Optimization of active coagulant agent extraction method from Moringa Oleifera seeds for municipal wastewater treatment

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ABSTRACT

An enhanced and different method for the active coagulant agent extraction from Moringa Oleifera seeds powder (MOSP) was established and compared to the conventional extraction method in distillate water. In the improved method, MOSP were extracted using sodium chloride as solvent at different concentrations to extract more coagulant agent from Moringa Oleifera and enhance coagulation activity. In this study, MOSP were initially processed and oil content was removed to minimize coagulant concentration usage (MOSP-EO). Moringa Oleifera seeds powder was characterized by both X-ray and FTIR analysis. Ultrasound treatment as well was considered as an additional treatment for MOSP-EO to investigate its effect on coagulant agent extraction process improvement. Coagulation/flocculation experiments were conducted to assess coagulant extraction performance realized through various conditions. The effect of coagulant dosage, solvent concentration and ultrasound exposition duration were investigated for a real effluent of municipal wastewater treatment. Among the three studied NaCl concentrations, 1.0 M was found to be the best solvent concentration for high turbidity removal of more than 97\% using 140 mg/L of MOSP-EO compared to extraction in distillate water 88\% using 170 mg/L of the same coagulant. NaCl 1.0 M demonstrated the best performance in biochemical oxygen demand (BOD\textsubscript{5}) removal as well, where more than 98\% of municipal wastewater initial BOD\textsubscript{5} was eliminated. Mixing MOSP-EO assisted with ultrasound waves at different treatment periods did decrease the active coagulant agent extraction and thus showed its inconvenient for Moringa Oleifera coagulation activity usage.

Key words: coagulant agent extraction, coagulation activity, Moringa Oleifera, Municipal wastewater, sodium chloride, turbidity

HIGHLIGHTS

- Application of different methods to extract active coagulant agent from Moringa Oleifera seeds.
- Biodegradable natural coagulant for municipal wastewater treatment.
- Use of physicochemical treatment process instead of biological treatment system.
- High performance of Moringa Oleifera seeds in municipal wastewater treatment.
1. INTRODUCTION

Water and wastewater treatment work for two key purposes, conserving fresh water resources and protecting the environment. In several regions in the world, municipal wastewater is considered as a complementary water source for numerous uses as landscaping, agriculture irrigation, and even for some industrial activities (Bukhari 2008). Improving current treatment technologies and come up with advanced ideas to treat municipal wastewater is of a great importance to keep and preserve the dependence of the formerly mentioned utilizations (Bukhari 2008; Chan et al. 2009). Most municipal wastewater plants are using biological treatment methods (Chan et al. 2009). However, using biological treatment has got a big disadvantage is that highly restricted control is required over the entire process. For example, in biological treatment process, it is required to create an optimally favorable environment, the system requires maintenance and management of microorganisms and/or physico-chemical pretreatment, slow process, poor decolorization, and possible sludge bulking and foaming (Crini & Lichtfouse 2019).

Coagulation/flocculation treatment offers the best alternative to treat municipal and industrial wastewater. The coagulant in question is usually being divalent or trivalent metallic salts or polymers that have little solubility in the pH range utilized (Wang et al. 2006; Suopajärvi et al. 2013). Metal salts hydrolyze quickly in wastewater to generate cationic species, that are adsorbed by negatively charged pollutant particles, causing instantaneous surface charge reduction (Li et al. 2006; Bouchareb et al. 2020). In the last decades, a large list of polymers has been used in the coagulation/flocculation process for coagulant concentrations optimization, reduction of sludge volume and ionic load of wastewater, and for cost purposes (Gao et al. 2002; Gao et al. 2003; Debora Peruço Theodoro et al. 2013). However, many natural-based coagulants are environmentally friendly and biodegradable,
with good coagulating ability (Muthuraman & Sasikala 2014; Kumar et al. 2017; Al-Saati et al. 2019; Ang & Mohammad 2020). In recent years, several studies were conducted on different plant materials that can be used as sources of natural coagulants (Abidin et al. 2013; Bouchareb et al. 2020). For instance, natural coagulants from acorn (Benalia et al. 2018), starch (Esparza-Soto et al. 2019), cactus (García-Morales et al. 2018), okra (Balaji et al. 2018), Gossypium herbaceum (Arulmathi et al. 2019), and Moringa Oleifera (Arulmathi et al. 2019; Bouchareb et al. 2020) have been investigated. The plant that has received the greatest interest is Moringa Oleifera seeds (Abidin et al. 2013; Bouchareb et al. 2020). However, until now, the acceptance and the extensive application of biocoagulants in water treatment industry is still low (Ang & Mohammad 2020). It is necessary to show the potential of using biocoagulants by illustrating the current development and the energies for improving natural coagulants, comprising showing the compatibility of biocoagulants with other treatment technologies. The improvement of extraction and purification methods for high purity of natural coagulants; and enhancing their treatment capability is a mandatory.

