JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2025, 26(11), 231–241
https://doi.org/10.12911/22998993/208043
ISSN 2299–8993, License CC-BY 4.0

Received: 2025.06.24
Accepted: 2025.08.01
Published: 2025.08.15

Correlating the polysaccharide and protein contents of five plant-derived coagulants with turbidity removal

Zahraa Hasan Mutar[1*], Siti Rozaimah Sheikh Abdullah[2], Israa Abdulwahab Al-Baldawi[3]

[1] Department of Architecture Engineering, College of Engineering, Wasit University, 52001 Wasit, Iraq
[2] Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia
[3] Department of Civil Engineering, College of Engineering, University of Baghdad, Baghdad, Iraq
* Corresponding author's e-mail: zahraa.mutar@uowasit.edu.iq

ABSTRACT

In an effort to address the issues related to chemical coagulants, the recent paradigm in wastewater treatment has shifted towards encouraging industries to adopt and implement sustainable solutions in their activities. This study aims to evaluate the polysaccharide and protein contents of five different plant coagulants derived from the leaves of cassava (*Manihot esculenta*), cow grass (*Axonopus compressus*), kaduk (*Piper sarmentosum*), moringa (*Moringa oleifera*) and petai belalang (*Leucaena leucocephala*) and compare their coagulation performances in turbidity removal in a kaolin suspension. Jar tests were conducted with varying biocoagulant doses (0–0.1 mg/L) and pH values (4–10). Results showed that moringa had the highest polysaccharide (8.65 ± 0.001g/L) and protein (2.89 ± 0.001 g/L) contents. After moringa, petai belalang had the next-highest polysaccharide content (7.08 ± 0.001 g/L) and kaduk had the next-highest protein content (1.28 ± 0.003 g/L). Under the optimal conditions of a dose ratio of 0.01 and pH of 4, the petai belalang and moringa coagulants achieved the highest turbidity removal rates, which exceeded 90% and 87%, respectively. These promising outcomes may encourage further exploration to examine the appropriate approach for the extraction of active ingredients and the optimal operation parameters.

Keywords: plant-derived coagulants, extraction, optimal dose, suspended solids.

INTRODUCTION

The United Nations predicts that the number of people living in areas of physical water scarcity will increase to 1.2 billion by 2025 with the deterioration of freshwater quality in conjunction with the marked decline in rainfall rates associated with climate change and unsustainable water consumption [1]. Water resources with deteriorated quality are typically characterised by high turbidity levels and abundant in natural organic matter, including pathogenic microorganisms, as well as precursors of potentially carcinogenic, teratogenic and mutagenic disinfection by-products [1, 2]. Poor-quality water increases disease rates worldwide, with 800 000 annual mortalities due to gastrointestinal diseases caused by unsafe drinking water [3]. Accordingly, wastewater generated from human activities requires comprehensive treatment to meet the global population's growing demand for clean water [1, 2]. The safe reuse of treated wastewater contributes to the circular economy and addresses food insecurity, particularly in areas transitioning between urban and rural, where effluent represents an essential nutrient-rich irrigation water resource [4].

Coagulation–flocculation is a fundamental process in water treatment that reduces turbidity and improves the transparency of water [5, 6]. This process involves the addition of certain chemical salts, such as aluminium sulphate or ferric chloride to reduce the turbidity of wastewater by destabilising the dissolved and suspended solid particles creating large, heavy flocs that easily settle [7]. However, the consequences of using chemical coagulants are grave; for instance, aluminium residues are not biodegradable, resulting in environmental complications related to the treatment and disposal of the generated sludge [8]. Therefore, strict standards for residual aluminium levels in drinking water have been established. According to the World Health Organisation, aluminium levels should not exceed 0.2 mg/L; moreover, they should not exceed 0.1 mg/L in Japan and 0.05 mg/L in the USA [9]. Therefore, an urgent need for other potential alternatives to minimise the use of aluminium salts has emerged [10].

Efforts to produce natural biocoagulants from plant residues are relentless [11–13]. Several biocoagulants have been derived from various plant materials, including wheat starch [10], chickpea (*Cicer arietinum*) [14], cotton seeds [15], prickly pear peels [16], Ceratophyllum [17], Garcinia kola [18], cassava (*Manihot esculenta*) peels [13, 19, 20] and Margaritarea discoidea [21].

The strength of plant-based coagulants is believed to lie in the selection of indigenous plants used by rural societies [22]. However, most previous studies have focused on specific species, particularly moringa (*Moringa oleifera*) [8, 23–34]. Therefore, further research can be conducted to discover other nontoxic plant species that can be used in mass production [22].

Identifying the active ingredients that determine the underlying mechanisms of coagulation is necessary to obtain a comprehensive understanding of the prevailing conditions during coagulation–flocculation using plant-based coagulants. Polysaccharides are polymers with skeletal frameworks containing monosaccharides and their derivatives. The polysaccharides' skeletal framework might be linear or branched, containing either one monosaccharide type (homopolysaccharides) or more than one monosaccharide type (heteropolysaccharides). This structure provides a sufficient number of active sites for particle adsorption and charge neutralisation in coagulation–flocculation operations [21]. Moreover, the chemical compounds present in plant-based coagulants, such as protein, may be responsible for coagulation activities in water clarification [10, 35].

This study focuses on determining the polysaccharide and protein contents of five tropical plant species, namely, cassava, cow grass (*Axonopus compressus*), kaduk (*Piper sarmentosum*) and moringa. Moreover, it evaluated the efficiency of the extracts of these plants as ecofriendly coagulants. It also aims to assess the effects of biocoagulant doses (0–0.1 mg/L) and pH values (4–10) on the removal of suspended solids and turbidity in synthetic kaolin wastewater.

MATERIALS AND METHODS

Source of turbid water

Synthetic turbid water (approximately 600 NTU) was prepared in accordance with Alnawajha et al. [36] by mixing kaolin powder with tap water at 60 rpm for 30 min to ensure the full hydration of kaolin and subsequent formation of colloidal particles.

