

Potential of *Moringa oleifera* Seeds as a Natural Coagulant for Turbidity and LAS Removal from Detergent Wastewater

Aulia Rhamdani Arfan^{*a}, Rahma Indah^a, Utami Irawati^a, Nawwal Hikmah^a

^a Chemistry Department, Faculty of Mathematics and Science, Lambung Mangkurat University, Jl. A. Yani Km. 35.8 Kota Banjarbaru, South Kalimantan, 70714

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ABSTRACT

Detergent wastewater contains high levels of surfactants, particularly Linear Alkylbenzene Sulfonate (LAS), which are persistent and harmful to aquatic ecosystems. This study evaluated the potential of *Moringa oleifera* seed (MOS) extract as a natural coagulant for detergent wastewater, compared with alum as a synthetic coagulant. The coagulation process was carried out using the jar test method at varying dosages (100–1500 mg/L) and pH levels (4–9). Turbidity was measured following SNI 06-6989.25-2005, while LAS concentration was analysed using the Methylene Blue Active Substances (MBAS) method. Under pH-optimized conditions, MOS achieved 95.63% turbidity removal and 71.31% LAS removal at pH 4 (1500 mg/L), whereas alum performed best at pH 6–7 (200 mg/L), reaching 52.79% turbidity removal (pH 6) and 38.79% LAS removal (pH 7). Validation on stored wastewater (1–3 nights) further indicated that MOS provided more consistent reductions in both turbidity and LAS than alum. An in-silico docking study also confirmed the molecular interaction between LAS and MOS proteins (binding energy –4.8 kcal/mol), supporting the observed experimental results. Overall, MOS demonstrates strong potential as a sustainable, effective, and low-cost alternative to alum for detergent wastewater treatment.

*e-mail: aulia.arfan@ulm.ac.id

*Telp: +6289529149959

Introduction

Detergent consumption in Indonesia has steadily increased, reaching about 18 kg per person per year in 2016 [1]. Most detergents contain surfactants, chemical compound that reduce surface tension and removes dirt through their hydrophobic and hydrophilic nature [2], [3]. Among these, Linear Alkylbenzene Sulfonate (LAS) is the most widely used globally [4], [5]. LAS concentrations in wastewater can reach 1000 – 10000 mg/L in domestic wastewater and up to

300 mg/L industrial discharge [5]. Although LAS could degrade naturally in water under aerobic or anaerobic condition, the process depends on specific environmental conditions. It might produce side products like sulphate, sulphite, and sulphide that has toxic properties [5]. Even at very low levels (< 15 µg/L), LAS has been reported to disrupt aquatic organism [6], highlighting the urgent need for effective treatment methods.

Coagulation-flocculation is one of the most common wastewater treatment approaches. This method involves addition of coagulants to

bind suspended particles and organic compounds, which separates the filtrate and precipitate. Synthetic coagulants such as alum, polyaluminum chloride (PAC), and cationic polyacrylamide (c-PAM) reported effectively in coagulation [2]. However, the continued use of synthetic coagulants poses various problems, such as increased sludge volume, residual metals in water, potential toxicity, and high operational costs [7], [8]. These issues have encouraged the search for natural coagulants as safer, sustainable alternatives.

Moringa oleifera seeds (MOS), are particularly promising due to their high content of water-soluble protein that capable of neutralizing colloidal charges content [9], [10]. MOS contain approximately 34% of these proteins and are effective within a neutral to alkaline pH range [11]. MOS has been applied successfully for turbidity reduction in various types of wastewater, including domestic, industrial, and even textile wastewater [12].

However, most previous studies focus on single surfactants such as Sodium Lauryl Ether Sulphate (SLES) [13], while the performance of MOS against LAS, which is more persistent and toxic, remains underexplored. Additionally, the specific interaction mechanism between the positively charged proteins of MOS and anionic surfactant molecules like LAS has rarely been discussed quantitatively or mechanistically.

This study addresses these gaps by evaluating MOS as a natural coagulant for detergent wastewater containing LAS, with alum used as a synthetic comparator. Beyond experimental coagulation tests, an in-silico molecular docking analysis was performed to examine the interaction between LAS and MOS proteins. This combined experimental and computational approach not only validates the coagulation performance but also provides molecular-level insight into the mechanism of LAS removal by MOS.

Materials and Methods

Materials

This study utilized analytical-grade chemicals and laboratory-grade equipment to ensure accuracy and reproducibility. The detergent wastewater sample was collected from household laundry discharge in

Banjarbaru, South Kalimantan, representing typical domestic wastewater. The sample was stored in a closed container and analyzed within 6 hours of collection. In this study, the focus was placed on turbidity and LAS as the main treatment indicators; therefore, other common wastewater parameters such as COD, BOD, and pH were not analyzed. This limitation is acknowledged, and future work should include a more comprehensive characterization to better contextualize the treatment performance.

The locally sourced MOS seed powder were de-shelled, air-dried, and ground with a dry blender. The resulting powder was sieved through a 60-mesh stainless steel screen to obtain a relatively uniform fraction. No defatting step was applied prior to grinding, as the present work focused on evaluating the coagulation performance of the seed powder in its natural state. For comparison, alum from $\text{Al}_2(\text{SO}_4)_3$ (Merck) was used as a synthetic coagulant. Other reagents used in this study included HCl, NaOH, phenolphthalein indicator, H_2SO_4 , methylene blue dye, chloroform, isopropyl alcohol, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, and 1 M NaCl. All reagents used in this study were of analytical grade and purchased from Merck (Germany). Distilled water (aquadest) was used as the solvent for all solution preparations and equipment cleaning.

