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Combination of alum and extracted *Moringa oleifera* bioactive molecules powder for municipal wastewater treatment

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ABSTRACT

Most of wastewater treatment stations use aluminum sulfate ($Al_2(SO_4)_3$, $18H_2O$) and ferric sulfate ($Fe_2(SO_4)_3$) despite their relatively high cost. High turbidity of wastewater constitutes also a limit for a good treatment and increases the cost of the treatment. *Moringa oleifera* (MO) seeds are well known to have the ability to reduce turbidity and Chemical Oxygen Demand (COD) of wastewater. Moreover, most of the studies focus on using seeds directly for that purpose increasing consequently organic matter content. One of the major objectives of this study is to proposes a contribution for municipal wastewater treatment using crude *MO* extracted protein powder (MOEP) for the first time for industrial applications. NaCl extraction of *MO* defatted seeds bioactive molecule is first performed with a yield of 34% and the protein precipitated with ammonium sulfate 60% (m/v) and dried for coagulation as classical protein precipitation. Parameters affecting the effectiveness of *MO* bioactive protein in wastewater treatment permits to operate at ambient temperature and a range of pH from 6 to 9. The proposed process uses alum and *MO* bioactive protein molecules powder as coagulant and as additive for classical municipal wastewater treatment. Within a ratio of 50:50 (w/w), more than 90% of the turbidity and 75% of COD. The results obtained with kaolin suspensions showed that MOEP is more efficient for higher turbid wastewater.

Keywords: *Moringa oleifera*, coagulant, aluminum sulfate, precipitation.

INTRODUCTION

New regulations governing the quality of the discharge water make it necessary for industrial and domestic wastewater to reuse or damp cleaner water in lot of countries such as Senegal.

Aluminum and ferric salts are the most commonly used coagulants in water treatment because of their high efficiency for the removal of colloids. Cost and environmental side effects of these compounds have increased interest in the use of organic coagulants derived from plant material (Chun-Yang, 2010; Fatombi et al., 2013) such as *Moringa oleifera* (MO) seeds (Jahn, 1988; Ghebremichael et al., 2005). However, most of the studies are focused on the use the seeds directly for turbidity removal (Bhatia et al., 2007), especially for drinking water (Ndabigengesere, Subba, 1998; Pritchard et al.; 2010; Natumanya, Oko-Okumu, 2015) Most of the studies concern the use of seeds directly for turbidity and COD removal, which increases consequently organic matter content (Poumaye et al., 2012; Tiea et al., 2015).

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Pritchard et al. (2010) compared the use of aluminum sulfate and powder obtained from MO seeds separately on purification of drinking water and showed that MO removed 84% turbidity and 88% *E. coli*, whereas alum removed greater than 99% turbidity and *E. coli*. Few studies focus on using a combination of MO and other coagulants such as arabic gum (Alang et al., 2011).

De Paula et al. (2014), by using a ratio of 20:80 (w/w) of MO seed powder and aluminum sulfate for concrete plant wastewater treatment for initial turbidity of about 120 NTU and pH 12.5, 90% of the turbidity was removed. The amount of aluminum sulfate was calculated in order to have a final pH ranging from 6 to 9 for water discharge or reuse in washing vehicles for example.

Bhuptawat and Folkard (2007), with aqueous extraction of the bioactive component of *Moringa oleifera*, treated a wastewater with initial turbidity of 13.5 NTU and 150 mg/L of COD and pH=7.2. The results indicated that by using the extracted bioactive component solution overall COD removal of 50% was achieved at both 50 and 100 mg/L. They showed that when 50 and 100 mg/L seed doses were applied in combination with 10 mg/L of alum for, COD removal increased to 58 and 64%, respectively and the majority of COD removal occurred during the filtration process.

Okuda et al. (1999) developed a method for the extraction of active coagulation component from *Moringa oleifera* seeds and compared it with the ordinary water extraction method. The use of 1 mol/L solution of sodium chloride and other salts were performed for extraction of the active coagulation component. The results showed better coagulation activity with dosages 7.4 times lower than that using ordinary water extraction method by distilled water for the removal of kaolinite turbidity.

