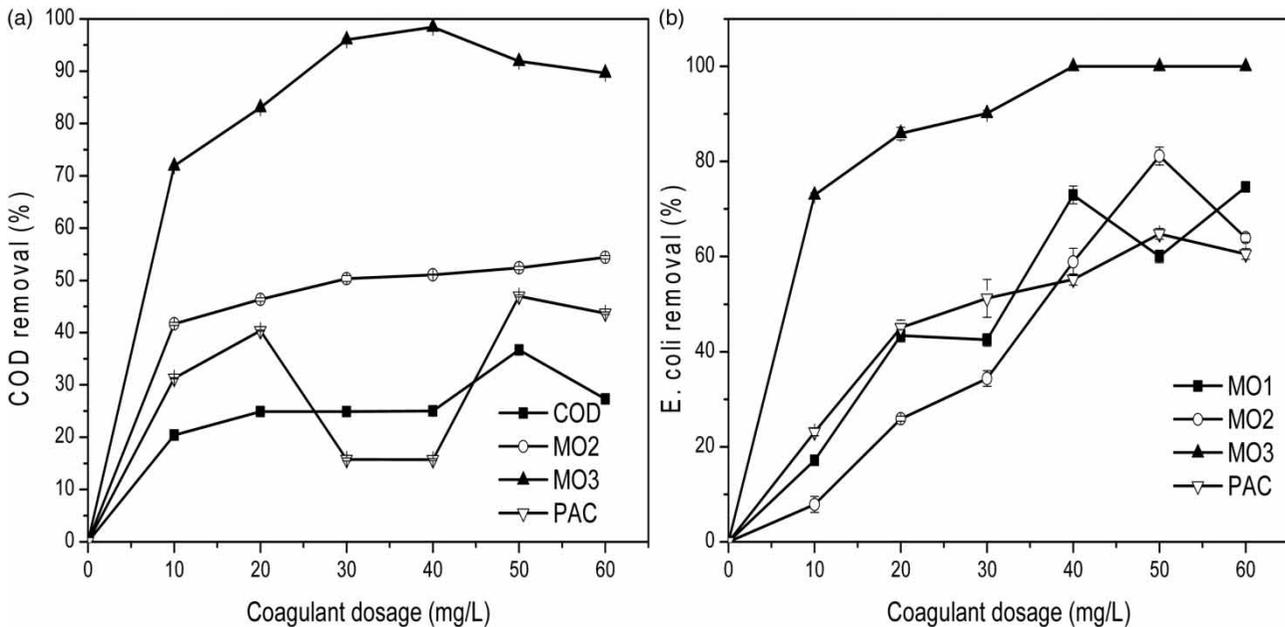


Figure 2 | (a) COD removal and (b) *E. coli* removal in lake water with *Moringa oleifera* seed extract flours and PAC and optimum coagulant dosages.

In contrast, substances produced from *Moringa oleifera* seeds are composed of the organic molecules produced and ready for application in the cationic form. The absence of chemical reaction between these cationic proteins and the alkalinity in water is also the reason why

the three substances do not decrease the pH of the water under treatment. The steady pH of *Moringa oleifera* extracted powder is an advantage as a replacement for the traditional coagulant PAC in water treatment, due to the reduction in the amount and cost of chemical agents

required to adjust the pH for the next water treatment process (Camacho *et al.* 2017).

The pH and alkalinity strongly influence the coagulation ability of inorganic coagulants like aluminum sulfate, iron sulfate and PAC. Each coagulant has a different optimal pH range, for example 3–9 for iron salts coagulant, and 5.5–8 for aluminum-based coagulants (Yan *et al.* 2008).

Effect of pH on aluminum hydroxide solubility (PAC)

pH effects aluminum hydroxide solubility as it is a typical amphoteric hydroxide. The pH of the water can be too basic or too acidic to make it dissolve, resulting in excess aluminum content in the water. pH also influences aluminum hydroxide particles. The charge of the colloidal particles in the water is related to the ion composition of the water, in particular the H^+ ion.

Influence of pH on organic matter in water (humic acid)

When the pH is neutral, the gel solution of humic acid is negatively charged so is easily degraded. PAC coagulant does not execute well for organic compound removal when $pH < 5.5$. This can be explained by the fact that when the solution condition is acidic, the carboxyl groups of organic compounds are hardly hydrolyzed because of the high concentration of H^+ ; furthermore, H^+ could react with organic molecules which compete with the complex reaction between aluminum pieces and organic matter. At higher pH, humic acid becomes completely soluble and is difficult to remove efficiently. In addition, formation of colloids are influenced by the pH value of water with regard to potential and the isoelectric point. If the alkalinity of the water is too low, it will not be sufficient to remove the acid produced by the hydrolysis of coagulants. As a result, the pH value of the water after the coagulation process decreases rapidly. Then the alkali is used to adjust the pH of the water and thus the effect of alkalinity to the coagulation is relative to the pH value.

According to Ndabigengesere & Narasiah (1996), the effect of pH was not significant to the coagulation process using *Moringa oleifera* extracted seeds. This is because the main protein of *Moringa oleifera* has a positive charge with an isoelectric value of around 10. This means the

protein remains positively charged as long as the pH is below 10. On the contrary, colloids in water are mostly negatively charged. Thus, the reactions between coagulant and colloids happen and are then removed from water in the settling process.