Moringa Oleifera is a multipurpose tree that grows widely throughout tropical regions. Moringa seeds contain up to 40% by weight of quality edible oil and yield proteins capable of acting as effective coagulants in water and wastewater treatment (Bhuptawat et al. 2007). Moringa Oleifera seeds powder has proved to be effective in eliminating suspended solids, removing chemical oxygen demand and generate lower sludge volumes compared to alum (Bhuptawat et al. 2007; Bouchareb et al. 2020). However, improving Moringa Oleifera applications have been, and continue to be, fruitful areas for researchers working on new methods of extraction and determining the coagulant agents containing in the seeds.

The aim of this study is to improve the extraction process of the coagulant agent from Moringa Oleifera seeds. As it is believed that the coagulant agent in Moringa Oleifera seeds is soluble protein (Okuda et al. 1999; Abidin et al. 2013), it is important to investigate coagulant agent extraction using different solvents as distillate water and NaCl and under different operating conditions.

2. MATERIALS AND METHODS

2.1. Water sampling

Raw municipal wastewater was collected from the inflow of Oued Athmania Sewage Treatment Plant. The main characteristics are illustrated in Table 1. Water samples were kept in the cold room at 4 °C for the subsequent experiments.

2.2. Moringa Oleifera seeds powder (MOSP) preparation

Moringa Oleifera seeds were acquired from a local supplier in Algeria. Seeds were carefully chosen and dehusked manually. The unshelled seeds were put in the oven for about 5 h at 35 ± 1 °C to remove humidity. Dry kernels were ground to fine powder with a laboratory mortal. Then, powder was sieved using a laboratory test sieve.

| Table 1 | Municipal wastewater characterization |
|---------|-------------------------------|
| Parameter | Measured Value | Unit |
| COD      | 201              | mg/L |
| BOD₅     | 150              | mg/L |
| Turbidity| 118.0            | NTU  |
| pH       | 7.4              | –    |
| Alkalinity (TA) | 260       | mg CaCO₃/L |
| Total Alkalinity (TAC) | 360       | mg CaCO₃/L |
| Total solid (TS) | 1.35       | g/L |
| Total volatile solid (TVS) | 1.41       | g/L |
| TVS/TS  | 95.74           | %    |
| Conductivity | 2,740.0     | μS/cm|
| Salinity | 1.3            | g/L  |
| TP      | 5.8             | mg/L |
| TN      | 48.0            | mg/L |

Uncorrected Proof
(Retsch ISO 3310-1 Body/Mesh S-Steel, 250 μm). The obtained uniform powder was used for the following experiments in this research study.

2.3. Moringa Oleifera seeds powder coagulant preparation

The overall process of this study is illustrated in Figure 1.

2.3.1. Oil extraction

The Moringa Oleifera seeds powder (MOSP) that was prepared previously was added to ethanol 95% for oil extraction at the ratio of 100 mL of ethanol for 10 g of MOSP as described in a previous study reported by Katata-Seru (Katata-Seru et al. 2018). The solution was mixed using a stirrer for 10 min. The solution was filtrated using a standard filter. Then, the retained powder was dried at room temperature for 24 hours and it is referenced as (MOSP-EO).