Researches over the last decades focused essentially on testing MO on raw water with low turbidity by using its defatted oil-free seeds powder or by using aqueous extracted MO bioactive component solutions. These methods are not useful in larger scale such as in classical wastewater treatment stations. In order to address this question, the objective of this work is to study the extraction of the MO bioactive molecule as it has been done since now and then examine the possibility of separating it from the raw extraction solution and precipitate it as classical protein and dry it for the first time. This work concerns municipal wastewater treatment with relatively high turbidity by *Moringa oleifera* extracted and precipitated bioactive seeds component and dried as coagulant and as adjuvant with alum for municipal wastewater and in a synthetic kaolin solutions treatment characterization purpose.

MATERIAL AND METHODS

Material

All the chemicals used were of analytical grade. The pH and the conductivity of the solution were monitored using a pH-meter *210-HANNA INSTRUMENTS and* a conductimeter *EC214-HANNA INSTRUMENTS.* Turbidity was monitored by using a turbid-meter *HYDRCURE HE9* and centrifugation was performed by using a centrifuge *UNIVERSAL 16A*.

Wastewater sample collection

Wastewater sample were collected from the National Office of wastewater Treatment of Senegal, West Africa, at Dakar in the primary settling before the biological treatment and refrigerated at 4 °C before the analysis. The National Office of Wastewater Treatment is in charge of collecting and treating domestic wastewater and pluvial water of Dakar where there are more than 5 million people. Typical wastewater characteristics are given in Table 1.

Coagulant used

Moringa oleifera used as coagulant comes from Senegal. The matured dry pods of *Moringa oleifera* were collected directly on the trees and shelled and the seeds stored until used. The seeds must be matured and dry in order to make sure that there is bioactive coagulant in the seeds (Ndabigengesere, 1995).

Preparation of *Moringa oleifera*

Dry seeds are shelled and the kernel ground in an electric grinder to achieve first, oil extraction and dissolution of active ingredients in a second step. Shelled seeds and kernel grinded are presented in Figure 1.

Oil extraction

Fourty grams of *Moringa oleifera* grinded kernel were fed to a lab-scale Soxhlet extractor fitted with 500 mL round-bottom flask and a condenser. Extraction was performed for 4 hours with 500 mL of hexane as solvent. A total reflux during four hours permits to extract most of the oil. After this time, the hexane is condensed and separated with the oil.

The extracted oil yield was expressed as percentage, which is defined as weight of oil extracted over weight of the sample taken.

Coagulant bioactive component extraction and precipitation

Ten grams of defatted seeds were mixed with 100 mL of sodium chloride (1M) using a magnetic stirrer for two hours. Then, the supernatant was separated by centrifugation (6000 rpm, 30 min) and filtered in order to make sure there are no seeds residues.

The proteins precipitation was performed by using ammonium sulfate 60% (m/v) for the protein coagulation and precipitation (Andre et al., 2009; Jeso, 1968; Dixon, 1953). The proteins are separated with the supernatant by filtration and dried at room temperature to form fine powder (Figure 2).

Coagulation with varying pH

In order not to increase the cost of wastewater treatment, it is important to determine the optimal pH with alum and *Moringa oleifera* extracted protein (MOEP). Initial wastewater pH was varied from 5 to 10 by using NaOH (1M) and concentrated sulfuric acid (18M) solutions for pH adjustments.

Coagulation with varying temperature

The efficiencies of MOEP and alum coagulation against a variety of temperatures were tested. Laboratory refrigerators were used to bring samples to the required temperature overnight. A range of temperatures varying from 20 to 40 °C with the optimized doses of MOEP and alum concentrations were tested.

Use of MOEP as additive with alum for municipal wastewater

The optimum dose of alum was used as reference value and the percentages of MOEP were varied from 0 to 100% by measuring residual turbidity and residual COD in order to determine the maximum amount of MOEP which can be used as additive to reduce the cost of the classical treatment with alum.