Quantification of the protein and polysaccharide contents of plant extracts

The protein and polysaccharide contents present in the structures of the plant extracts were quantified by using extracts with concentrations of 10–100 g/L. Each concentration was prepared separately by accurately weighing the required amount of the dried powder of each plant and dissolving it in 100 mL of distilled water. The resulting solution was homogeneously mixed for 1 h, centrifuged at 4000 rpm for 7 min (5810, Eppendorf, Germany) and decanted through a Buchner funnel lined with 0.45 μm filter paper (Whatman, Germany). Protein was quantified in accordance with the Bradford protein assay adopted by Shah et al. [13]. In brief, 0.5 mL of each concentration of the freshly extracted plant solution was agitated at room temperature with 2.5 mL of Bradford reagent for 5 min to obtain a colorimetric protein solution. Protein content was measured by quantifying the intensity of the blue protein–dye complex solution with a UV spectrophotometer (DR3900, HACH Company, the USA) at a wavelength of 595 nm, and the results were compared with the bovine serum albumin standard curve. The polysaccharide content of the plant extracts was determined in accordance with the acid phenol method by following Alnawajha et al. [12]. Equal volumes (1 mL) of freshly prepared plant solutions and phenol (5%) were mixed with 5 mL of concentrated H_2SO_4. The mixture was left for 10 min prior to the measurement of absorbance (488 nm). The results were compared with the standard curve of glucose solution.

Preparation of coagulant stock solutions

Branches of kaduk, cow grass, cassava, moringa and petai belalang (Leucaena leucocephala) were collected from Bangi, Selangor, Malaysia. Leaves were washed and oven-dried (BS100, Protech, Malaysia) at 40 °C for three days. The dried leaves were ground by using a blender (Panasonic, Malaysia) into fine powders, sieved through 200–500 μm meshes, then stored in confined containers for subsequent use. Coagulants with a dose of 10 g/L (Figure 1) were freshly prepared before every jar test experiment by dissolving 5 g of the dry powder of each plant separately in 500 mL of distilled water. The solution was homogeneously mixed for 1 h, centrifuged (5810, Eppendorf, Germany) and decanted through a Buchner funnel lined with a 0.45 μm filter paper (Whatman, Germany).

Jar test experiments

Coagulation–flocculation experiments were conducted by using a jar test flocculator (VELP, Malaysia) with six 500 mL beakers (Iwaki, Germany). The one-variable-at-a-time approach was conducted to optimise the dose ratio and pH value with fixed operation parameters, including a rapid mixing gradient of 200 rpm for 1 min, slow mixing gradient of 15 rpm for 30 min and settling time of 30 min. The dose ratio represents the mass ratio of a coagulant to wastewater [37]. Accordingly, each beaker was filled with 450 mL of wastewater and 50 mL of a coagulant in a dose ratio between 0 to 0.1 in accordance with Equation 1:

$$Dose\ ratio = \frac{C_C \times V_C}{TSS_{ww} \times V_{ww}} \quad (1)$$

where: C_C is the concentration of the biocoagulant, V_C is the volume of the biocoagulant, TSS_{ww} is the initial measured value of the total suspended solids (TSS) in wastewater and V_{ww} is the volume of wastewater.

Analytical methods

The performance of the plant-based coagulants was evaluated by measuring the turbidity, TSS and colour of wastewater. The turbidity of wastewater was measured with a turbidimeter (2100AN, HACH, China). TSS and colour were analysed by using a spectrophotometer (DR3900, HACH Company, the USA). The removal rates for turbidity, TSS and colour were calculated in accordance with Equation 2 [38]:

$$Removal\ (\%) = \frac{C_i - C_t}{C_i} \times 100\% \quad (2)$$

where: C_i represents the initial concentration of wastewater, and C_t is the final concentration of the treated wastewater.

Statistical analysis using ANOVA

The results of this study were statistically analysed by using SPSS software version 21 (IBM, the USA) with a confidence interval of 95%. One-way ANOVA followed by a post hoc test (Turkey's HSD) was conducted to determine the significant effects of coagulant dose and pH

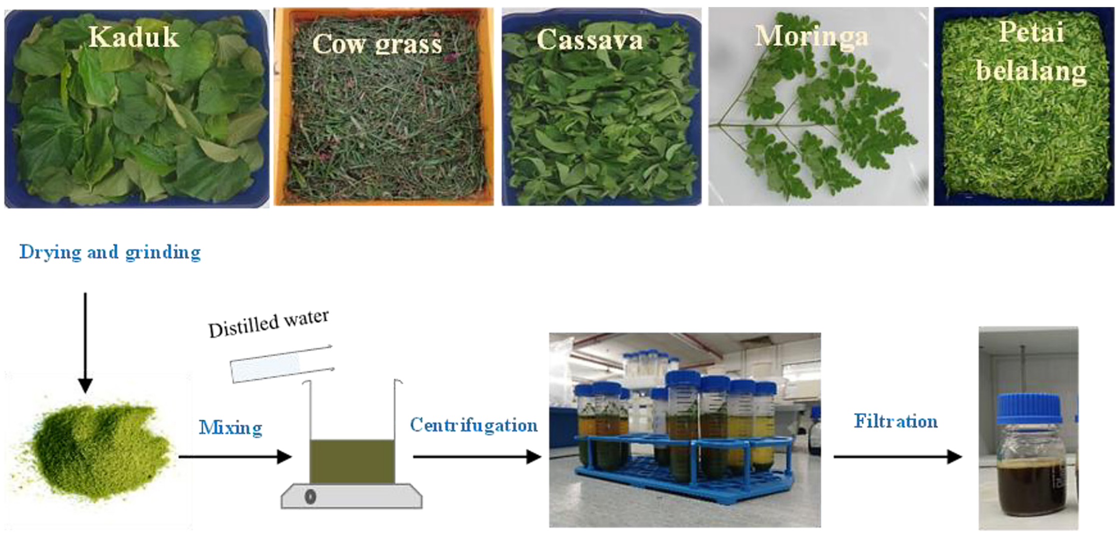

Figure 1. Preparation of plant-based coagulants

on coagulation performance, which was represented by the removal rates for turbidity, TSS and colour [39].

RESULTS AND DISCUSSIONS

Analysis of the protein and polysaccharide contents of plants

The extracted solutions of plant leaves were characterised by quantifying the protein and polysaccharide contents of extracts of different concentrations (10–100 g/L), as presented in Figure 2. As an initial observation, all tested plant extracts had higher polysaccharide contents than protein contents. This finding appears to be consistent with the presence of diverse polysaccharides, such as cellulose, hemicellulose and pectin, in the cell wall. Miscellaneous polysaccharides are often the most abundant macromolecules in plants. Polysaccharides have distinct structures and functions that contribute to wall mechanics and influence plant morphogenesis [13, 40, 41]. By contrast, proteins are usually minimally involved in plant cell walls because they are limited to structural roles or function as enzymes [35, 42].