This study did not investigate the effect of pH on the coagulation process extensively (these results were not displayed). We agree that the optimum pH for PAC coagulant is 5.5–8 and for *Moringa oleifera* extracted powders pH is not a significant factor for coagulation capability. Based on the analysis, the optimum pH obtained using *Moringa oleifera*, a bio-coagulant, is pH 6–8. At this point, amino acids are ionized to form a carboxylate ion and proton, and directly attract electrons (colloids) to form neutral groups and produce floc so as to remove the contaminants from the water (Ndabigengesere & Narasiah 1996).

From Figure 1(b) it can be seen that coagulants extracted from *Moringa oleifera* significantly change the highest turbidity of the lake water at 59.89%, 87.04% and 99.32% for optimal dosages of coagulation agents of 60 mg/L, 50 mg/L and 40 mg/L, respectively. In comparison, the turbidity decreased considerably at 91.67% of the optimum PAC dose of 50 mg Al/L. Thus, the coagulation ability of *Moringa oleifera* seeds depends on the quality of the cationic protein and the oil in the seed. While the cationic protein has the role of crosslink to colloids of water, as a means of neutralizing their negative charge and weakening the electrostatic double layer rejection such that van der Waals forces reduce turbidity, the oil of the *Moringa oleifera* seed was put into the water to prevent connection between the coagulant and flocs. It is more likely that the cationic proteins adsorb the kaolin particles.

Compared with previous studies which treated surface water with low turbidity, this MO3 powder coagulated more effectively. Vo *et al.* (2012) illustrated removal of only 50% of river water where turbidity was 44 NTU. Or Baptista *et al.* (2017) reported on the fractionation protein by n-hexane 1% and 5% *Moringa oleifera* seeds to reduce turbidity by 89% in the low turbid water (<50–100 NTU).

The COD of the influence of Van Quan lake was 93.77 mg/L. After treatment, the following results were obtained: 59.37, 42.75, 1.44 and 49.7 mg/L for the optimum dosage of MO1, MO2, MO3, and PAC, respectively, as

shown in Figure 2(a). Most results were less than 30 mg/L which was standardized at class B1 (water quality for navigation purposes) of the National Technical Regulation on Surface Water Quality (QCVN 08-MT:2015/BTNMT). Treated water can be used for water supply purpose (<10 mg/L) after utilizing MO3 coagulant.

Many authors have reported on the mechanism of removing COD by the coagulated ion process. First of all, COD removal was combined with the decrease of turbidity in water. Following on from this, adsorption was one of the main mechanisms for the coagulation process by *Moringa oleifera* seeds extract. Real-Olvera *et al.* (2015) found that the interaction between protein/amino acids and ions consisting of organic compounds from slaughter wastewater fit the second-order kinetics, while Sivakumar (2013) reported that the adsorption of COD in leachate wastewater followed the Elovich kinetic models when using crushed *Moringa oleifera* seeds.

The concentrations of residual *E. coli* are illustrated in Figure 2(b). It was clear that using the *Moringa oleifera* extracted powder was better than utilizing PAC for removing *E. coli*. Simple crushed *Moringa oleifera* seed reduced *E. coli* to 30 CFU/mL while PAC only removed it to 42 CFU/mL after water treatment process. In addition, both *Moringa oleifera* extracted powder by fatty extraction and fully protein fraction removed 100% of *E. coli*. The reasons for this are as follows. Firstly, coagulation processes can remove microorganisms through the coagulation and precipitation process associated with turbidity and suspended solids in water. Secondly, according to Shebek *et al.* (2015), this *Moringa oleifera* cationic protein can destroy the inner and outer membranes of *E. coli* cells by antioxidant compound, α_L -rhamnosyloxy-benzyl isothiocyanate (Eilert *et al.* 1981). Therefore, coagulants of *Moringa oleifera* play an important role in water disinfection (Bichi 2013). Madsen *et al.* (1987) observed 80–99.5% turbidity reduction and 90–99.99% bacterial removal during 1–2 hours of treatment with *Moringa oleifera* seed extracted in treating Sudanese water. Ghebremichael *et al.* (2005) investigated the coagulation and antimicrobial ability of the purified protein for surface water treatment (the River Meuse and Delft Canal waters). The results were up to 97% and 86% turbidity removal for high turbid and low turbid waters, respectively, and *E. coli* removed completely. Poumaye

et al. (2012) tested *Moringa oleifera* dried powder for purifying the river M'Poko surface water. *Streptococci*, *Clostridium*, and *E. coli* were reduced by 62%, 95%, and 47%, respectively. Dasgupta *et al.* (2016) illustrated detailed antimicrobial agents of the seed extract of *Moringa oleifera* to remove a maximum of 93.2% for *E. coli* and 96.2% for *Bacillus subtilis*. Nguyen (2016) also found that it is possible to remove the whole of the *coliform* bacteria from Hong River, belonging to an *Escherichia coli* family, by using the simple crushed *Moringa oleifera* seeds.