Comparison of MOEP and alum as coagulant in kaolin suspensions

Experiments were carried out in samples of trouble water by adding kaolin particles in potable water. Kaolin was purchased in local market, grounded in fine particles. 10 g of the powder are then dissolved in a flask of 1 L and mixed during 24 h with a magnetic stirrer. After that, the suspension is settled during 24h again in order to complete kaolin hydration (Olsen, 1987). This operation permits to settle non colloidal particles. The colloidal solution is then used for turbidity removal tests.

RESULTS

Oil yield extraction

Results obtained with *Soxhlet* oil extraction with hexane as solvent depended on time of extraction. Maximum oil yield extraction was 34.73% after 4 hours of extraction.

Optimal contact time

In order to determine the optimal contact time for turbidity removal, based on literature, a concentration of 200 mg/L of MOEP was chosen for the tests. Addition of the powder is followed by a rapid mixing of the solution (500 rpm) during 2 min, followed by a smaller mixing rate (100 rpm) for 10 min.

The results (Figure 3) indicated that settling time of about 2 h is need for the solution for 95% of turbidity removal.

Optimal coagulant concentrations of MOEP and alum

In order to determine the optimal coagulant concentration, reduction of turbidity versus coagulation concentration for both MOEP and alum are plotted as shown in Figure 4 for initial turbidity of 145 NTU.

COD removal of MOEP and alum

Figure 5 shows the influence of COD removal with MOEP and alum concentrations. The results indicate that as concentrations of both MOEP and alum increase, reduction in COD increases. The maximum COD reduction occurs at concentrations of 200 mg/L and 300 mg/L for MOEP and alum respectively as for the turbidity reduction. When the maximum are reached, increase in coagulant concentrations causes decrease in COD reduction.

Optimal pH treatment

The data show that maximum turbidity removal occurs at pH=6.2 for alum and pH=9 for MOEP. However, it can be seen that for MOEP, over 50% turbidity removal was achieved with pH ranging from 6 to 9 (Figure 6).

Final conductivity of treated wastewater with MO coagulant concentration

The conductivity of treated municipal wastewater with MOEP does not increase the conductivity as shown in Figure 7. In contrast, the treatment with alum decreases the conductivity.

Turbidity removal depending on temperature

The optimal temperature for both alum and MOEP are comprised between 25 and 30 °C as shown in Figure 8.

Use of MOEP as additive with alum for municipal wastewater

The optimal dose of alum (200 mg/L) is used as reference and MOEP weight concentration adjusted to match that concentration. The results indicate that 50% of MOEP can be used as additive for turbidity removal. COD removal decreases when MOEP concentration is increased due to increase of organic matter (Figure 9).

Use of MOEP as coagulant in kaolin suspensions

Three solutions of 195, 105 and 55 NTU were prepared by diluting kaolin initial solution. The solutions are stirred frequently in order to avoid settling of colloids and maintain initial concentration. Coagulation with MOEP is first performed and then with alum by varying coagulant concentrations. Results are presented in Figure 10.

Table 1: Indicative wastewater characteristics at 20 °C.

	pH	Conductivity (mS/cm)	Turbidity (NTU)	COD (mg/L)
Wastewater sample	8,2	4,63	145	528

(a) (b)

Figure 1 : *Moringa oleifera* shelled seeds (a) and ground kernel (b).

Figure 2: *Moringa oleifera* coagulant protein powder.