Methods

Preparation of MOS Extract and Alum Solution

The MOS extract preparation followed a modified method from previous studies (Taiwo et al., 2020; Andrade et al., 2021). Seeds were de-shelled, dried at room temperature, and ground with a dry blender to obtain a fine powder. A 1% (w/v) natural coagulant solution was prepared by dissolving 10 g of seed powder in 1000 mL of 1 M NaCl, stirring with a magnetic stirrer for 15 min, and filtering through Whatman No. 42 filter paper. The resulting filtrate served as the coagulant solution.

For comparison, a 1% (w/v) alum solution was prepared by dissolving 10 g of aluminium sulfate ($\text{Al}_2(\text{SO}_4)_3$) in 1000 mL of distilled water. The mixture was stirred with a magnetic stirrer for 15 min until homogeneous before use in the coagulation process.

Evaluation of Turbidity and LAS Removal Efficiency

Detergent wastewater (1000 mL) was placed into five beakers and treated with MOS extract at 100, 200, 500, 1000, and 1500 mg/L concentrations. Coagulation was performed using a standard jar test apparatus, with rapid mixing at 100 rpm for 1 min followed by slow mixing at 40 rpm for 10 min. The mixtures were then allowed to settle undisturbed for 30 min to facilitate floc formation and sedimentation.

Following the settling phase, the supernatant (clear liquid above the sediment) was carefully withdrawn using a pipette for further analysis. Two main parameters, turbidity and residual concentration of LAS, were evaluated. Turbidity was measured according to the Indonesian National Standard (SNI) 06-6989.25-2005 for water and wastewater analysis. The efficiency of turbidity removal was expressed as Turbidity Removal Efficiency (TRE) and calculated using the following formula [14]:

$$TRE (\%) = \left(\frac{T_o - T_t}{T_o} \right) \times 100\% \quad (1)$$

T_o is the initial turbidity (NTU), and T_t is the turbidity after treatment. To determine the concentration of residual LAS, the Methylene Blue Active Substances (MBAS) method was used, employing a UV-Vis spectrophotometer at a maximum wavelength of 652 nm. LAS removal efficiency was calculated using the following equation:

$$LAS \text{ Removal Efficiency } (\%) = \left(\frac{C_o - C_t}{C_o} \right) \times 100\% \quad (2)$$

where C_o is the initial LAS concentration (mg/L) in the untreated sample (control), and C_t is the residual LAS concentration (mg/L) after treatment.

In addition to removal efficiency, the adsorption capacity (q) of the coagulants for LAS was calculated to evaluate the mass-based effectiveness of LAS removal. The following standard adsorption equation was used [13]:

$$q = \frac{(C_o - C_t) \times V}{W} \quad (3)$$

Where q is the adsorption capacity (mg LAS per mg coagulant), V is the volume of wastewater treated (L) and W is the mass of coagulant added (mg).

A similar procedure was applied to assess the performance of $Al_2(SO_4)_3$ using the same dosage variations as MOS for direct comparison. As a negative control, measurements were also taken from untreated wastewater samples to establish baseline values for turbidity and LAS concentration and assess the actual impact of coagulation treatment.

Determination of Optimum Coagulation pH

A 1000 mL detergent wastewater sample was placed in a beaker, and its pH was adjusted to the desired value (4–9) using 1 M NaOH or 1 M HCl. The MOS coagulant solution was then added at the optimum dose determined from the previous experiment.

The coagulation process was carried out using a jar test with rapid stirring (100 rpm) for 1 minute, followed by slow stirring (40 rpm) for 10 minutes. The mixture was settled for 30 minutes to allow floc sedimentation. The supernatant was then carefully withdrawn using a pipette for subsequent analysis. The measured parameters were turbidity and residual LAS concentration. As a negative control, these parameters were also assessed in detergent wastewater samples without coagulant addition.

Comparison of Coagulant Effectiveness

Three types of detergent wastewater with varying degradation levels were used. Each 500 mL sample was placed in a beaker, and its pH was adjusted to the optimum value (determined in the previous experiment) using 1 M NaOH or 1 M HCl.

Coagulant solutions were then added at the optimal dose obtained from prior trials, using either the natural coagulant (MOS seed extract) or the synthetic coagulant (alum). Coagulation was performed by rapid mixing at 100 rpm for 1 minute, followed by slow mixing at 40 rpm for 10 minutes. The mixtures were allowed to settle undisturbed for 30 min to facilitate floc sedimentation.

After sedimentation, the upper part of the solution (supernatant) is carefully collected using a pipette for analysis. The parameters measured are turbidity and anionic surfactant (LAS) concentration to evaluate the effectiveness of each coagulant.

Result and Discussion

Extraction and Coagulation Mechanism of MOS

This section describes the mechanism by which proteins from MOS are extracted using NaCl solution and how they function as coagulants for anionic surfactants, particularly Linear Alkylbenzene Sulfonate (LAS). Previous studies have confirmed that MOS seeds are effective as natural coagulants for binding anionic surfactants. In this study, sieved MOS powder (60 mesh) was dissolved in 1 M NaCl solution. NaCl was used to extract globulin-type proteins, which are positively charged, salt-soluble, and exhibit strong coagulation activity [15], [16]. Hadadi et al. also reported that MOS extracted with NaCl exhibited higher coagulation efficiency compared to water-based extraction [17].