Considerable variation in polysaccharide and protein contents were observed amongst the plant extracts within the same concentration range. Moringa had a higher polysaccharide content (8.65 ± 0.001g/L) than petai belalang (7.08 ± 0.001 g/L), kaduk (6.29 ± 0.01 g/L), cassava (5.28 ± 0.001 g/L) and cow grass (3.28 ± 0.063 g/L). The amount of polysaccharides extracted from the plants gradually increased as the extract concentration increased from 10 g/L to 70 g/L, then insignificantly fluctuated at high concentrations (> 70 g/L). The polysaccharide contents of kaduk, grass, cassava, moringa and petai belalang peaked at concentrations of 100, 70, 100, 90 and 90 g/L, respectively.

Moringa had higher protein contents (2.89 ± 0.001 g/L) than kaduk (1.28 ± 0.003 g/L), grass (0.93 ± 0.005 g/L), cassava (0.48 ± 0.003 g/L) and petai belalang (0.37 ± 0.001 g/L). The protein contents of kaduk, grass, cassava, moringa and petai belalang peaked at concentrations of 80, 70, 100, 80 and 70 g/L, respectively. Shah et al. [13] reported a similar result given that the protein contents of cassava steadily increased from 5 g/L (340 ± 14.1 mg/L) to 80 g/L (5833.3 ± 1130.3 mg/L). Protein content (6151.7 ± 274.6 mg/L) peaked at 70 g/L then significantly dropped (1576.9 ± 88.7 mg/L) at 90 g/L. Shah et al. [13] attributed this phenomenon to the hydration effect. The concentration of salts, especially calcium, markedly increases in conjunction with the increase in plant extract concentration. Calcium in leaf extracts is preferentially hydrated and adsorbed onto negative surfaces over other positively charged ions. Therefore, the increasing salt level enhances the aggregation and precipitation of proteins due to their decreasing solubility [13, 43, 44].

Effect of dose ratio

In this study, the effect of varying dose ratios on the removal of turbidity, TSS and colour in synthetically prepared wastewater at pH 7.4 was investigated. Five types of water-extracted biocoagulants were screened to identify the most efficient coagulant. The comparison of the performances of

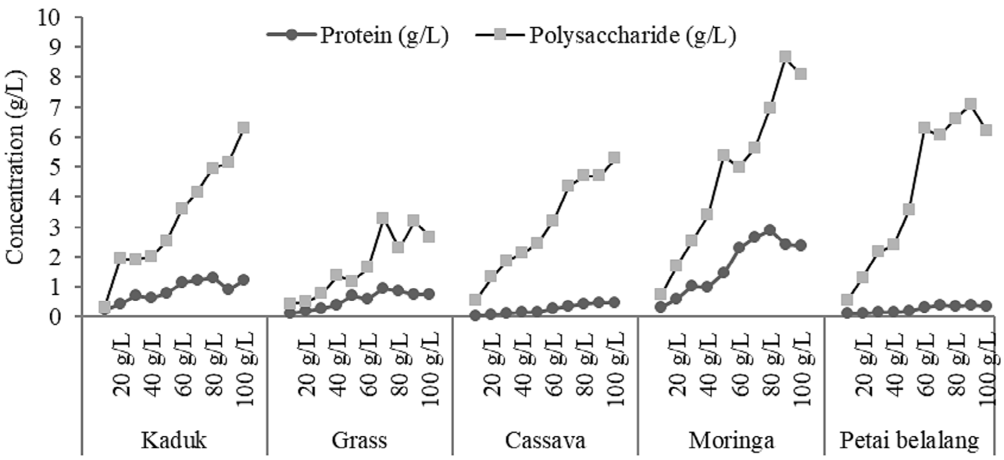

Figure 2. Protein and polysaccharide contents of plants

the prepared biocoagulants is illustrated in Figure 3. Jar test experiments showed that compared with the control, the tested coagulants achieved a statistically significant reduction in turbidity, TSS and colour levels. However, the moringa-based coagulant had no statistically significant effect on colour reduction. The cow grass coagulant achieved the highest turbidity removal rates, which ranged from 27% to 37%, followed by the cassava (23–32%), kaduk (18–28%), moringa (15–22%) and petai belalang (16–22%) coagulants. The results clearly revealed that all coagulants achieved the highest turbidity removal rates at the lowest dose ratio (0.01). Petai belalang, which exhibited