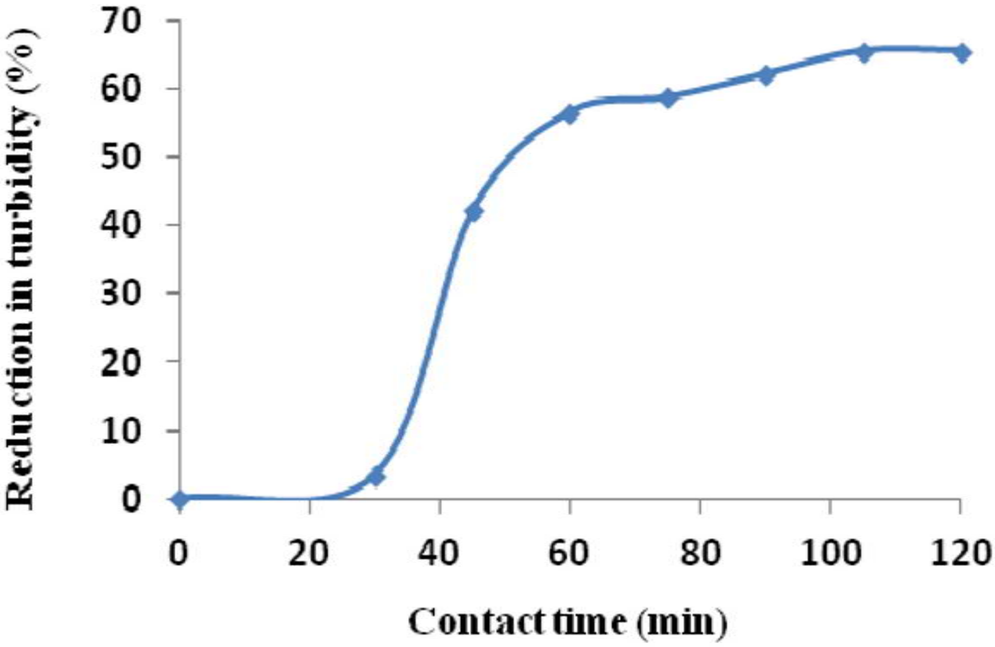


Figure 3: Turbidity removal depending on contact time.

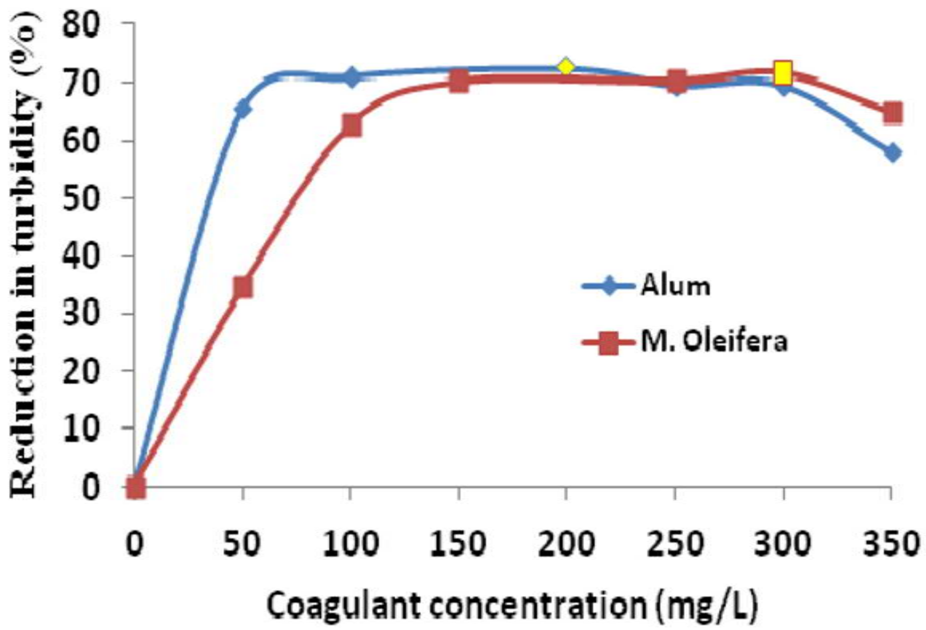


Figure 4: Turbidity removal depending on alum and MOEP concentration.