When MOS powder is dissolved in NaCl solution, a "salting-in" effect occurs, chloride ions (Cl^-) interact with positively charged protein sites disrupting intermolecular associations and enhancing protein solubility [16], [18]. This process facilitates the release of salt-soluble globulin proteins that act as active coagulants. When introduced to detergent wastewater, the weakly bound Cl^- is replaced by sulfonate groups ($-\text{SO}_3^-$) from LAS molecules, resulting in charge neutralization and particle destabilization. At the same time, hydrophobic interactions between the LAS alkyl chains and nonpolar protein regions promote floc formation and sedimentation [19]. This dual mechanism explains the strong coagulation performance of MOS observed in the experiments.

Evaluation of Turbidity Removal Efficiency at Varying Coagulant Dosages

The coagulation performance of MOS was assessed through its effectiveness in turbidity reduction. It has been reported that the use of detergent-based surfactants contributes to

increased TSS in wastewater [20]. As TSS increases, the amount of light scattered by suspended particles (measured as NTU) also increases. This phenomenon is visually apparent, as detergent wastewater typically appears greyish with visibly suspended particles, which may include surfactants and various contaminants. The added coagulant is expected to bind these suspended impurities and reduce turbidity, where a lower final turbidity indicates better coagulation performance. In this study, MOS extract, prepared in 1 M NaCl solution, was tested at different dosages: 100, 200, 500, 1000, and 1500 mg/L. Each dosage was applied to 1 L of domestic detergent wastewater with an initial turbidity of 107 NTU.

For comparison, alum was also applied at identical dosages to evaluate the relative coagulation performance of MOS in removing anionic compounds like LAS, commonly found in detergent wastewater [21].

Table 1. Turbidity Removal Efficiency (TRE) of detergent wastewater treated by MOS and alum at various dosages

Coagulant (mg/L)	$T_0 = 107 \text{ NTU}$			
	Alum		MOS	
	T_t (NTU)	TRE (%)	T_t (NTU)	TRE (%)
100	19.9	81.40	94	12.15
200	11.5	89.25	120	-12.15
500	20.8	80.56	115	-7.48
1000	70.1	34.49	10.4	90.28
1500	73.6	31.17	8.5	92.06

Table 1 presents a comparative summary of turbidity reduction achieved by each coagulant. The results clearly demonstrate a sharp contrast between the two coagulants. At a dosage of 100 mg/L, alum reduced turbidity by 81.04%, while MOS only achieved 12.15%. At 200 and 500 mg/L, the performance of MOS declined even further, as final turbidity values exceeded the initial value, showing an ineffective coagulation.

A marked improvement in MOS performance was observed only at higher dosages, with the highest turbidity removal (92.06%) observed at 1500 mg/L. In contrast, the

effectiveness of alum decreased beyond 200 mg/L, with its peak efficiency (89.25%) observed at that dosage, suggesting that 200 mg/L is its optimum. This performance pattern is consistent with the findings of Andrade et al., who reported that both alum and MOS extract are highly effective in reducing turbidity in domestic wastewater, although MOS requires a higher dosage to achieve comparable results [12]. As shown in Figure 1, alum follows a bell-shaped performance curve, while MOS displays a steadily increasing trend, indicating that higher dosages may still yield improved performance. Further testing at dosages beyond 1500 mg/L is therefore recommended for future studies.

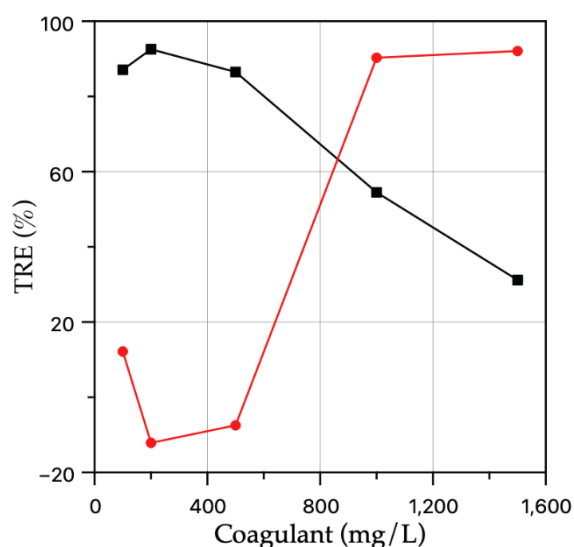


Figure 1. Comparison of turbidity removal efficiency between MOS seed extract (●) and alum (■) across various coagulant concentrations ranging from 100 to 1500 mg/L

Several factors may explain the low coagulation efficiency of MOS at lower dosages. First, the use of NaCl as the solvent could have contributed to this issue. According to Du et al., NaCl concentrations exceeding the optimal threshold (~0.6 M) may trigger a salting-out effect, where salt ions draw water molecules away from the protein surface [16]. This reduces protein solubility and causes premature aggregation, which can hinder the availability of active binding sites and, in turn, reduce coagulation effectiveness, or even worsen turbidity.