Treatment of municipal wastewater by different *Moringa oleifera* extract powders

Figures 3 and 4 illustrate the efficiency in removing pH (Figure 3(a)), the turbidity (Figure 3(b)), organic pollutants through parameter COD (Figure 4(a)) and *E. coli* (Figure 4(b)) as a function of coagulant concentrations of 20, 40, 60, 80, 100 and 120 mg/L for four coagulants.

As with lake water, protein fractionation of *Moringa oleifera* seeds had the best results in treating municipal wastewater for turbidity, COD, and *E. coli*. These results were 95.44%, 82.4% and 99.1%, respectively, at the optimum dosage of 80 mg/L (except for *E. coli* removal at 120 mg/L) of coagulant MO3. In fact, Hendrawati *et al.* (2016) described how when *Moringa oleifera* extract exceeded the optimum dosage, turbidity and COD reduced because all colloids in water had been neutralized, then formed bigger particles. Therefore, the overwhelming coagulant dosage caused the increase of turbidity and COD in water as shown in Figures 3(b) and 4(a) as they did not react with oppositely charged colloidal particles. Based on this, treated water may discharge to a water resource used for supplied water for navigation without a secondary treatment process (QCVN 14:2015/BTNMT class B2).

The lowest effect for treating domestic wastewater was the *Moringa oleifera* integral powder. According to Figure 3(b), the turbidity removal efficiency increased with the rise of the dosage of *Moringa oleifera* seed simple extracted powder by around 62.65% (turbidity decreased from 81.67 NTU to 30.48 NTU). Compared with MO3 (where turbidity in water after the treatment process was 3.72 NTU), the turbidity in wastewater after the coagulation process using MO1 was more than 26 NTU. Therefore, the

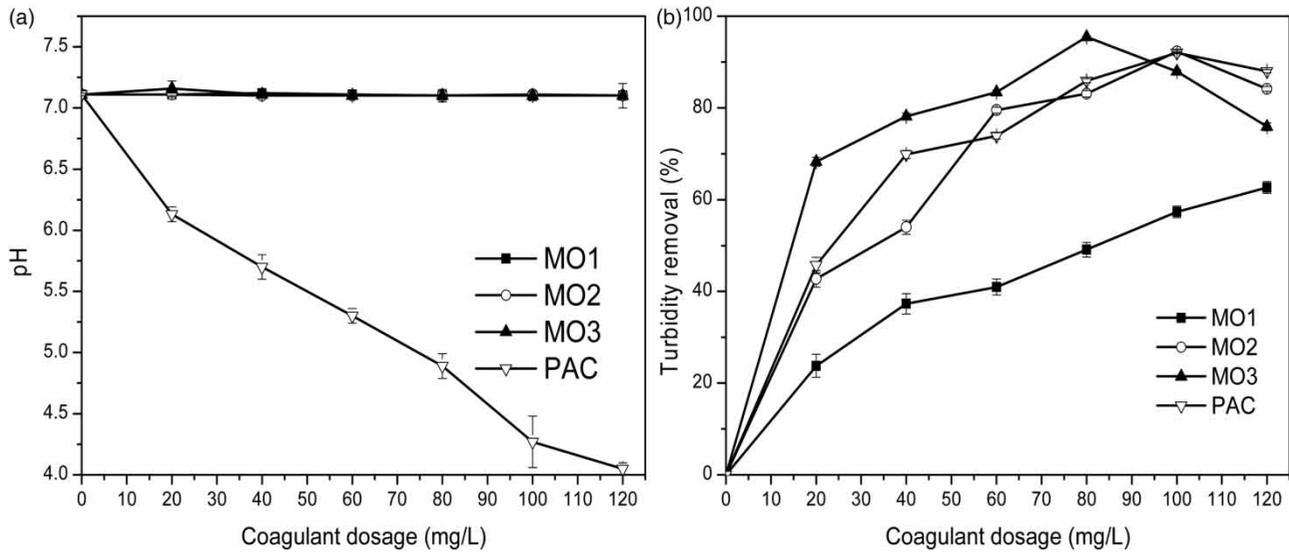


Figure 3 | (a) pH residual and (b) turbidity removal in municipal wastewater with *Moringa oleifera* seed extract flours and PAC and optimum coagulant dosages.