2.3.2. Conventional blending extraction method

The MOSP-EO prepared previously was mixed with distillate water using magnetic stirrer for 80 min. The optimum mixing duration was established in a previous study by the authors herein after protein dosage determination in function of mixing time. The mixture was filtrated and the clear solution is preserved in 4 °C cold room for next usages. The obtained coagulant is referenced as MOP-01.

Another solvent, sodium chloride NaCl, was used for coagulant extraction at different concentrations: 0.5, 1.0 and 1.5 M. The solvent concentration selection was based on earliest laboratory results. The suspension was filtered through standard filter paper and the filtrate solution was used in the subsequent jar test experiments. To prevent any ageing effects, such as changes in pH, viscosity and coagulation activity due to microbial
decomposition of organic compounds during storage, the prepared coagulants were kept at 4 °C of temperature. The corresponding coagulants to 0.5, 1.0 and 1.5 M of NaCl concentrations are noted as MOP-02, MOP-03 and MOP-04.

2.3.3. Ultrasound-assisted extraction method
After determining the optimum concentration among the prepared four (04) coagulants: MOP-01, MOP-02, MOP-03 and MOP-04 for municipal wastewater treatment and after determining the best solvent and its concentration, powder (MOSP-EO) and solvent mixture were immersed in an ultrasound cleaning bath (Model: VWR xtra TT Heavy-Duty Ultrasonic Cleaners) for 10, 20 and 30 min. This experiment was conducted at room temperature ±20 °C using one default ultrasound wavelength of 42 kHz. The resulting suspension was filtered through a standard filter paper. Again the filtrate was used in a subsequent jar test experiments. The corresponding coagulants to 10, 20 and 30 min of ultrasound treatment are noted as MOP-05, MOP-06 and MOP-07.

2.4. Moringa Oleifera seeds powder characterization
In order to evaluate the presence of crystalline phases existing in the examined powder, an X-ray diffraction patterns were obtained using a diffractometer (Malvern Panalytical Empyrean). Samples were exposed to X-ray with 2θ angle between 3° and 70°.

The functional groups containing in the Moringa Oleifera seeds powder were characterized using a Fourier transform Infrared (FT-IR) spectrometer (Shimadzu, IRAffinity-1S, Tokyo, Japan). The spectral range varied from 4,000 to 500 cm⁻¹, with twenty-eight scans and a resolution of 4 cm⁻¹.

2.5. Coagulation/flocculation treatment
All coagulation/flocculation experiments were implemented using JP SELECTA six-agitators jar tester, with digital mixing rods rotation control. Every coagulant dose is added to a sample of 500 mL wastewater then agitated at rapid mixing speed of 150 rpm for 3 min then by slow mixing speed at 50 rpm for 24 min. Then, beakers were left for 30 min for sedimentation (process shown in Figure 2). Next, two parameters were selected to investigate the efficiency of the coagulation/flocculation process. As municipal wastewater presents high bacteria levels, pathogens, or particles that can shelter harmful organisms, turbidity and BOD₅ were the two considered parameters.

3. RESULTS AND DISCUSSION
3.1. Moringa Oleifera seeds powder characterization
Figure 3 shows X-ray diffraction patterns of MOSP and MOSP-EO. Since Moringa seeds contain high amount of oils and proteins, around 69% by weight (Araújo et al. 2010; Katata-Seru et al. 2018), the X-ray pattern shows a poorly resolved peak that indicates a predominance of amorphous material. Hence, a heterogeneous and complex matrix with numerous substances, including proteins, lipids, and to a smaller amount of carbohydrates and ash was observed. It is possible to separate two broad peaks at 2θ equal to 21° and 24° for MOSP XRD pattern (Figure 3(a)). The presence of these peaks is probably associated with diffraction of the constituent oils surrounding the other components that have more amorphous nature. Theses peaks disappeared in MOSP-EO as shown in Figure 3(b).