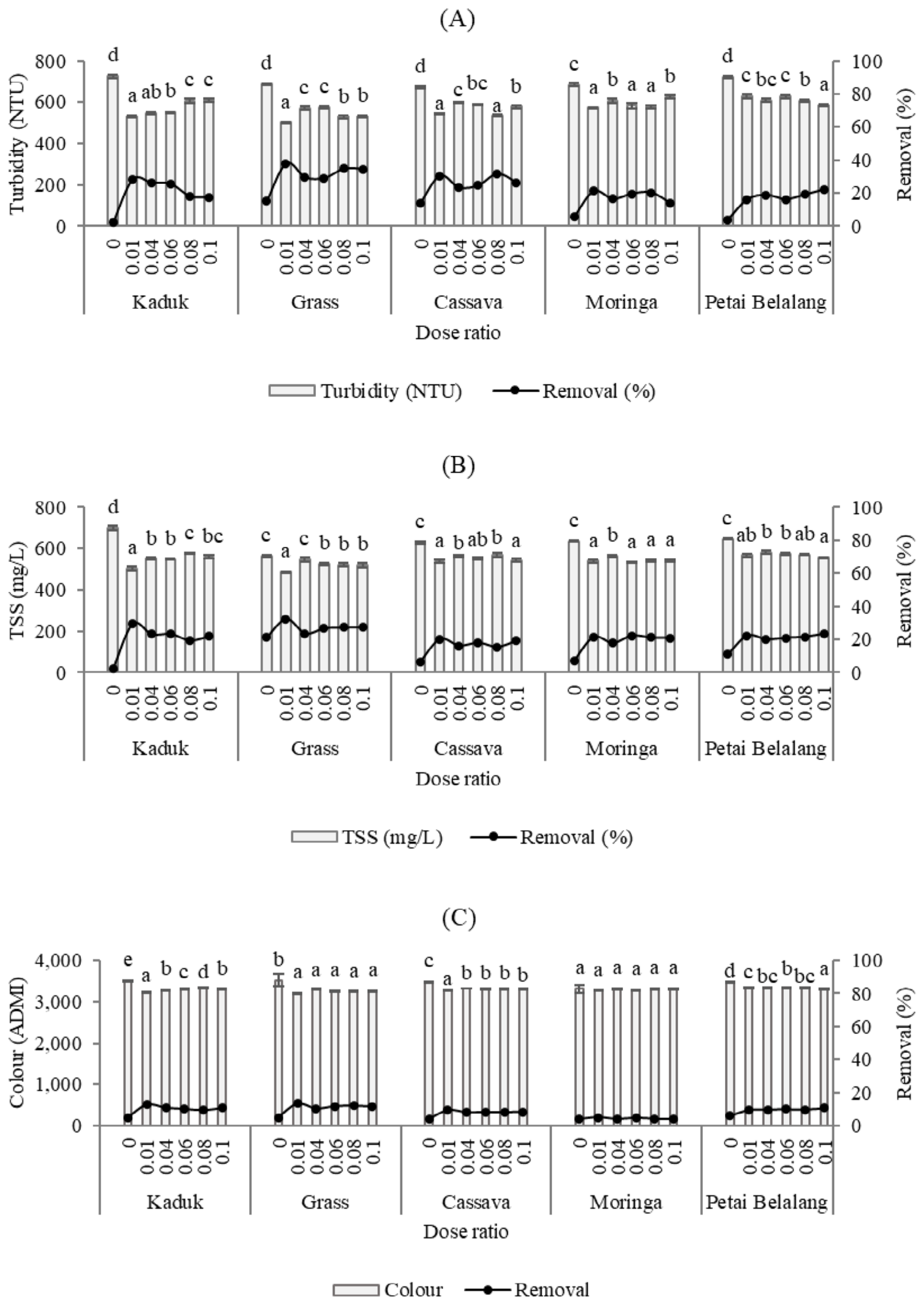

Figure 3. Effect of the mass ratio of the tested plant-based coagulants on the removal of (A) turbidity, (B) TSS and (C) colour. Different letters represent statistically significant differences in the turbidity removal ability of each coagulant at each concentration ($p < 0.05$)

the highest turbidity removal rate at the highest dose ratio (0.1), was the sole exception (Figure 3A). The cow grass coagulant obtained the highest removal rates for TSS (23.57–32.35%), followed by the moringa (28.24–32.18%), cassava (26.35–30.49%), kaduk (19.30–29.37%) and petai belalang (20.05–23.48%) coagulants. All the tested plant-derived coagulants significantly reduced the levels of TSS at the dose ratio of 0.01. However, the levels of TSS significantly increased when the dose ratio of the kaduk and grass coagulants increased (Figure 3B). These results are in line with the results reported by Putra et al. [45], who reported that increasing the dose of biocoagulant extracted from jackfruit seed initially enhanced the removal of turbidity; however, the removal declined in conjunction with dose increasing beyond the optimal dose. It is believed that overdosing causes particle restabilization which can hinder floc formation and sedimentation [45]. TSS levels clearly oscillated when the cassava, moringa and petai belalang coagulants were used at dose ratio > 0.01. Increasing the dose ratio of the kaduk and cassava coagulants (> 0.01) had a negative effect on colour levels (Figure 3C), whereas increasing those of the grass and moringa coagulants from 0.01 to 0.1 had no significant effect on colour levels. The results also revealed that the petai belalang coagulant followed different trends, wherein increasing its dose ratio from 0.01 to 0.1 had a positive effect on colour reduction.

Effect of pH

In this study, the plant-based coagulants were tested at the dose ratio of 0.01 and different pH values ranging from 4 to 10. Figure 4 depicts the removal rates and final concentrations of turbidity, TSS and colour in wastewater at different pH values. The initial pH value of wastewater had a strong effect on turbidity removal rates and other parameters to different extents. The results revealed that an extremely acidic condition (pH 4) significantly enhanced the removal of turbidity, TSS and colour (90.33% ± 0.40%, 90.52% ± 0.41% and 88.26% ± 2.16%, respectively) by the petai belalang coagulant relative to the neutral (15.33% ± 3.04%, 21.07% ± 0.19 and 5.70% ± 0.81%, respectively) and alkaline conditions (9.42% ± 2.12%, 22.12% ± 0.15% and 14.94% ± 0.81%, respectively). The moringa, grass and kaduk coagulants followed a pattern similar to that of the petai belalang coagulant but had low removal rates. The reduction in turbidity achieved by the moringa, grass and kaduk coagulants at pH 4 was significantly higher (87.28% ± 0.53%, 59.11% ± 0.15% and 47.82% ± 1.34%, respectively) than that at pH 7.4 (18.88% ± 1.61%, 7.911% ± 2.59% and 19.20% ± 3.36%, respectively) and pH 10 (4.88% ± 2.39%, 9.11% ± 0.27% and 21.95% ± 2.15%, respectively). The moringa, grass and kaduk coagulants significantly reduced the levels of TSS at pH 4 (83.18% ± 0.13%, 57.98% ± 0.15% and 50.74% ± 2.92%, respectively) relative to at pH 7.4 (18.38% ± 0.26%, 18.84% ± 0.07% and 24.33% ± 0.07%, respectively) and pH 10 (22.81% ± 0.26%, 18.57% ± 0.07% and 26.94% ± 0.07%, respectively). The reductions in colour levels by the moringa, grass and kaduk coagulants at pH 4 were significant (78.53% ± 0.27%, 52.79% ± 1.11% and 44.97% ± 0.83%, respectively) relative to those at pH 7.4 (9.23% ± 0.27%, 12.29% ± 1.67% and 19.27% ± 0.27%, respectively) and pH 10 (9.78% ± 2.05%, 13.96% ± 1.11% and 18.99% ± 5.58%, respectively). According to Nigussie and Habtu [50], the acidic conditions (pH 4) improve the protonation of functional groups, including carboxyl and amine groups, of biocoagulants, raising their capacity to bind with negatively charged particles [50]. Notably, the alteration in pH conditions during the jar test had no significant effect on the reduction in turbidity, TSS and colour by the cassava coagulant.