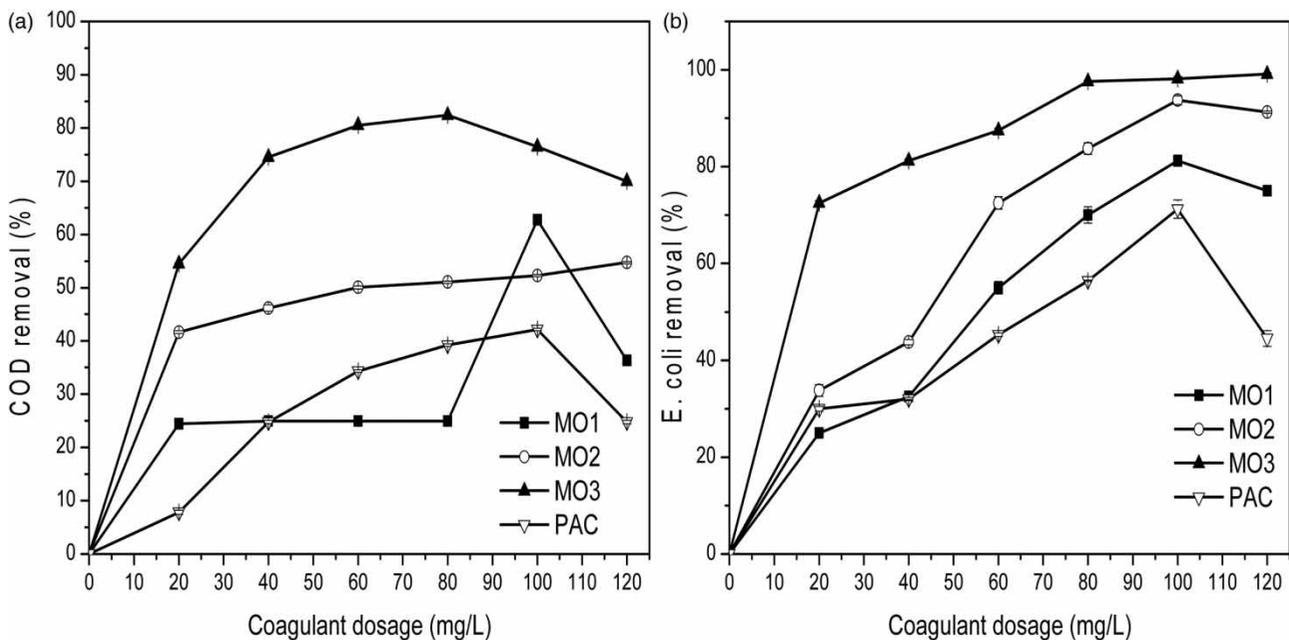


Figure 4 | (a) COD removal and (b) *E. coli* removal in municipal wastewater with *Moringa oleifera* seed extract flours and PAC and optimum coagulant dosages.

purified protein in *Moringa oleifera* seeds extraction is affected by coagulation availability, whereas the reduction of COD in the wastewater is shown in Figure 4(a). The COD removal reached its maximum of 62.72% for a MO1 dosage of 100 mg/L. At the same with an optimal dosage of coagulant MO1 at 100 mg/L, residual *E. coli* were reduced by about 81.25%. Furthermore, the safety of

utilizing *Moringa oleifera* in water treatment also received some attention. *Muyibi & Evison (1995)* recommended that further investigations need to be carried out to guarantee the complete safety of utilizing *Moringa oleifera* in water treatment and ensure there is no toxic effect on human health. In addition, it was also found to possess an antimicrobial activity and was able to clear water of several

waterborne human pathogens. Compared with PAC, a conventional coagulant, the effect of treating municipal wastewater was lower than both MO2 and MO3. The results were only shown with 92% for turbidity removal, 42.16% for COD removal and 71.25% for *E. coli* reduction. Also, the optimum dosage of PAC was more than fractionation protein MO3 and equal to the optimal of MO2 coagulants at 100 mg Al/L. Thus, *Moringa oleifera* seeds from the oil extract and the fractionated protein by ECAS solution were extremely efficient for clarifying turbidity, degradation of COD, infected microorganisms and stable pH of water, whereas the decrease in pH reduced the efficiency of the coagulant PAC in the municipal wastewater treatment process (Yarahmadi *et al.* 2009). Kane *et al.* (2016) discussed the coagulating and antibacterial effects of the coagulant *Moringa oleifera* extract to remove pollutants from domestic wastewaters. The results showed that the combination usage between alum and *Moringa oleifera* protein molecules powder as coagulant within a ratio of 50:50 (w/w) provided a more than 90% decrease of turbidity and 75% COD removal. Adeniran *et al.* (2017) used completely randomized design (CRD) experimental design to investigate the potential of a new bio-coagulant protein purified from *Moringa oleifera* to treat the municipal wastewater. The turbidity value was increased drastically after treating. COD reduced from 81.6 mg/L to 72 mg/L after the coagulation process. Generally, the above results showed that the higher the quantity of three *Moringa oleifera* seeds extracted as applied to sewage, the better the purification of the sewage.

Cost of protein extracted from *Moringa oleifera* seeds and sustainable development

Moringa oleifera is a common plant in the middle-south of Vietnam. This plant is reported as a vegetable with high nutritional value and medicinal properties. Each tree can produce around 15,000 to 25,000 seeds/year with a weight of 0.3 g/seed. The kernel-to-hull ratio is 75:257 (Ganatra *et al.* 2012). Thus, it helps to reduce the cost for producing bio-coagulants process. According to cost estimation, the cost of producing the bio-coagulant from *Moringa oleifera* seeds includes: the cost of raw materials, chemical compounds (n-hexane), the electrochemically activated solution, energy consumption, labor costs and cost for storage to keep the substance. In