Second, as explained by Rasheed et al., effective coagulation depends heavily on the

electrostatic balance between negatively charged colloidal particles and the positively charged functional groups of MOS proteins [14]. If the dosage is too low, insufficient positive charge is present to neutralize colloidal particles, leaving them dispersed in solution. Conversely, excessive coagulant dosage may lead to charge reversal, creating new repulsive forces and re-stabilizing the suspension, preventing or breaking up floc formation. A third factor to consider is the absence of a defatting process prior to extraction. As noted by Skaf et al. MOS that still contain natural oils may form a thin film on the surface of the water [18]. This film can interfere with contact between coagulant molecules and suspended particles, thereby reducing floc formation efficiency. Moreover, the flocs that do form may be weak and prone to disintegration.

Effect of Coagulant Dosage on Residual LAS Concentration

The effectiveness of MOS as a natural coagulant was also evaluated specifically for the removal of anionic surfactants, particularly LAS, using the MBAS method. In this method, any uncoagulated LAS remaining in the supernatant interacts electrostatically with methylene blue cations (MB⁺), forming a blue-colored ion-association complex. This complex is then quantified using a UV-Vis spectrophotometer at a wavelength of 652 nm (Wahid et al., 2021). The absorbance values obtained represent the amount of residual LAS that formed complexes with methylene blue. These absorbance values were then converted to LAS concentrations using a linear calibration equation:

$$y = 0,2341x - 0.013 \quad (4)$$

where y is the absorbance and x is the concentration of LAS (mg/L). This calibration equation has coefficient of determination $R^2 = 0.9952$ indicating an excellent linear relationship between LAS concentration and the corresponding absorbance values. This means that 99.52% of the variability in absorbance can be explained by the variation in LAS concentration, confirming the reliability and

accuracy of the calibration curve for quantifying residual LAS in the samples.

Table 2. Residual LAS concentration and removal percentage in detergent wastewater after coagulation with varying dosage of MOS and alum

Coagulant (mg/L)	$C_0 = 6,9594 \pm 1,03$			
	Residual LAS with Alum treatment (mg/L)	LAS Removal Efficiency with Alum (%)	Residual LAS with MOS treatment (mg/L)	LAS Removal Efficiency with MOS (%)
100	3,8782 $\pm 0,30$	44,27	5,9401 $\pm 0,48$	14,65
200	2,9338 $\pm 0,11$	57,84	6,094 $\pm 0,17$	12,43
500	2,0406 $\pm 0,29$	70,68	4,6901 $\pm 0,61$	32,61
1000	3,5684 $\pm 0,08$	48,73	1,3098 $\pm 0,03$	81,18
1500	3,1474 $\pm 0,07$	54,77	1,0321 $\pm 0,11$	85,17

The residual LAS concentrations remaining after coagulation with either MOS or alum are presented in **Table 2**. The data show that coagulant dosage significantly affects the reduction of LAS concentration. As the dosage of MOS increases, the residual LAS concentration steadily decreases. In contrast, alum demonstrates a substantial reduction at lower dosages and reaches its optimum performance at 500 mg/L, after which its effectiveness declines with increasing dosage.

To support these observations, a two-way ANOVA was conducted (**Table 3**). The results indicate that both the type of coagulant (MOS or alum) and the coagulant dosage have a statistically significant effect on the residual LAS concentration ($p < 0.05$). Furthermore, the interaction between coagulant type and dosage was also highly significant ($F = 83.77$, $p = 1.19 \times 10^{-7}$), suggesting that the effectiveness of each coagulant is strongly influenced by the dosage applied. For instance, MOS tends to be more effective at higher dosages (≥ 1000 mg/L), whereas alum performs more consistently at low to moderate dosages.

Table 3. Two-Way ANOVA analysis for LAS residual concentration

Source of Variation	DF	Mean Square	F Value	P Value
Coagulant	1	2,44678	28,88119	$3,13 \times 10^{-4}$
Concentration	4	6,13934	72,46716	$2,41 \times 10^{-7}$
Interaction	4	7,09687	83,76963	$1,20 \times 10^{-7}$

Note: Values with $p < 0.05$ are considered statistically significant

To determine which specific treatment pairs differ significantly, post hoc comparisons were conducted using the Bonferroni and Tukey HSD tests (**Table 4**). The Bonferroni test revealed a significant difference between the two coagulants, with alum producing statistically lower residual LAS concentrations than MOS ($p = 0.0003$). Increasing the dosage also had a significant effect, particularly at 1000 and 1500 mg/L, where the reduction in LAS concentration was more pronounced. The Tukey HSD test confirmed these findings and highlighted that significant differences mainly occurred in high-dosage MOS treatments compared to low-dosage alum. For example, the difference between MOS at 1500 mg/L and alum at 100 mg/L was highly significant ($p < 0.0001$), indicating the strong performance of MOS as a natural coagulant at higher dosages. However, at 100 mg/L, there was no significant difference between MOS and alum ($p > 0.86$), suggesting that the statistical effectiveness of MOS only becomes evident at higher dosages. Overall, the post hoc analysis reinforces that LAS reduction effectiveness is influenced not only by the type of coagulant but also by its dosage. To further understand the performance of each coagulant, LAS removal efficiency was examined alongside adsorption capacity as an indicator of mass-based efficiency.