The improvement in turbidity removal and other parameters under the neutral and alkaline conditions observed in this study agrees with the results reported by Saritha et al. [46], who found that sago coagulant achieved the highest reduction in turbidity and other parameters at pH 7. Similar findings were also reported by Shah et al. [13], who reported that the removal of TSS and turbidity by plant-based coagulants was greater at pH 7 than at the original pH of 3.4. Lek et al. [14] suggested that the protein solubility of a plant-based coagulant (chickpea) increased after pH 4, accounting for the observed improvement in palm oil mill effluent treatment at high pH values.

Comparison with previously reported coagulants

Several studies have discussed the performance of most of the plant extracts involved in this study, except for grass, in the treatment of wastewater with different origins, as

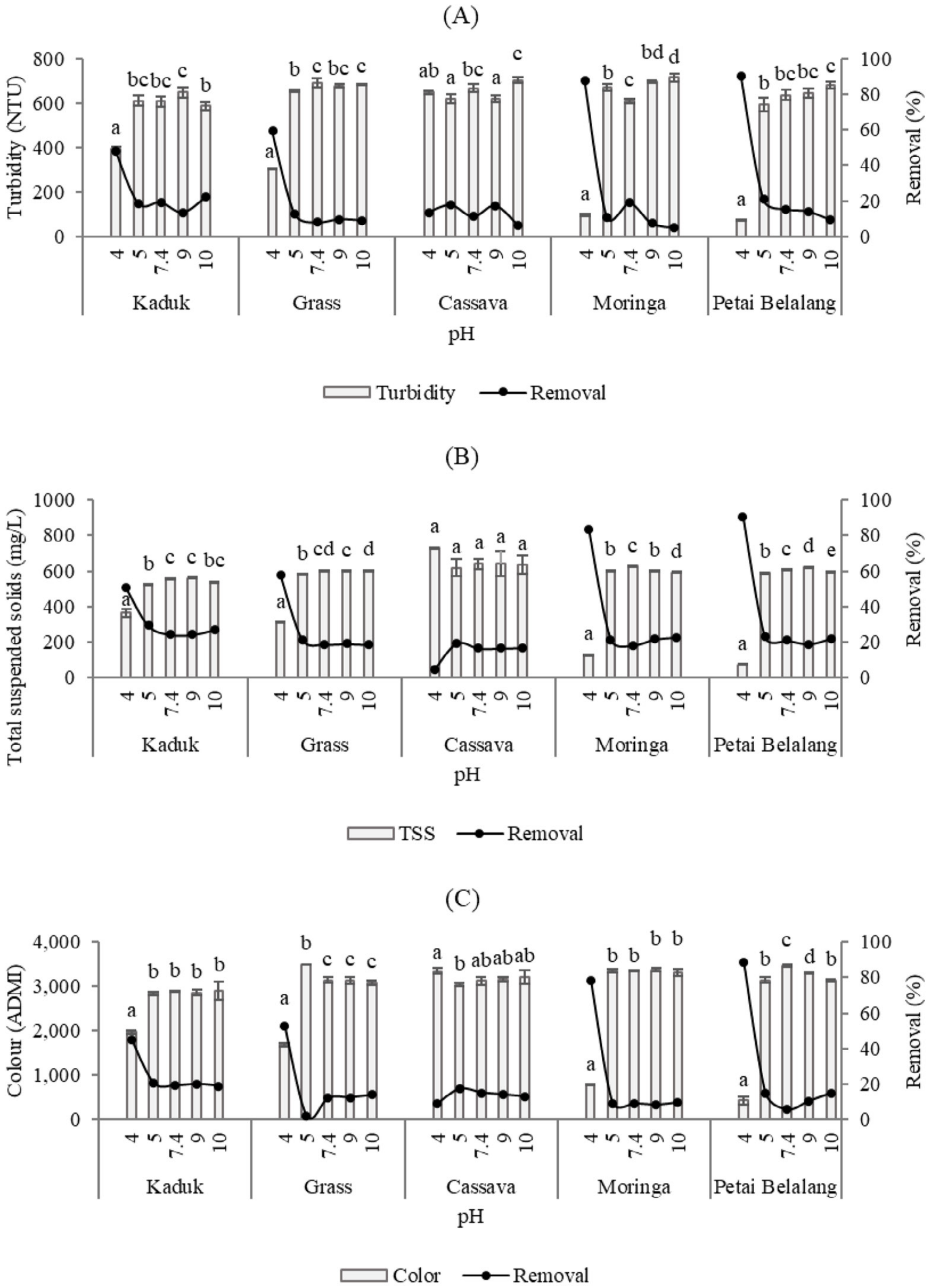

Figure 4. Effect of the initial pH on the removal rates for (A) turbidity, (B) TSS and (C) colour. Different letters represent statistically significant differences in the ability of each concentration of each plant coagulant to remove turbidity ($p < 0.05$)

summarised in Table 1. Protein in plant extracts contributes to charge neutralisation and bridging mechanisms in removing colloidal particles, whereas polysaccharides contribute to the bridging mechanism [36]. The results of this study indicate a strong correlation between the polysaccharide content and coagulation performance of the five plant extracts. The two plant extracts (petai belalang and moringa) that showed the highest coagulation performance also possessed the highest polysaccharide content, suggesting that high polysaccharide content enhances adsorption and bridging coagulation and charge neutralisation [21]. Alnawajha et al. [47] reported on the good performance of petai belalang seed extract, which reduced the turbidity, TSS

Table 1. Performance of plant-based coagulants derived from kaduk, cassava, moringa and petai belalang in previous studies