this case, we can save raw material and substance storage prices. The reason is that *Moringa oleifera* seeds are a solid waste from cultivating *Moringa oleifera* trees. Recently, the planted area of *Moringa oleifera* has been expanding rapidly throughout Vietnam. Thus, it is the greatest resource for producing the bio-coagulant. Another reason is that using ECAS promotes the bio-coagulants preservation effectively. This means that the coagulant is more resistant to attack by bacteria or moulds. In addition, we can gain income from the sale of oil extracted from *Moringa oleifera* seeds and the fatty decade for reuse in composting. The cost benefits of *Moringa oleifera* seed in Vietnam are employed to the net-zero cost of its press cake. However, in Malaysia, the growth cost of 1 kg of *Moringa oleifera* was evaluated to be approximately US\$2. Similarly, in south Iran, the cultivation cost of every kilogram of *Moringa oleifera* seed was also US\$2 (Yarahmadi *et al.* 2009), whereas the cost of 1 kg of seeds has been estimated to be more expensive at US\$27, higher than the production cost of poly aluminum chloride. Thus, Vietnam has the potential to develop the bio-coagulant from *Moringa oleifera* seeds because of abundant and cheap raw material. Hence, the cost for protein fraction from 1 kg (5,000 seeds) of *Moringa oleifera* is approximately 8,000 VND (0.4 US\$). This is cheaper than PAC (approximately 10,000 VND (0.5 US\$) per 1 kg). And on top of that, protein extract of *Moringa oleifera* seeds is of more advantage to communities in terms of health, ecology, and economy.

Using a bio-coagulant based on plants is an eco-friendly operation that often involves energy efficiency, recycling, safety and health concerns, and renewable resources. The aim is that this study informs developments in this currently growing field with a focus on sustainability, in other words meeting the needs of society in ways that can continue indefinitely into the future while protecting the environment and sustainable use of natural resources.

Coagulants from *Moringa oleifera* seeds have been investigated to generate not only a much smaller (five times) sludge volume (Ndabigengesere *et al.* 1995) but also a higher organic sludge value. As such, sludge after the coagulation process can biodegrade, has lower toxicological residue and handling costs are cheaper, making it safer for human health, aquatic or river life, and consequently having a smaller environmental impact (Choy *et al.* 2014; Olyae *et al.* 2014). Another advantage of using renewable

materials, such as reusing locally available solid waste, is not only a low-cost alternative to chemical coagulants, but also their minimal net effect on global warming. Moreover, using *Moringa oleifera* seed extract is observed as environmentally friendly behavior: the process does not consume alkali, so pH adjustments can be limited, thus resulting in a lower number of chemical agents entering into aquatic life, and is therefore more sustainable (Wang *et al.* 2014). The seed extracts are also non-corrosive, which eliminates concerns about pipe destruction (Choy *et al.* 2014). The *Moringa oleifera* seed extracts are easy to use as coagulants require less processing and can provide a sustainable means of treatment.

CONCLUSION

The method of protein fractionation from *Moringa oleifera* seed discussed in this study is simple, and easy to apply on an industrial scale. Using hexane to remove oil and extracting the protein with electrochemically activated solution is a novel method of producing new bio-coagulant. In comparison with PAC coagulant, the most popular coagulation agent, fractionation protein MO3 was not only more effective for removing turbidity and COD, but also *E. coli* in treating lake and domestic wastewater. It has the potential to become a commercial product – bio-coagulant to replace common chemical coagulants (PAC) – with many benefits such as improved water treatment efficiency, more eco-friendliness, less sludge formation, biodegradability and lower costs.

In this study, the challenge presented by the coagulation process is the complexity of scaling-up the process on the water treatment plants. Different parameters of coagulation–flocculation technology require accurate control to attain the efficiency of the process. Thus, bench-scale experiment or trial and error testing is always required to determine the most efficient coagulant, pH range and alkalinity value for particular wastewater at a certain dosage.

ACKNOWLEDGEMENTS

The authors thank the Environmental Engineering Laboratory at Thuyloi University and Institute of Marine

Biochemistry, VAST for providing the Jar Test procedure and access to their facilities.