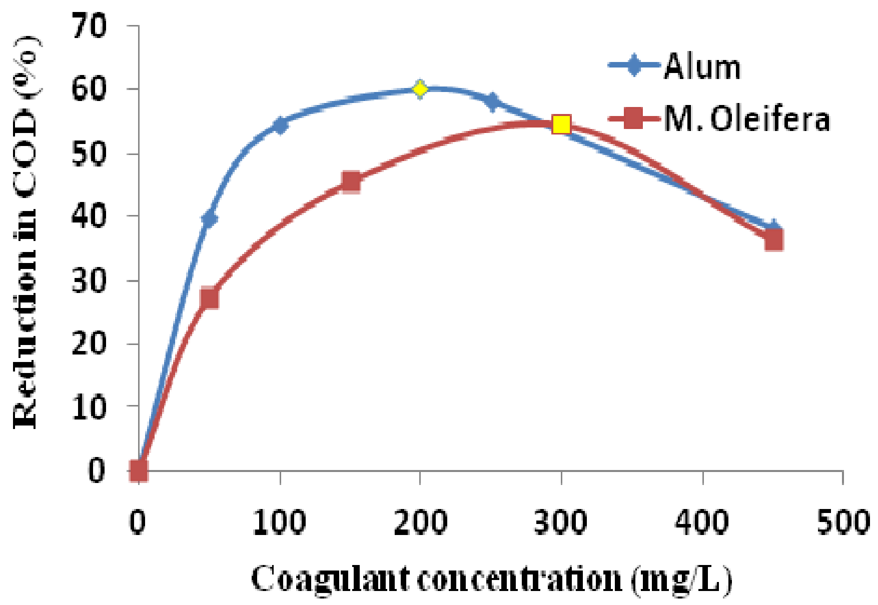


Figure 5: Reduction in COD depending coagulant concentration for alum and MOEP.

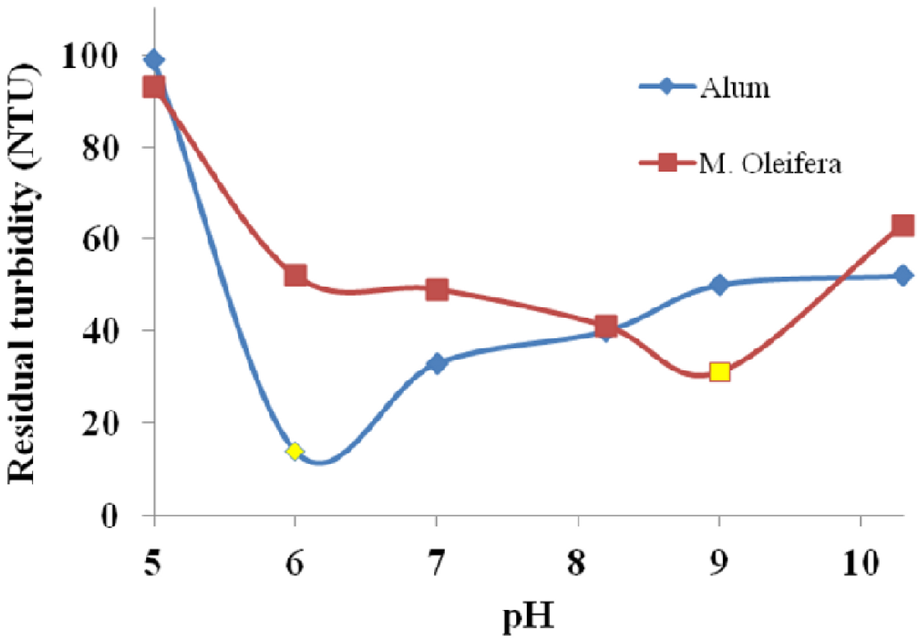


Figure 6 : Turbidity variation with pH.