Plant-based coagulant	Type of wastewater	Removal rate	Reference
Kaduk leaves	Coffee effluent	51.0% (TSS) 66.9% (turbidity)	[13]
Cassava leaves	Domestic wastewater	High TSS removal	[48]
	Landfill leachate	6.7% (aluminium content removal)	[49]
	Coffee effluent	>60% (turbidity)	[13]
Cassava peel starch	Institutional wastewater	77.48% (turbidity) 77.34% (TSS) 56.89% (COD)	[19]
Moringa (seeds and leaves)	Kaolin suspension	36.7% (TSS) 34.8% (turbidity)	[11]
Moringa seeds	Raw water	98.83% (turbidity)	[50]
	Bentonite suspension	93% (turbidity)	[27]
	Municipal sewage	~90% (coagulation performance)	[31]
	Dairy industry	96.8% (turbidity) 90.3% (colour)	[51]
Petai belalang seeds	Kaolin suspension	93.05% (turbidity)	[36]
Petai belalang seeds	Aquaculture effluent	96.32% (turbidity) 92.85% (TSS) 86% (colour)	[47]
Kaduk leaves	Kaolin suspension	47.82% (turbidity) 50.74% (TSS) 44.97% (colour)	Present study
Cassava leaves		17.78% (turbidity) 19.42% (TSS) 17.39% (colour)	
Cow grass		59.11% (turbidity) 57.98% (TSS) 52.79% (colour)	
Moringa leaves		87.28% (turbidity) 83.18% (TSS) 78.53% (colour)	
Petai belalang leaves		90.33% (turbidity) 90.52% (TSS) 88.26% (colour)	

and colour of aquaculture effluent by 96.32%, 92.85% and 86%, respectively. They found that the promising performance of the coagulant was correlated with the protein and polysaccharide contents (1001 and 1803 mg/L, respectively) of the extract. In another study by Alnawajha et al. [36], petai belalang seed extract, with protein and polysaccharide contents of approximately 1082 and 2257 mg/L, respectively, reduced the turbidity of a kaolin suspension by 93.05%. Shah et al. [13] reported that kaduk removed 51.0% and 66.9% of TSS and turbidity, respectively, in coffee effluent.

CONCLUSIONS

This study focused on natural alternatives for coagulation by screening five plant-derived coagulants. These coagulants were extracted from cassava leaves, cow grass, kaduk leaves, moringa leaves and petai belalang leaves by using water. All tested plants had considerably higher polysaccharide contents than protein contents most likely due to the involvement of polysaccharides, including cellulose, hemicellulose and pectin, in the cell wall. Compared with the other coagulants, the petai belalang and moringa coagulants exhibited superior performance in the removal of turbidity, TSS and colour of the prepared kaolin suspension, especially under acidic conditions. Coagulation performance was influenced by coagulant dose, wherein increasing the dose ratio to beyond 0.01 caused a marked decline in the coagulation efficiency of all screened biocoagulants, except for petai belalang, which achieved the highest removal efficiency for turbidity and TSS at a dose ratio of 0.1.

Acknowledgements

The authors would like to thank Universiti Kebangsaan Malaysia for supporting this research project through the DIP-2024-001 grant. The first author would like to acknowledge and thank Wasit University and the Iraqi Ministry of Higher Education and Scientific Research for their support.

REFERENCES

1. Chellam, S., Sari M.A. (2016). Aluminum electrocoagulation as pretreatment during microfiltration of surface water containing NOM: A review of fouling, NOM, DBP, and virus control. *J Hazard Mater, 304*, 490–501. https://doi.org/10.1016/j.jhazmat.2015.10.054
2. Mutar, Z.H., Mohammed, A.A., Al-Baldawi, I.A. (2022). Optimization of acetaminophen and methylparaben removal within subsurface batch constructed wetland systems. *J Ecol Eng, 23*(1), 228–239. https://doi.org/10.12911/22998993/143934
3. Al-Baldawi, I.A., Mohammed, A.A., Mutar, Z.H., Abdullah, S.R.S., Jasim, S.S., Almansoory, A.F., Ismail, N.I. (2021) Application of phytotechnology in alleviating pharmaceuticals and personal care products (PPCPs) in wastewater: source, impacts, treatment, mechanisms, fate, and SWOT analysis. *J Clean Prod, 319*, 128584. https://doi.org/10.1016/j.jclepro.2021.128584
4. The United Nations Human Settlements Programme (UN-Habitat) and the World Health Organization (WHO) (2024). *Progress on the proportion of domestic and industrial wastewater flows safely treated – Mid-term status of SDG*. Licence: CC BY-NC-SA 3.0 IGO.
5. Al-Baldawi, I.A., Hussain, A. (2025) Nutrient fate after the coagulation–flocculation process of fish-farm water using green coagulant. *J Ecol Eng, 26,* 65–72. https://doi.org/10.12911/22998993/200480
6. Alnawajha, M.M., Abdullah, S.R.S., Hasan, H.A., Othman, A.R., Kurniawan, S.B., Jusoh, H.H.W., Ismail, A. (2025). Water vs salt extraction of biocoagulant from Leucaena leucocephala pods for removing turbidity from aquaculture effluent. *J Indian Chem Soc, 102*, 101784. https://doi.org/10.1016/j.jics.2025.101784
7. Mutar, Z.H., Abdullah, S.R.S., Al-Baldawi, I.A. (2025). Flocculating suspended solids in palm oil mill effluent using Leucaena leucocephala extract: performance, kinetics and characterisation of the recovered sludge. *Clean Techn. Environ. Policy.* https://doi.org/10.1007/s10098-025-03235-4
8. 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. *Chem Eng J, 313*, 226–237. https://doi.org/10.1016/j.cej.2016.12.031
9. Jiao, R., Xu, H., Xu, W., Yang, X., Wang, D. (2015). Influence of coagulation mechanisms on the residual aluminum – The roles of coagulant species and MW of organic matter. *J Hazard Mater, 290*, 16–25. https://doi.org/10.1016/j.jhazmat.2015.02.041
10. Choy, S.Y., Prasad, K.N., Wu, T.Y., Raghunandan, M.E., Ramanan, R.N. (2016). Performance of conventional starches as natural coagulants for turbidity removal. *Ecol Eng, 94*, 352–364. https://doi.org/10.1016/j.ecoleng.2016.05.082
11. Jaganathan, J.S., Abdullah, S.R.S., Ismail, N.‘I, Sharuddin, S.S.N. (2022). Coagulation-flocculation process of nutrient-rich suspended solids from aquaculture effluent using bioflocculant. *JOBIMB, 10*, 46–56. https://doi.org/10.54987/jobimb.v10iSP2.728
12. Alnawajha, M.M., Abdullah, S.R.S., Hasan, H.A., Othman, A.R., Kurniawan, S.B. (2024). Effectiveness of using water-extracted Leucaena leucocephala seeds as a coagulant for turbid water treatment: Effects of dosage, pH, mixing speed, mixing time, and settling time. *Biomass Convers Biorefinery, 14*, 11203–11216,. https://doi.org/10.1007/s13399-022-03233-2
13. Shah, R.A.R., Abdullah, S.R.S., Hasan, H.A., Othman, A.R., Ismail, N.‘I. (2023). Prospective plants as biocoagulant-flocculants for removal of total suspended solids in coffee effluent treatment. *Chem Eng Res Des, 198*, 282–295. https://doi.org/10.1016/j.cherd.2023.09.002
14. Lek, B.L.C., Peter, A.P., Chong, K.H.Q., Ragu, P., Sethu, V., Selvarajoo, A., Arumugasamy, S.K. (2018). Treatment of palm oil mill effluent (POME) using chickpea (*Cicer arietinum*) as a natural coagulant and flocculant: Evaluation, process optimization and characterization of chickpea powder. *J Environ Chem Eng, 6*, 6243–6255. https://doi.org/10.1016/j.jece.2018.09.038
15. DePaolis, M., Respino, S.D., Samineni, L., Brightona, S., Kumar, M. (2023). Cottonseed extract as a coagulant for water treatment. *Environ Sci, 2*, 227–234. https://doi.org/10.1039/D2VA00205A.
16. Flores, A., Mauricio, M., Miranda, O.E.R., Castillo, N.A.M., Vázquez, V.Á., Mata, O.S., Bañuelos, R.V., Murillo, C.E.C. (2024). Evaluation of the potential of a biocoagulant produced from prickly pear peel waste valorization for wastewater treatment. *Water, 16*, 1444. https://doi.org/10.3390/w16101444
17. Hussain, A., Al-Baldawi, I.A. (2025). Hybrid system using local plant as sustainable coagulation-flocculation process. *J Ecol Eng, 26*, 286–293. https://doi.org/10.12911/22998993/195471