REFERENCES

- Adeniran, K. A., Akpenpuun, T. D., Akinyemi, B. A. & Wasiiu, R. A. 2017 Effectiveness of *Moringa oleifera* seed as a coagulant in domestic wastewater treatment. *African Journal of Science, Technology, Innovation and Development* **9**, 323–328. doi:10.1080/20421338.2017.1327475.
- Aider, M., Gnatko, E., Benali, M., Plutakhin, G. & Kastyuchik, A. 2012 Electro-activated aqueous solutions: theory and application in the food industry and biotechnology. *Innovative Food Science and Emerging Technologies* **15**, 38–49. doi: 10.1016/j.ifset.2012.02.002.
- AOAC 2000 *Official Methods of Analysis of the Association of Agricultural Chemists*, 17th edition. AOAC, Washington, DC, USA.
- Baptista, A. T. A., Silva, M. O., Guttierrez Gomes, R., Bergamasco, R., Vieira, M. F. & Vieira, A. M. S. 2017 Protein fractionation of seeds of *Moringa oleifera* Lam and its application in superficial water treatment. *Separation and Purification Technology* **180**, 114–124. doi:10.1016/j.seppur.2017.02.040.
- Barrado-Moreno, M. M., Beltran-Heredia, J. & Martín-Gallardo, J. 2016 Microalgae removal with *Moringa oleifera*. *Toxicon* **110**, 68–73. doi:10.1016/j.toxicon.2015.12.001.
- Bichi, M. H. 2013 A review of the applications of *Moringa Oleifera* seeds extract in water treatment. *Civil and Environmental Research* **3**, 1–11.
- Brilhante, R. S. N., Sales, J. A., Pereira, V. S., Castelo-Branco, D. d. S. C., Cordeiro, R. d. A., Sampaio, C. M. d. S., Paiva, M. d. A. N. & Santos, J. B. F. d. 2017 Research advances on the multiple uses of *Moringa oleifera*: a sustainable alternative for socially neglected population. *Asian Pacific Journal of Tropical Medicine* **10**, 621–630. doi: 10.1016/j.apjtm.2017.07.002.
- Camacho, F. P., Sousa, V. S., Bergamasco, R. & Teixeira, M. R. 2017 The use of *Moringa Oleifera* as a natural coagulant in surface water treatment. *Chemical Engineering Journal* **313**, 226–237. doi:10.1016/j.cej.2016.12.031.
- Cao, T. H., Nguyen, T. T. O., Nguyen, T. M. H., Le, N. T. & Razumovskaya, R. G. 2017 Characteristics and physicochemical properties of gelatin extracted from scales of Seabass (*Lates calcarifer*) and Grey Mullet (*Mugil cephalus*) in Vietnam. *Journal of Aquatic Food Product Technology* **26**, 1293–1302. doi:10.1080/10498850.2017.1390026.
- Choy, M., Dubé, C. M., Patterson, K., Barnes, S. R., Maras, P., Blood, A. B., Hasso, A. N., Obenaus, A. & Baram, T. Z. 2014 A novel, noninvasive, predictive epilepsy biomarker with clinical potential. *J. Neurosci.* **34**, 8672–8684. doi: 10.1523/JNEUROSCI.4806-13.2014.
- Dasgupta, S., Gundaa, N. S. K. & Mitra, S. K. 2016 Evaluation of the antimicrobial activity of *Moringa oleifera* seed extract as a