The FT-IR technique was used to determine the main functional groups present in Moringa Oleifera seeds. This technique allows some important functional groups, which are capable of coagulating wastewater pollutants, to be identified. Figure 4 reports the corresponding FTIR spectra of Moringa Oleifera seeds (MOSP) and Moringa

![Figure 2](http://iwaponline.com/wst/article-pdf/doi/10.2166/wst.2021.234/899792/wst2021234.pdf?by guest)
Oleifera seeds extracted oil (MOSP-EO). The spectra of both substances showed identical bands with different broad bands intensity revealing oil extracted from Moringa Oleifera seeds. The figure displays a rich set of broad IR absorption bands located in the intervals: 3,039–3,645 cm\(^{-1}\), 2,792–3,024 cm\(^{-1}\), 1,906–1,770 cm\(^{-1}\) and 1,292–1,472 cm\(^{-1}\).

**Figure 3** | XRD of (a) Moringa Oleifera seeds powder (MOSP) and (b) Moringa Oleifera seeds powder extracted oil (MOSP-EO).

**Figure 4** | FTIR of Moringa Oleifera seeds powder (MOSP) and Moringa Oleifera seeds powder extracted oil (MOSP-EO).
401–763 cm\(^{-1}\) spectral regions. A broad band centered at 3,290 cm\(^{-1}\) assigned to O–H stretching. This functional group appears mainly in the fatty acid and protein structures existent in Moringa Oleifera seeds. Due to the high content of protein present in Moringa Oleifera seeds, there is also an involvement in this region from the N–H stretching of amide groups. The peaks at 2,924 cm\(^{-1}\) and 2,854 cm\(^{-1}\) are allocated to symmetrical and asymmetrical stretching of the C–H of CH\(_2\) groups present in fatty acids. In the region between 1,485 and 1,770 cm\(^{-1}\), there are three intense bands assigned to C=O bond stretching. The carbonyl group is present in the fatty acid and protein structures. In this case, the spectra show two bands at 1,651 and 1,743 cm\(^{-1}\) associated with fatty acids and a band at 1,543 cm\(^{-1}\) associated with the amide group in the protein. The presence of a peak at 1,481 cm\(^{-1}\) can be assigned to C–N stretching and/or N–H deformation. The presence of this band confirms the protein structure in the Moringa seeds. Stretching vibrations located at around 401–763 cm\(^{-1}\) represent C–H, C=O and N–H.

3.2. Alum and MOP-01 coagulants dose effect

In order to make a comparison between a chemical coagulant and a natural one, aluminum sulfate (Alum) coagulant was used in addition to the processed natural coagulant under different extraction methods.

Figure 5(a) shows the effect of alum concentration on the turbidity removal of municipal wastewater. The investigation was conducted at 80, 110, 140, 170 and 200 mg/L. Turbidity removal efficiency increases as coagulant dose increases until optimum concentration of 140 mg/L, where the maximum removal efficiency was 99.05%.

Moringa seeds powder (MOP-01) was largely used in previous research studies (Okuda et al. 1999; Arulmathi et al. 2019; Bouchareb et al. 2020) for different wastewater treatment. In this study, 170 mg/L of MOP-01 removed up to 88.98% of the initial municipal wastewater turbidity as illustrated in Figure 5(b).

Figure 6 illustrates both used coagulants, alum and Moringa Oleifera extracted in distillate water, at optimum concentrations 140 and 170 mg/L subsequently, on BOD\(_5\) and final treated water pH. Alum has highly removed biochemical oxygen demand and decreased it from 150 to 15 mg/L. However, the final obtained pH is relatively low, which requires additional addition of alkali to have a suitable pH between 6.5 and 7.5. In the other hand, though MOP-1 did not remove BOD\(_5\) efficiently, the obtained water had an appropriate pH of 6.9.

3.2. Effect of using different concentrations of NaCl for extracting the coagulant agent

In order to study the improvement of extraction process and therefore the quantity of coagulant extracted from seeds powder, 1 M of sodium chloride molar concentration was used.