Table 4. Summary of Post Hoc Comparison Results (Bonferroni and Tukey Tests) of MOS vs. Alum

Comparison	Mean Diff	p (Bonferroni)	p (Tukey)
MOS vs Alum	0.6995	0.0003	0.0003
1500 mg/L vs 100 mg/L	-2.82	8.33×10^{-7}	1.33×10^{-6}

Comparison	Mean Diff	p (Bonferroni)	p (Tukey)
1000 mg/L vs 100 mg/L	-2.47	2.91×10^{-6}	2.23×10^{-6}
MOS 1500 vs Alum 100	-4.91	5.77×10^{-7}	2.43×10^{-7}
MOS 100 vs Alum 100	0.81	0.8610	0.8863

Note: Values with $p < 0.05$ are considered statistically significant

The LAS removal efficiency (%) and adsorption capacity (q) of each coagulant are presented in Figure 2. The application of alum at dosages ranging from 100 to 1500 mg/L was able to reduce LAS concentration in detergent wastewater by 44–70%, with the highest removal efficiency observed at 500 mg/L (70.68%). Meanwhile, MOS exhibited a broader removal range of 14–85% at the same dosage interval, with the highest efficiency recorded at 1500 mg/L (85.17%) and is predicted to increase further at higher dosages. Further evaluation is needed to determine the optimum dosage of MOS.

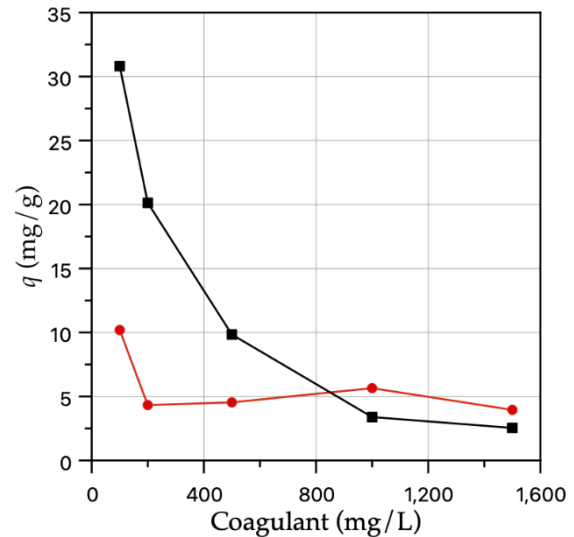
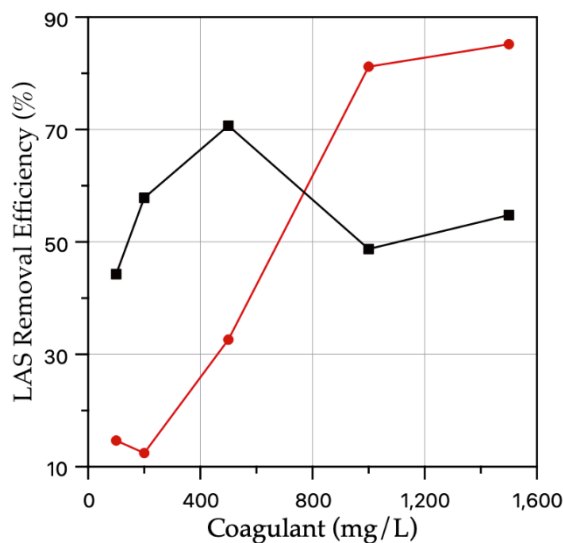


Figure 2. LAS removal efficiency (%) and adsorption capacity (q) using MOS extract (●) and alum (■) at various coagulant concentrations.

To assess the mass efficiency of the coagulants, adsorption capacity (q) was calculated. The value of q represents the amount of LAS bound per gram of coagulant (mg/g), thus reflecting the relative adsorption efficiency of each material. A higher q value indicates better performance in removing LAS from wastewater. Alum demonstrated the highest q at low dosage (30.8 mg/g), which sharply declined with increasing dosage, reaching the lowest point (2.04 mg/g) at 500 mg/L, which was previously identified as the optimum dosage in terms of LAS removal efficiency.

In contrast, MOS showed generally lower but more stable q values. At a dosage of 100 mg/L, MOS achieved the highest q value of 10.19 mg/g, which then declined and stabilized within the range of 3.95–5.65 mg/g from 200 to 1500 mg/L, with the lowest q (3.95 mg/g) at 1500 mg/L. These differences suggest that MOS is effective at high dosages for total LAS removal but exhibits lower mass-based efficiency compared to alum at lower dosages. This pattern is consistent with the findings of Beltrán-Heredia et al. who reported that the highest q values were obtained at the lowest coagulant dosages, while increasing dosages led to a decline in q due to excess coagulant mass that was not fully utilized during the adsorption process [13].

Evaluation of Coagulation Efficiency at Varying pH Levels

Coagulation efficiency is highly dependent on the charge interactions between surfactant molecules and the coagulants used, such as MOS seed extract and alum. The pH of the solution can influence the surface charge of both the coagulant and the organic or inorganic impurities present in the sample [22]. Therefore, an evaluation was conducted to assess the effect of pH on the turbidity removal efficiency of the coagulation process.