- sustainable solution for potable water. *RSC Adv.* **6**, 25918–25926. doi:10.1039/C6RA04011 J.
- Eilert, U., Wolters, B. & Nahrstedt, A. 1981 The antibiotic principle of seeds of *Moringa oleifera* and *Moringa stenopetala*. *Planta Med.* **42**, 55–61.
- Eri, I. R., Hadi, W. & Slamet, A. 2018 Clarification of pharmaceutical wastewater with *Moringa Oleifera*: optimization through response surface methodology. *Journal of Ecological Engineering* **19**, 126–134. doi:10.12911/22998993/86148.
- Galal-Gorchev, H., Chambon, P., Lund, U. & Ohanian, E. 1998 Aluminium in Drinking-Water: Background Document for Development of WHO Guidelines for Drinking-Water Quality. Report WHO/SDE/WSH/03.04/53, Health criteria and other supporting information, WHO, Geneva, Switzerland.
- Ganatra, T. H., Joshi, U. H., Bhalodia, P. N., Desai, T. R. & Targar, P. R. 2012 Therapeutic and prophylactic values of *Moringa oleifera* Lam. *International Research Journal of Pharmacy* **3**, 1–7.
- Garcia-Fayos, B., Arnal, J. M., Sancho, M. & Rodrigo, I. 2016 *Moringa oleifera* for drinking water treatment: influence of the solvent and method used in oil-extraction on the coagulant efficiency of the seed extract. *Desalination and Water Treatment* **57**, 23397–23404. doi:10.1080/19443994.2015.1137144.
- Garde, W. K., Buchberger, S. G., Wendell, D. & Kupferle, M. J. 2017 Application of *Moringa Oleifera* seed extract to treat coffee fermentation wastewater. *Journal of Hazardous Materials* **329**, 102–109. doi:10.1016/j.jhazmat.2017.01.006.
- Ghebremichael, K. A., Gunaratna, K. R., Henriksson, H., Brumer, H. & Dalhammar, G. 2005 A simple purification and activity assay of the coagulant protein from *Moringa oleifera* seed. *Water Resource* **39**, 2338–2344. doi: 10.1016/j.watres.2005.04.012.
- Grabow, W. O. K., Slabert, J. L., Morgan, W. S. G. & Jahn, S. A. A. 1985 Toxicity and mutagenicity evaluation of water coagulated with *Moringa oleifera* seed preparations using fish, protozoan, bacterial, enzyme and *Ames Salmonella* assays. *Water SA* **11**, 9–14.
- Gregory, J. & Duan, J. 2001 Hydrolyzing metal salts as coagulants. *Pure and Applied Chemistry* **73** (12), 2017–2026. doi: 10.1351/pac200173122017.
- Hendrawati, Y. I. R., Rohaeti, E., Effendi, H. & Darusman, L. K. 2016 The use of *Moringa oleifera* seed powder as coagulant to improve the quality of wastewater and ground water. *IOP Conference Series: Earth and Environmental Science* **31**. doi:10.1088/1755-1315/31/1/012033.
- Jung, Y., Jung, Y., Kwon, M., Kye, H., Abrha, Y. W. & Kang, J. W. 2018 Evaluation of *Moringa oleifera* seed extract by extraction time: effect on coagulation efficiency and extract characteristic. *Journal of Water & Health* **16**. doi:10.2166/wh.2018.078.
- Kane, C., Bâ, A., Mahamat, S. A. M., Ayessou, N., Mbacké, M. K. & Mar Diop, C. G. 2016 Combination of alum and extracted *Moringa oleifera* bioactive molecules powder for municipal wastewater treatment. *International Journal of Biological and Chemical Sciences* **10**, 1918–1929. doi: 10.4314/ijbcs.v10i4.39.
- Lin, L., Huang, C., Dempsey, B. & Hu, J. Y. 2014 Fate of hydrolyzed Al species in humic acid coagulation. *Water Res.* **56**, 314–324. doi: doi.org/10.1016/j.watres.2014.03.004.
- Madrona, G. S., Serpelloni, G. B., Salcedo Vieira, A. M., Nishi, L., Cardoso, K. C. & Bergamasco, R. 2010 Study of the effect of saline solution on the extraction of the *Moringa oleifera* seed's active component for water treatment. *Water Air Soil Pollution* **211**, 409–415. doi:10.1007/s11270-009-0309-0.
- Madrona, G. S., Branco, I. G., Seolin, V. J., Filho, B. A. A., Fagundes-Klen, M. R. & Bergamasco, R. 2012 Evaluation of extracts of *Moringa oleifera* Lam seeds obtained with NaCl and their effects on water treatment. *Acta Scientiarum* **34**, 289–293. doi:10.4025/actascitechnol.v34i3.9605.
- Madsen, M., Schlundt, J. & Omer, E. F. 1987 Effect of water coagulation by seeds of *Moringa oleifera* on bacterial concentrations. *The Journal of Tropical Medicine and Hygiene* **90**, 101–109. doi: 10.1016/0378-8741(88)90285-1.
- Muyibi, S. A. & Evison, L. M. 1995 Optimizing physical parameters affecting coagulation of turbid water with *Moringa oleifera* seeds. *Water Research* **29**, 2689–2695.
- Muyibi, S. A., Noor, M. J. M. M., Leong, T. K. & Loon, L. H. 2010 Effects of oil extraction from *Moringa oleifera* seeds on coagulation of turbid water. *International Journal of Environmental Studies* **59**, 243–254. doi:10.1080/00207230210924.
- Ndabigengesere, A., Narasiah, K. S. & Talbot, B. G. 1995 Active agents and mechanism of coagulation of turbid waters using *Moringa oleifera*. *Water Research* **29**, 703–710.
- Ndabigengesere, A. & Narasiah, K. S. 1996 Influence of operating parameters on turbidity removal by coagulation with *Moringa oleifera* seeds. *Environmental Technology* **17**, 1103–1112. doi: 10.1080/09593331708616479.
- Ndabigengesere, A. & Narasiah, K. S. 1998 Quality of water treated by coagulation using *Moringa oleifera* seeds. *Water Research* **32**, 781–791. doi: 10.1016/S0043-1354(97)00295-9.
- Nguyen, T. H. 2016 Use of *Moringa oleifera* seeds as natural coagulant for water purification in Viet Nam. *Young Scientist* **132**, 127–132.
- Nordmark, B. A., Przybycien, T. M. & Tilton, R. D. 2016 Comparative coagulation performance study of *Moringa oleifera* cationic protein fractions with varying water hardness. *Journal of Environmental Chemical Engineering* **4**, 4690–4698. doi:10.1016/j.jece.2016.10.029.
- Oladoja, N. A. 2014 Headway on natural polymeric coagulants in water and wastewater treatment operati. *Journal of Water Process Engineering* **6**, 174–192. doi: 10.1016/j.jwpe.2015.04.004.
- Olyae, E., Banejad, H., Chau, K. W. & Melesse, A. M. 2014 A comparison of various artificial intelligence approaches performance for estimating suspended sediment load of river systems: a case study in United States. *Environmental Monitoring and Assessment* **187**, 187–189. doi: 10.1007/s10661-015-4381-1.