Figure 7 displays the effect of Moringa Oleifera concentration extracted in 1 M NaCl solution on turbidity removal of municipal wastewater. The same previous investigated concentrations were used in order to check how the extraction method can improve turbidity removal compared to results obtained using MOP-01. From
Figure 7, it is found that the optimum concentration for the best turbidity removal of municipal wastewater decreased from 170 to 140 mg/L after extraction in 1 M NaCl solution. This means that the quantity of coagulant agent which was extracted and dissolved in sodium chloride solution was more than the one extracted and dissolved in distillate water. This phenomenon is known as salting-in effect (Abidin et al. 2013). As the coagulant agent is protein (Bhuptawat et al. 2007), at salt presence the solubility of the coagulant agent and hence its concentration in the solution increase. More coagulant agent means more coagulation activity may occur, consequently less coagulant concentration is necessary to obtain better pollutant removal efficiency.

Figure 8 demonstrates the effect of using different NaCl concentrations (0.5, 1.0 and 1.5 M) on the turbidity, BOD$_5$ and water salinity at 140 mg/L of MOP as determined previously.

Turbidity removal was found to increase as the salt concentration increases till 1 M, then decreased back at 1.5 M. When salt concentration increased, both turbidity and BOD$_5$ removal improved up to 1 M of sodium chloride concentration. Above 1 M, the studied pollutant concentrations increased back. The was attributed to the salting-out effect where the solubility of proteins decreases with salt concentration above the optimum (Abidin et al. 2013). For MOP-03 at 140 mg/L of concentration dissolved in 1.0 M of NaCl, turbidity decreased from 118 NTU to 3.37 NTU with a removal efficiency of 97.14%. A similar trend was observed for BOD$_5$ removal,
where it was decreased from 150 to 5 mg/L (96.67% of removal efficiency). Therefore, the optimum concentration to improve the coagulant agent solubility from Moringa Oleifera seeds was 1.0 M and this conducted to an improvement of coagulation activity. For the three investigated NaCl concentrations, the final water salinity is in the range recommended by WHO (WOH 2006), which is less than 3.0 g/L until crops are not affected.

3.3. Effects of using ultrasound-assisted extraction method

Figure 9 shows the effect of using combined salt extraction in 1.0 M NaCl concentration and ultrasound for extracting the active coagulant agent from Moringa Oleifera seeds at different exposing times: 10, 20 and 30 min. From Figure 9, the longer the mixture was subjected to the combination treatment, the more turbidity removal obtained from municipal wastewater. However, the obtained results were not as performant as without ultrasound treatment. Therefore, it was found that ultrasound treatment did not increase Moringa Oleifera coagulant agent solubility, nevertheless, coagulation activity decreased as illustrated in Figure 10. In contrary, ultrasound treatment enhanced coagulant agent extraction of other natural coagulants (Opuntia ficus-indica or barbary fig, and Jatropha curcas seeds) as reported in (Abidin et al. 2013; Adjeroud-Abdellatif et al. 2020). This effect can be explained by the destruction coagulant agent structure and eventually its ability to act in the coagulation process caused by ultrasound waves. Prolonged exposure to ultrasound treatment helped to generate a force for cell disturbance to extraction method and created greater amount for the coagulant agent to emerge

Figure 8 | Effect of NaCl solution concentration on municipal wastewater BOD₅, turbidity and treated water salinity using 140 mg/L of MOP concentration.

Figure 9 | Effect of MOP concentration extracted in 1M NaCl solution and ultrasound treatment duration on municipal wastewater turbidity.
from Moringa Oleifera seeds and dissolve in the solution. However, this technique of extraction did not reach sodium chloride as a unique solvent extraction performance efficiency for Moringa Oleifera seeds powder.

4. CONCLUSION

The main objective of this study was to enhance the active coagulant agent extraction from Moringa Oleifera seeds powder. The coagulant agent extracted using sodium chloride of 1.0 M concentration found to be more efficient and hence enhanced turbidity and biochemical oxygen demand removal performance compared to extraction using other NaCl doses and distillate water as solvent. The extraction using 1.0 M NaCl solution has given a turbidity and BOD$_5$ removals of more than 97 and 96% subsequently for just 140 mg/L of MOSP-E0 concentration. The application of ultrasound waves did not give an additional extraction of coagulant agent for Moringa Oleifera seeds powder whatever the exposure duration.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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