18. Igwegbe, C.A., Ighalo, J.O., Iwuozor, K.O., Onukwuli, O.D., Okoye, P.U., Al-Rawajfeh, A.E. (2022). Prediction and optimisation of coagulation-flocculation process for turbidity removal from aquaculture effluent using *Garcinia kola* extract: Response surface and artificial neural network methods. *Cleaner Chem Eng, 4*, 100076. https://doi.org/10.1016/j.clce.2022.100076
19. Kumar, V., Al-Gheethi, A., Asharuddin S.M., Othman N. (2021). Potential of cassava peels as a sustainable coagulant aid for institutional wastewater treatment: Characterisation, optimisation and techno-economic analysis. *Chem Eng J*, 420, 127642. https://doi.org/10.1016/j.cej.2020.127642
20. Kusuma, H.S., Amenaghawon, A.N., Darmokoesoemo, H., Neolaka, Y.A.B., Widyaningrum, B.A., Onowise, S.U., Anyalewechi, C.L. (2022) A comparative evaluation of statistical empirical and neural intelligence modeling of Manihot esculenta-derived leaves extract for optimized bio-coagulation-flocculation of turbid water. *Indus Crops Prod, 186*, 115194. https://doi.org/10.1016/j.indcrop.2022.115194
21. Oladoja, N.A., Unuabonah, E.I., Amuda, O.S., Kolawole, O.M. (2017). Mechanistic Insight into the Coagulation Efficiency of Polysaccharide-based Coagulants. In: *Polysaccharides as a Green and Sustainable Resources for Water and Wastewater Treatment.* Springer Briefs in Molecular Science. Springer, Cham. https://doi.org/10.1007/978-3-319-56599-6_2
22. Yin C.Y. (2010). Emerging usage of plant-based coagulants for water and wastewater treatment. Process Biochemistry, 45, 1437-1444. https://doi.org/10.1016/j.procbio.2010.05.030
23. Nordmark, B.A., Przybycien, T.M., Tilton, R.D. (2016). Comparative coagulation performance study of Moringa oleifera cationic protein fractions with varying water hardness. *J Environ Chem Eng, 4*, 4690-4698. https://doi.org/10.1016/j.jece.2016.10.029
24. Shan, T.C., Matar, M.A., Makky, E.A., Ali, E.N. (2017). The use of *Moringa oleifera* seed as a natural coagulant for wastewater treatment and heavy metals removal. *Appl Water Sci, 7*, 1369–1376. https://doi.org/10.1007/s13201-016-0499-8
25. Barbosa, A.D., da Silva, L.F., de Paula, H.M., Romualdo, L.L., Sadoyama, G., Andrade, L.S. (2018). Combined use of coagulation (*M. oleifera*) and electrochemical techniques in the treatment of industrial paint wastewater for reuse and/or disposal. *Water Res, 15*, 153–161. https://doi.org/10.1016/j.watres.2018.08.022
26. Adelodun, B., Ogunshina, M.S., Ajibade, F.O., Abdulkadir, T.S., Bakare, H.O., Choi, K.S. (2020). Kinetic and prediction modeling studies of organic pollutants removal from municipal wastewater using *moringa oleifera* biomass as a coagulant. *Water, 12*, 2052. https://doi.org/10.3390/w12072052
27. Saleem, M., Sami, A. J., Bachmann, R.T. (2020). Characterisation and coagulant activity screening of fractionated water-soluble seed proteins from *Moringa oleifera*. *Materials Today: Proceedings, 31*, 207–210. https://doi.org/10.1016/j.matpr.2020.03.700
28. Sera, P.R., Diagboya, P.N., Akpotu, S.O., Mtunzi, F.M., Chokwe, T.B. (2021). Potential of valourized Moringa oleifera seed waste modified with activated carbon for toxic metals decontamination in conventional water treatment. *Bioresour Technol Rep, 16*, 100881. https://doi.org/10.1016/j.biteb.2021.100881
29. Deepa, D., Keerthana, R., Kumar, R.P., Suryaprakash, R. (2022). Primary treatment of dairy wastewater using bio based natural coagualnts. *Materials Today: Proceedings, 60*, 616–621. https://doi.org/10.1016/j.matpr.2022.02.125
30. Murali, A., Hillstead, K.D., Wrobel, B.S., Thomas, D.J., Gonety, R., Tarabara, V.V. (2022). Moringa oleifera-derived coagulants for water treatment: Floc structure, residual organics, and performance trade-offs. *Environ Sci Pollut Res, 29*, 24381–24389. https://doi.org/10.1007/s11356-022-19071-8
31. Varsani, V., Vyas, S.J., Dudhagara, D.R. (2022). Development of bio-based material from the Moringa oleifera and its bio-coagulation kinetic modeling–A sustainable approach to treat the wastewater. *Heliyon, 8*, e10447. https://doi.org/10.1016/j.heliyon.2022.e10447.
32. Madjene, F., Benhabiles, O., Boutra, A., Benchaib, M., Bouchakour, I. (2023). Coagulation/flocculation process using Moringa oleifera bio-coagulant for industrial paint wastewater treatment: optimization by D-optimal experimental design. Int J Environ Sci Technol, 20, 12131–12140. https://doi.org/10.1007/s13762-023-04808-w.
33. Nzeyimana, B.S., Mary A.D.C. (2024). Sustainable sewage water treatment based on natural plant coagulant: Moringa oleifera. *Discov Water, 4*, 15. https://doi.org/10.1007/s43832-024-00069-x
34. Jammeli L., Louhichi, G., Khouni, I. (2025). Effectiveness of using Moringa oleifera seeds as a green coagulant for soya oil refinery wastewater ecological treatment: Box–Behnken design optimization. *Euro-Mediterr J Environ Integr*. https://doi.org/10.1007/s41207-024-00729-3
35. Kurniawan, S.B., Imron, M.F., Noramalina, C.E., Chik, C.E., Owodunni, A.A., Ahmad, A., Alnawajha, M., Rahim, N.F.M., Said, N.S.M., Abdullah, S.R.S., Kasan, N.A., Ismail, S., Othman A.R., Hasan H.A. (2022). What compound inside biocoagulants/bioflocculants is contributing the most to the