Table 5. Effect of pH variation on initial and final turbidity (T_c and T_f), and turbidity removal efficiency (TRE) of coagulation using alum and MOS extract

pH	$T_0 = 164$ NTU				
	Alum (200 mg/L)			MOS (1500 mg/L)	
	T_c (NTU)	T_f (NTU)	TRE (%)	T_f (NTU)	TRE (%)
4	252	193	23,41	11	95,63
5	124	140	-12,90	11,9	90,40
6	233	110	52,79	10,7	95,41
7	202	125	38,12	10,1	95,00
8	137	116	15,33	9,98	92,72

The test was performed using the previously determined optimum dosages: 1500 mg/L for MOS and 200 mg/L for alum. Five pH values were examined, ranging from pH 4 to pH 8. This range was selected based on the isoelectric point of the active proteins in MOS, which lies between pH 6.5 and 7.5 [17]. Extremely low pH values were avoided, as they may approach the point of zero charge (PZC) of certain organic or inorganic compounds in the sample, potentially leading to colloidal stabilization rather than destabilization, and thus reducing coagulation efficiency [22].

Initially, the turbidity of the detergent wastewater sample was 164 NTU. However, after pH adjustment using HCl and NaOH, the turbidity levels changed, as shown in Table 5. This change is attributed to the protonation or deprotonation of functional groups present in the wastewater [22], which can alter the electrostatic interactions between particles. These interactions may shift from repulsive to

attractive, leading to either agglomeration (resulting in reduced turbidity) or restabilization (increased turbidity).

Since the addition of acid or base directly altered the turbidity of the wastewater samples, the control values at each pH condition were used as the T_0 for calculating the TRE. This approach allows for an accurate assessment of coagulant performance under each specific pH condition, independent of the pH-induced changes in the raw turbidity. The influence of different pH into the turbidity removal efficiency could be seen in Figure 3.

From the data shown in Figure 3, the best pH for MOS in this study was pH 4, achieving a turbidity removal efficiency of 95.63%. MOS proteins have an isoelectric point in the range of 6.5–7.5 [17]. When the pH is below the isoelectric point, MOS proteins carry strong positive charges, making them more effective in attracting the negatively charged of surfactants molecules in the wastewater sample. At acidic pH 4, the high concentration of hydrogen ions from HCl maximized the protonation of the active sites of MOS proteins, further enhancing their ability to bind negatively charged particles. This result is consistent with Desta and Bote, who reported that hydrogen ions at acidic pH (pH 3) can significantly improve turbidity removal efficiency [23].

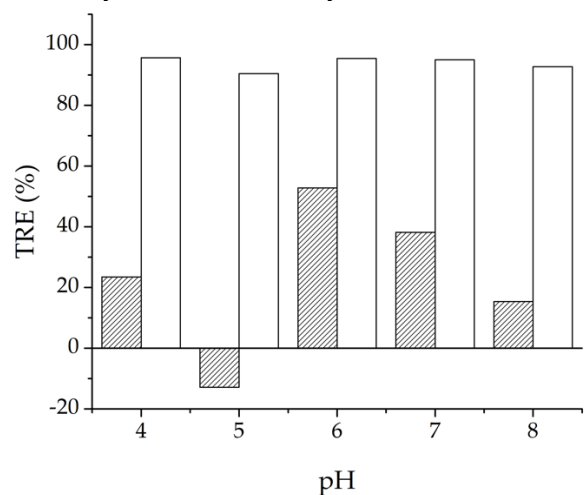


Figure 3. Effect of pH on turbidity removal efficiency (TRE) using alum (200 mg/L, black striped bars) and MOS extract (1500 mg/L, white bars)

In contrast, alum showed its highest turbidity removal performance at pH 6, with a

TRE of 52.79%. This can be explained by Kishimoto et al. who reported that aluminum hydroxide flocs carry positive charges at pH values below 7.4, thereby enhancing their effectiveness in coagulating wastewater [24]. This trend is further supported by the specific evaluation of LAS concentration removal by alum and MOS *within* a similar pH range, as shown in **Table 5**. The data in **Table 5** indicate that changes in pH had only a modest effect on LAS concentration. The lowest residual LAS concentration for MOS *was* observed at pH 4, while for alum it occurred at pH 7.

Table 6. Residual LAS concentrations before and after treatment with alum and MOS at varying pH levels

pH	LAS without any coagulation (mg/L)	Residual LAS with Alum treatment (mg/L)	Residual LAS with MOS treatment (mg/L)
4	3.7094	3.0214	1.0641
5	3.7778	2.4103	1.2222
6	3.7949	2.8376	1.094
7	3.9103	2.3932	1.4957
8	3.7265	2.2863	1.59401

Zhang et al. reported that when acid (HCl) is added to the wastewater, hydrogen ions interact with the hydrophilic groups of LAS molecules, neutralizing their negative charges [25]. This reduces electrostatic repulsion and increases the zeta potential toward zero. As a result, LAS becomes less soluble, and larger aggregates form, leading to decrease LAS concentration. In contrast, when a base is added, positively charged metal ions (particularly multivalent cations like Ca^{2+}) can interact with the negatively charged sulfonate groups on LAS. These cations can bridge multiple sites and promote aggregation, thereby reducing turbidity.