- Okuda, T., Baes, A. U., Nishijima, W. & Okada, M. 1999 Improvement of extraction method of coagulation active components from *Moringa oleifera* seed. *Water Research* **33**, 3373–3378. doi: 10.1016/S0043-1354(99)00046-9.
- Petersen, H. H., Petersen, T. B., Enemark, H. L., Olsen, A. & Dalsgaard, A. 2016 Adsorption of organic pollutants from slaughterhouse wastewater using powder of *Moringa oleifera* seeds as a natural coagulant. *Food and Waterborne Parasitology* **3**, 1–8. doi: 10.1016/j.fawpar.2016.03.002.
- Popa, I., Papastavrou, G. & Borkovec, M. 2010 Charge regulation effects on electrostatic patch-charge attraction induced by adsorbed dendrimers. *Physical Chemistry, Chemical Physics* **12**, 4863–4871. doi: 10.1039/B925812D.
- Poumaye, N., Mabingui, J., Lutgen, P. & Bigan, M. 2012 Contribution to the clarification of surface water from the *Moringa oleifera*: case M'Poko River to Bangui, Central African Republic. *Chemical Engineering Research and Design* **90**, 2346–2352. doi:10.1016/j.cherd.2012.05.017.
- QCVN 2015 Quy chuẩn kỹ thuật quốc gia về chất lượng nước mặt 2015 (National Technical Regulation on Surface Water Quality) QCVN 08:2015/BTNMT. Ministry of Natural Resources and Environment, Viet Nam.
- Real-Olvera, J., Rustrian-Portilla, E., Houbron, E. & Landa-Huerta, F. J. 2015 Adsorption of organic pollutants from slaughterhouse wastewater using powder of *Moringa oleifera* seeds as a natural coagulant. *Desalination and Water Treatment* **57**, 9971–9981. doi:10.1080/19443994.2015.1033479.
- Shebek, K., Schantz, A. B., Sines, I., Lauser, K., Velegol, S. & Kumar, M. 2015 The flocculating cationic polypeptide from *Moringa oleifera* seeds damages bacterial cell membranes by causing membrane fusion. *Langmuir* **31**, 4496–4502. doi:10.1021/acs.langmuir.5b00015.
- Sivakumar, D. 2013 Adsorption study on municipal solid waste leachate using *Moringa Oleifera* seed. *International Journal of Environmental Science and Technology* **10**, 113–124. doi: 10.1007/s13762-012-0089-8.
- Tie, J., Jiang, M., Li, H., Zhang, S. & Zhang, X. 2015 A comparison between *Moringa oleifera* seed presscake extract and polyaluminum chloride in the removal of direct black 19 from synthetic wastewater. *Industrial Crops and Products* **74**, 530–534. doi: 10.1016/j.indcrop.2015.04.004.
- USEPA 2014 Method 1603: *Escherichia Coli* (*E. Coli*) in Water by Membrane Filtration Using Modified Membrane-Thermotolerant *Escherichia Coli* Agar (Modified mTEC). United States Environment Protection Agency, Washington, DC, USA.
- Valverde, K. C., Nishi, L., Vieira, A. M. S., Bongiovani, M. C., Coldebella, P. F., Madrona, G. S., Santos, O. A. A. d. & Bergamasco, R. 2014 Optimization of process conditions in water treatment through coagulation diagrams, using *Moringa oleifera* Lam and aluminium sulphate. *Desalination and Water Treatment* **56**, 1787–1792, doi: 10.1080/19443994.2014.960470.
- Viera, G. H., Mourão, J. A., Angelo, A. M., Costa, R. A. & Vieira, R. H. S. F. 2010 Antibacterial effect (*in vitro*) of *Moringa oleifera* and *Annona muricata* against Gram positive and Gram negative bacteria. *Rev. Inst. Med. Trop. Sao Paulo* **52**, 129–132. doi: 10.1590/S0036-46652010000300003.
- Vilaseca, M., López-Grimau, V. & Gutiérrez-Bouzán, C. 2014 Valorization of waste obtained from oil extraction in *Moringa oleifera* seeds: coagulation of reactive dyes in textile effluents. *Materials* **7**, 6569–6584. doi:10.3390/ma7096569.
- Villaseñor-Basulto, D. L., Astudillo-Sánchez, P. D., Real-Oliver, J. & Bandalac, E. R. 2018 Wastewater treatment using *Moringa oleifera* Lam seeds: a review. *Journal of Water Process Engineering* **23**, 151–164. doi: 10.1016/j.jwpe.2018.03.017.
- Vo, H. T., Hoang, H. & Luong, M. K. 2012 Nghiên cứu sử dụng hạt Chùm Ngây (*Moringa oleifera*) để làm trong nước tại Việt Nam (Study on water clarification using *Moringa oleifera* seeds in Viet Nam). *Hue University Journal of Science* **75A**, 153–164.
- Wang, W. C., Xu, D. M., Chau, K. W. & Lei, G. J. 2014 Assessment of river water quality based on theory of fuzzy sets and fuzzy binary comparison method. *Water Resources Management* **28**, 4183–4200. doi: 10.1007/s11269-014-0738-4.
- Wei, N., Zhang, Z., Liu, D., Wu, Y., Wang, J. & Wang, Q. 2015 Coagulation behavior of polyaluminum chloride: effects of pH and coagulant dosage. *Chinese Journal of Chemical Engineering* **23**, 1041–1046. doi: 10.1016/j.cjche.2015.02.005.
- Yan, M., Wang, D., Yu, J., Ni, J., Edwards, M. & Qu, J. 2008 Enhanced coagulation with polyaluminum chlorides: role of pH/alkalinity and speciation. *Chemosphere* **71**, 1665–1673. doi: 10.1016/j.chemosphere.2008.01.019.
- Yarahmadi, M., Hoseini, M., Bina, B., Mahmoudian, M. H., Naimabadi, A. & Shsavani, A. 2009 Application of *Moringa oleifera* seed extracted and polyaluminum chloride in water treatment. *World Applied Sciences Journal* **7**, 962–967.
- Yu, J., Wang, D., Yan, M., Ye, C., Yang, M. & Ge, X. 2007 Optimized coagulation of high alkalinity, low temperature and particle water: pH adjustment and polyelectrolytes as coagulant aids. *Environmental Monitoring Assessment* **131**, 377–386. doi: 10.1007/s10661-006-9483-3.

First received 31 May 2018; accepted in revised form 9 July 2018. Available online 19 September 2018