coagulation and flocculation processes? *Sci Total Environ, 806*, 150902. https://doi.org/10.1016/j.scitotenv.2021.150902

36. Alnawajha, M.M., Kurniawan, S.B., Imron, M.F., Abdullah, S.R.S., Hasan, H.A., Othman, A.R. (2022). Plant-based coagulants/flocculants: characteristics, mechanisms, and possible utilization in treating aquaculture effluent and benefiting from the recovered nutrients. *Environ Sci Pollut Res, 29*, 58430–58453. https://doi.org/10.1007/s11356-022-21631-x
37. Ahmad, A., Abdullah, S.R.S., Hasan, H.A., Othman, A.R., Ismail, N.'I. (2021). Plant-based versus metal-based coagulants in aquaculture wastewater treatment: Effect of mass ratio and settling time. *J Water Process Eng, 43*, 102269. https://doi.org/10.1016/j.jwpe.2021.102269
38. Mutar, Z.H., Al-Baldawi, I.A., Mohammed, A.A., Hameed, H.K., Abdullah, S.R.S., Ismail, N.I. (2023). *IOP Conf Ser: Earth Environ Sci, 1215* 012002. https://doi.org/10.1088/1755-1315/1215/1/012002
39. Salem, A.K., Almansoory, A.F., Al-Baldawi, I.A. (2023). Potential plant leaves as sustainable green coagulant for turbidity removal. *Heliyon, 9*, E16278. https://doi.org/10.1016/j.heliyon.2023.e16278
40. Barnes, W.J., Koj, S., Black, I.M., Archer-Hartmann, S.A., Azadi, P., Urbanowicz, B.R., Peña, M.J., O'Neill, M.A. (2021). Protocols for isolating and characterizing polysaccharides from plant cell walls: a case study using rhamnogalacturonan-II. *Biotechnol. Biofuels, 14*, 142. https://doi.org/10.1186/s13068-021-01992-0
41. Mohammed, A.S.A., Naveed, M., Jost, N. (2021). Polysaccharides: classification, chemical properties, and future perspective applications in fields of pharmacology and biological medicine (a review of current applications and upcoming potentialities). *J Polym Environ, 29*, 2359–2371. https://doi.org/10.1007/s10924-021-02052-2
42. Voiniciuc, C., Pauly, M., Usadel, B. (2018). Monitoring polysaccharide dynamics in the plant cell wall. *Plant Physiology, 176*, 2590–2600. https://doi.org/10.1104/pp.17.01776
43. Choi, J., Choi, S.Q., Park, K., Han, Y., Kim, H. (2016). Flotation behaviour of malachite in mono- and di-valent salt solutions using sodium oleate as a collector. *Int J Miner Process, 146*, 38–45. https://doi.org/10.1016/j.minpro.2015.11.011
44. Benalia, A., Derbal, K., Panico, A., Pirozzi, F. (2018). Use of acorn leaves as a natural coagulant in a drinking water treatment plant. *Water (Switz.), 11*, 1–12. https://doi.org/10.3390/ w11010057
45. Putra, R.S., Female, N.Z., Sinta, D. (2022) Dosage effect of biocoagulant from jackfruit seed (*Artocarpus heterophyllus* L.) on the wastewater treatment of chemical laboratory. *AIP Conf. Proc. 2638*(1), 100003.
46. Saritha, V., Srinivas, N., Vuppala, N.V.S. (2017). Analysis and optimization of coagulation and flocculation process. *Appl Water Sci, 7*, 451–460. https://doi.org/10.1007/s13201-014-0262-y
47. Alnawajha, M.M., Kurniawan, S.B., Abdullah, S.R.S., Hasan, H.A., Othman, A.R. (2023). Performance of Leucaena leucocephala and alum in treating aquaculture effluent: Effect of dosage, rapid speed and settling time. *Inter J Environ Sci Technol, 20*, 9981–9994. https://doi.org/10.1007/s13762-022-04682-y
48. Ormaza J., Vélez M., Banchón, C. (2025). Natural coagulant from cassava peel (*Manihot esculenta*) for domestic wastewater. *Res Soc Dev, 14*, e4514248161. https://doi.org/10.33448/rsd-v14i2.48161
49. Mohd-Salleh, S.N., Mohd-Zin, N.S., Othman, N., Gopalakrishnan, Y., Abu-Bakar, N. (2021). Treatability of Manihot esculenta Peel Extract as Coagulant Aid for Stabilised Leachate. *Pertanika J Sci Technol, 29*, 1503–1516.
50. Nigussie, Z., Habtu, N.G. (2023). Performance evaluation of biocoagulant for the effective removal of turbidity and microbial pathogens from drinking water. *J Water Health, 21*, 1158. https://doi.org/10.2166/wh.2023.059
51. Trigueros, D.E.G., Hinterholz, C.L., Fagundes-Klen, M.R., Veit, M.T., Formentini-Schmitt, D.M. (2023). Statistical evaluation of the coagulation-flocculation process by using Moringa oleifera seeds extract to reduce dairy industry wastewater turbidity. *Bioresour Technol Rep, 23*, 101579. https://doi.org/10.1016/j.biteb.2023.101579