However, in this study, NaOH was used as the base, which provides monovalent Na^+ ions. These ions carry only a single positive charge and are not as effective in bridging negatively charged sites, so the formation of large aggregates is limited. This explains the relatively minimal decrease in LAS

concentration observed. Ultimately, the outcome depends on the balance of positive and negative charges in the system. When the charges are well balanced, aggregation occurs and LAS concentration decreases. If the positive charge is insufficient, particles remain dispersed, while an excess of positive charge may lead to charge reversal and restabilization, potentially increasing LAS concentration [18]. This phenomenon showed clearly illustrated in **Figure 4** shows that pH 4 was the optimum pH for MOS with LAS removal efficiency of 71.31%, while at pH 7 produced nearly identical value 61.75%. In contrast, alum achieved its highest LAS removal efficiency at pH 7 (38.79%), whereas at pH 4 it only achieved 18.55%.

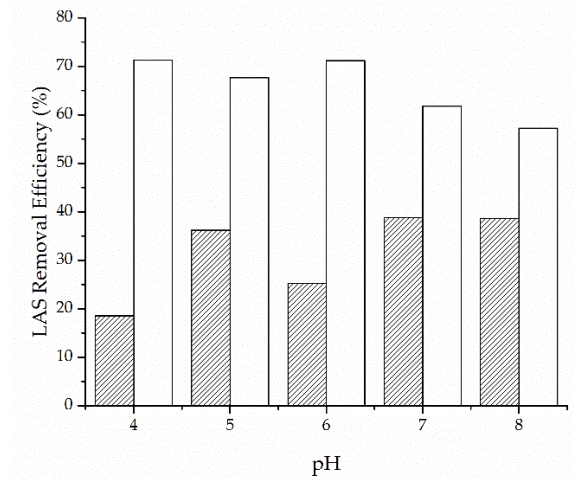


Figure 4. Effect of pH on LAS removal efficiency for alum and MOS, using dosages determined from prior turbidity optimization tests (1500 mg/L for MOS, black striped bars and 200 mg/L for alum, white bars).

These results indicate that MOS proteins are more active under acidic conditions, while alum shows better LAS removal closer to neutral conditions. The high performance of MOS at acidic conditions can be explained by the protonation of its active protein, which increases their positive charge and enhances electrostatic attraction toward the negatively charged LAS molecule at pH levels below isoelectric point (6.5 – 7.5) [17].

Validation of Coagulant Performance on Wastewater Samples with Different Degradation Levels

Following the determination of optimum coagulation conditions (MOS: 1500 mg/L at pH 4; alum: 200 mg/L at pH 6), both coagulants were further evaluated for their effectiveness in reducing turbidity and LAS concentration in three detergent wastewater samples with different degradation levels: Sample 1 (stored for 1 night), Sample 2 (stored for 2 nights), and Sample 3 (stored for 3 nights)

Table 7. Turbidity removal efficiency (TRE) of alum (200 mg/L, pH 6) and MOS (1500 mg/L, pH 4) in detergent wastewater samples stored for different durations.

Sample	T ₀	Alum		MOS	
		T _f (NTU)	TRE (%)	T _f (NTU)	TRE (%)
1	306	280,5	8,33	105,7	65,46
2	94,7	1,99	97,90	4,76	94,96
3	76	5,41	92,88	6,5	91,45

Based on the results shown in **Table 7**, the initial turbidity of the wastewater samples decreased with longer storage duration: from 306 NTU in the sample stored for one night, to 94.7 NTU after two nights, and 76 NTU after three nights. This corresponds to a natural turbidity reduction of about 69.1% and 75.2% after two and three nights of storage, respectively, even without the addition of external coagulants. Such a decrease suggests that suspended solids in the wastewater underwent partial self-coagulation or sedimentation during storage. This observation is consistent with the findings of Shaikh & Ahammed, who reported that turbidity in greywater decreased by up to 41% after 24 hours of natural settling [26]. These results highlight that natural sedimentation can gradually reduce turbidity in detergent wastewater, although the process is relatively slow and incomplete compared to coagulant-assisted treatment.

When coagulants were applied, further clarification occurred, leading to a decrease in turbidity. On sample 1 (initial turbidity 306 NTU), MOS achieved a higher turbidity reduction (65.46%) compared to alum (8.33%)

(**Figure 5**). However, the final turbidity of this sample still did not meet the detergent wastewater quality standard (≤ 100 NTU) as regulated by the Ministry of Environment Decree No. 51/MENLH/10/1995. In contrast, samples 2 and 3 already had lower initial turbidities (94.7 NTU and 76 NTU, respectively), which were below the quality standard. Consequently, only slight reductions were observed after coagulation, with TRE values of 1.99–4.76% for alum and 4.76–6.50% for MOS. These differences may be attributed to the self-sedimentation effect: after two nights of storage, sufficient suspended solids remained for the coagulant to act on, as seen in sample 2, while in sample 3 most solids had already settled naturally, leaving less material for removal. Meanwhile, the relatively lower TRE of MOS in sample 1 compared to alum may suggest that higher initial turbidity conditions require a different MOS dosage than that optimized in earlier experiments (at ~100 NTU).

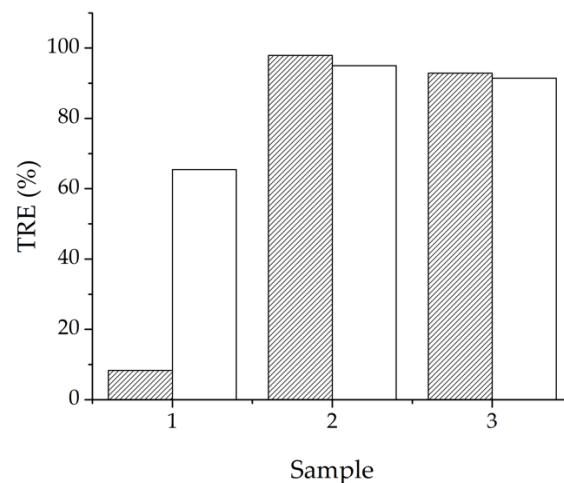


Figure 5. Comparison of TRE (%) of alum (200 mg/L, black stripped bars) and MOS (1500 mg/L, white bars) in detergent wastewater samples stored for one, two, and three nights.