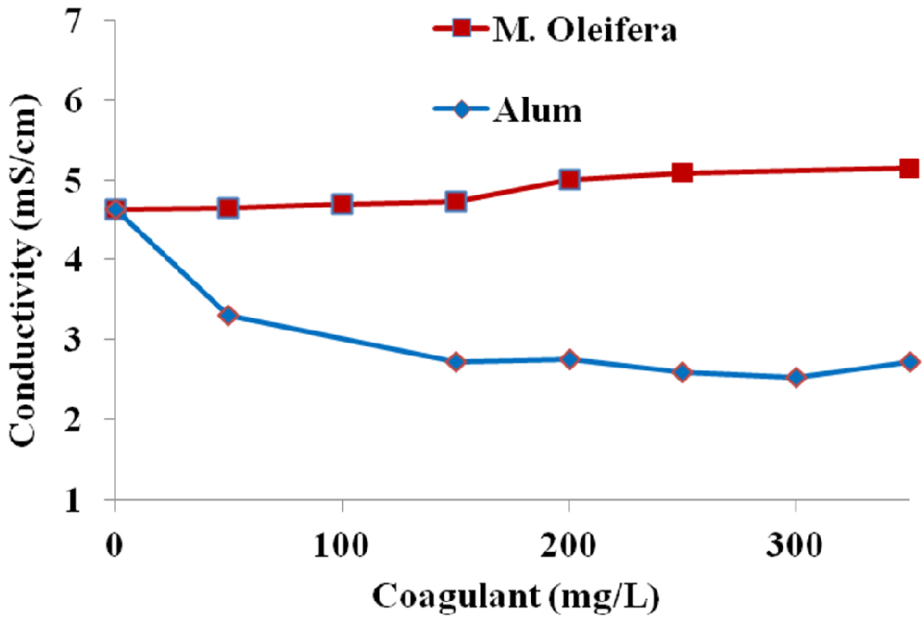


Figure 7: Residual conductivity depending on coagulant concentrations.

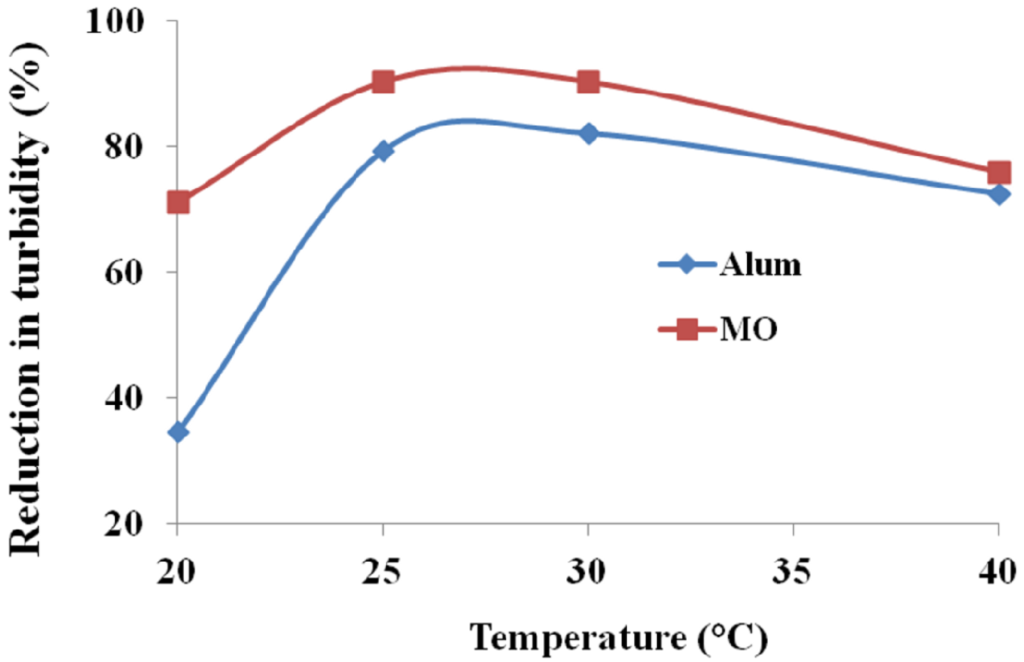


Figure 8: Optimal temperature for turbidity removal.

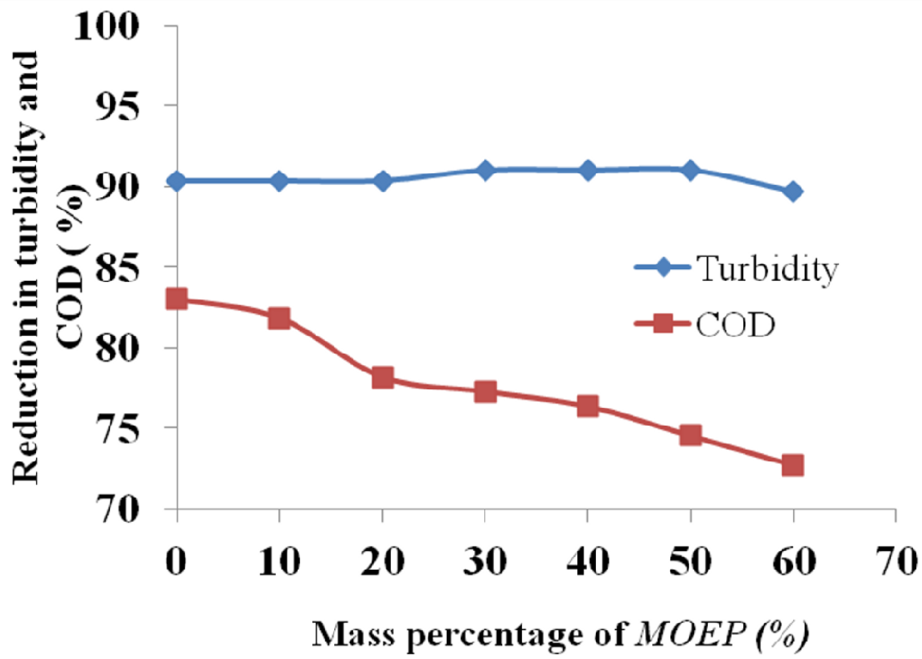
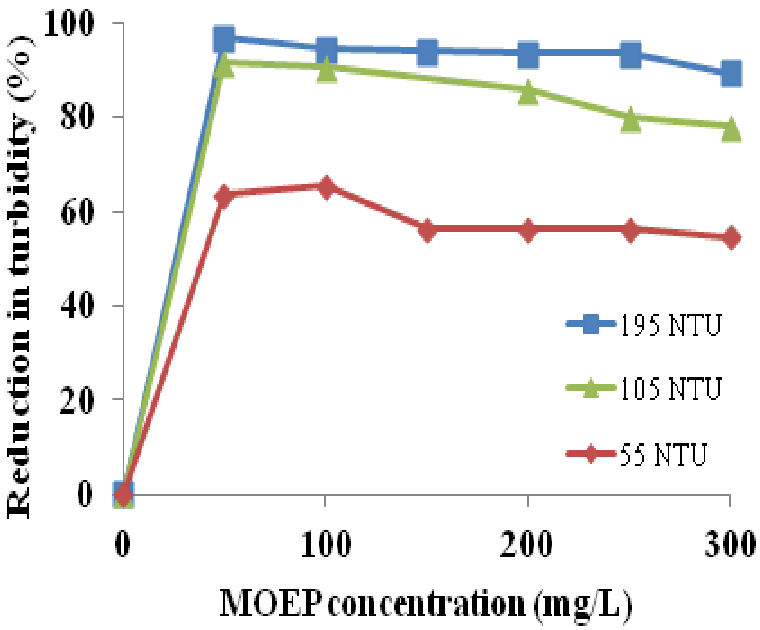
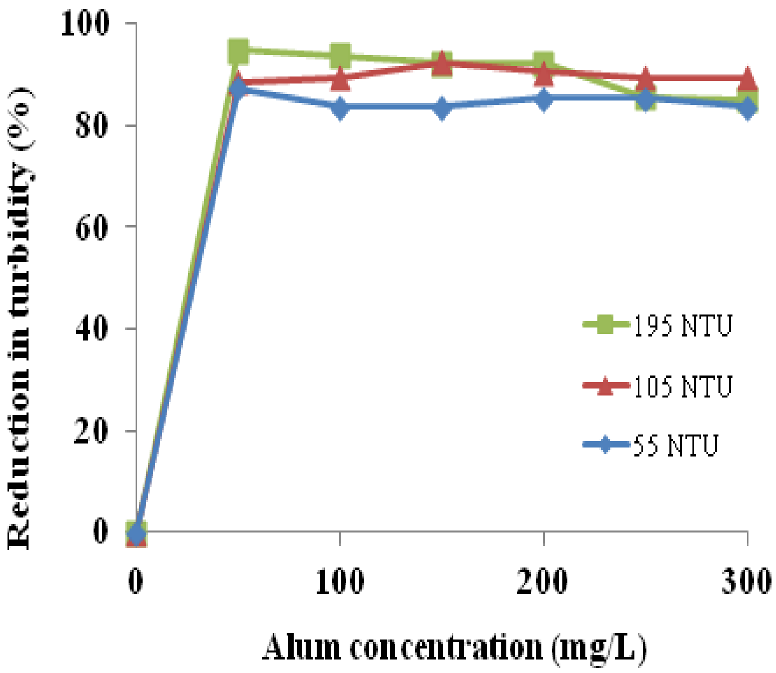