However, MOS remains a promising natural coagulant because it is environmentally safe and does not significantly alter the pH of water. Hounsinou et al. reported that the addition of MOS to lake water did not affect its pH, whereas alum shifted the pH toward acidic conditions [27]. This highlights MOS as a sustainable alternative to conventional chemical coagulants, especially in environmentally sensitive applications.

Table 8. Residual LAS concentrations before and after treatment with alum and MOS in detergent wastewater samples stored for different durations.

Sample	$C_0 = 5.82 \text{ mg/L}$	
	Residual LAS with Alum treatment (mg/L)	Residual LAS with MOS treatment (mg/L)
1	5.0179	1.7589
2	1.0572	0.8223
3	1.7407	0.5842

The concentration of LAS was also evaluated based on the duration of wastewater storage. All three samples initially had an LAS concentration of 5.82 mg/L. After treatment with MOS and alum, the LAS concentrations in all samples met the detergent wastewater quality standard established by the Ministry of Environment Regulation No. KEP-51/MENLH/10/1995, except for sample 1 treated with alum (**Table 8**).

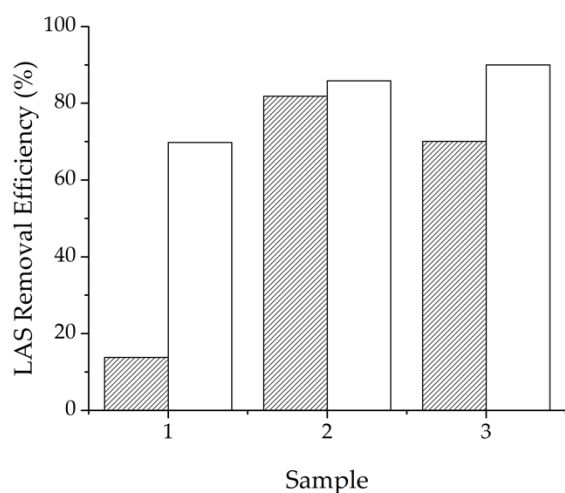


Figure 6. Comparison of LAS Removal Efficiency (%) of alum (200 mg/L, black striped bars) and MOS (1500 mg/L, white bars) in detergent wastewater samples stored for one, two, and three nights.

For sample 1, there was already a clear difference between the residual LAS after treatment with MOS and alum. As the

wastewater was stored longer before adding the coagulants, this difference became even more obvious. Alum showed unstable performance, with only 13.78% removal in sample 1, rising to 81.83% in sample 2, and then dropping again to 70.09% in sample 3. In contrast, MOS consistently improved, starting from 69.77% in sample 1 and reaching 85.87% and 89.96% in samples 2 and 3 (**Figure 6**). Although some bacteria are known to degrade LAS, this usually requires more than a week and would need further testing to confirm. What stands out is that even after three nights of storage, MOS still managed to notably reduce LAS levels. This highlights MOS as a promising natural alternative to alum, easy to use, readily available, and more environmentally friendly

In-Silico Docking of MOS Proteins with LAS

To further explore the potential molecular mechanism underlying the interaction between MOS proteins and LAS, an in-silico docking study was performed using PyRx. The protein model used was 2S albumin (UniProt ID: W5S2K0), a salt-soluble protein known for its coagulating activity, obtained via AlphaFold. The LAS molecule (PubChem CID: 15593874) was docked as ligand.

The binding energy was found to be -4.8 kcal/mol, indicating a spontaneous and thermodynamically favourable interaction. While preliminary, this result suggests that LAS may interact directly with active proteins in MOS, complementing electrostatic mechanisms and supporting the observed reduction in LAS concentration during coagulation. These findings provide a molecular rationale for the dual function of MOS in both turbidity and LAS removal.

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Conclusions

Our findings show that *Moringa oleifera* seed extract (MOS) can serve as a reliable natural coagulant for detergent wastewater. At higher dosages, MOS steadily improved its performance, reaching more than 92% turbidity reduction and about 85% LAS removal at 1500 mg/L, while alum peaked earlier at lower dosages with slightly lower efficiencies. When pH was adjusted, MOS performed best under acidic conditions, removing 95.63% of turbidity and 71.31% of LAS at pH 4. Alum, on the other hand, worked closer to neutral conditions but with far lower removal rates.

Importantly, MOS proved to be more stable than alum when applied to wastewater samples that had been stored for several days, still removing close to 90% of LAS when alum's performance became inconsistent. While alum showed a higher adsorption capacity at very low doses, MOS delivered steadier results across a broader range. The docking study further supported these observations, showing that LAS molecules can directly bind to MOS proteins (binding energy -4.8 kcal/mol), giving a molecular explanation for the experimental outcomes. Taken together, these results highlight MOS as a simple, affordable, and environmentally safer alternative to alum, and point to its potential for sustainable wastewater treatment at larger scales.

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