Figure 9: Turbidity and COD removal depending in mass percentage of MOEP as additive.



(a)



(b)

Figure 10 : Reduction in turbidity by using MOEP (a) and alum (b) for kaolin initial turbidity of 195NTU, 105NTU and 55NTU.

DISCUSSION

The results of the oil extraction are in accordance with literature. According to the previous studies *Moringa oleifera* seeds have 30-35% (w/w) of oil content (Premi and Sharma, 2013).

The two graphs in Figure 4 for turbidity removal show a similar trend. When the coagulant concentration is increased, reduction of turbidity increases and at about 50 mg/L of alum, the maximum turbidity reduction is reached while for MOEP, the maximum reduction in turbidity is reached for a concentration of 150 mg/L.

When the coagulant concentration is increased at the maximum reduction in turbidity (71%), there is no influence until the concentration equals 200 mg/L and 300 mg/L for alum and MOEP respectively. At these concentrations, reduction in turbidity decreases.

In general, for COD removal, when the coagulant concentration is increased in order to reduce the turbidity, organic matters negatively charged are also removed by a sweep coagulation mechanism (Packham, 1965; Fedala, 2015). However, when all the charges are neutralized, increase of coagulant concentrations may change the charge of the colloids; releasing again adsorbed organic matter, which reduces COD reduction. The same trend is observed for both alum and MOEP.

Moreover, the results for pH optimization for MOEP are similar to those obtained with MO grinded raw seeds coagulant for drinking water treatment (MPr10).

The temperature influences on turbidity removal show that in developing countries such as Senegal, theses temperatures are average ambient temperature. At relatively low temperature (<25 °C), reduction in turbidity decreases due to increase in viscosity which decreases the settling time. This phenomenon increase generation of micro-flocks. The increase decrease in turbidity removal when the temperature is higher than 40 °C may be due to MOEP degradation. These results are similar to those obtained with the crude seeds in the literature (Ndabigengesere and Narasiah, 1996). The same trend is observed with alum. The phenomena can be explained with the reduction of viscosity and density.

The Use of MOEP as coagulant in kaolin suspensions show that these results are similar to those for natural water (Jahn and Dirar, 1979) treated with raw *Moringa oleifera* seeds. However, treated wastewater with alum for smaller or higher turbid solutions has shown no significant differences.

Results obtained with MOEP show that using *Moringa oleifera* as coagulant for turbidity removal is more efficient for relatively higher turbid wastewater (>100NTU).

Conclusion

The present study clearly shows that classical protein precipitation can be performed for *Moringa oleifera* protein precipitation. Optimum operating parameters such as pH and temperature for municipal wastewater treatment have been determined in the present study by using *Moringa oleifera* extracted protein with ammonium sulfate 60% (m/v). The results have shown almost the same effect than alum for turbidity removal, especially for higher turbid water. In order to reduce the cost of wastewater treatment, *Moringa oleifera* extracted protein can be used as additive with alum with a maximum mass concentration of 50%. It seems that the active bioactive molecule of *Moringa oleifera* is a protein. More studies are needed for industrial production of *Moringa oleifera* protein powder.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

CK and NA designed and supervised the study. AB was the principal investigator; she conducted all the experiments in this study. SAMM and MKM contributed in conducting the experiments. CGM/D is the head of the Laboratory.

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