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Research Article

Performance and Mechanisms of Activated Natural Coagulants for Efficient *Microcystis* Removal via Coagulation–Flocculation in Thailand

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Abstract

Thailand produces fruit peels and seeds rich in natural polymers, which can serve as eco-friendly coagulants to control algal blooms. In this study, banana peel (*Musa* spp.), orange peel (*Citrus sinensis*), basil seed (*Ocimum basilicum*) mucilage, and chia seed (*Salvia hispanica*) mucilage were used. These activated natural coagulants with aluminum were compared with nonactivated and commercial coagulants (ferric sulfate, ferrous sulfate, and polyaluminum chloride) for *Microcystis* removal. Phytochemical composition analysis, Fourier transform infrared (FTIR) spectroscopy, and oxidation–reduction potential (ORP) analysis were conducted. The results revealed that banana and orange peels contain phenolic compounds, whereas galacturonic acid was detected in basil and chia seed mucilage. Phytoplankton contain amino, carboxyl, and phosphate groups with a net negative surface charge. Compared with commercial coagulants, activated natural coagulants achieved 90% chlorophyll-a removal, whereas nonactivated forms reached 50–60% efficiency. ORP measurements and floc analysis confirmed effective precipitation. Mechanistically, phytoplankton acted as reducing agents, whereas banana and orange peels served as oxidizing agents through phenolic–aluminum interactions. Consequently, activated natural coagulants destabilize the phytoplankton, leading to flocculation. Similarly, aluminum ions in basil and chia mucilage bind to galacturonic acid, destabilizing the cells. The polymeric chains of polygalacturonic acid further facilitated interparticle bridging, thereby promoting flocculation. These mechanisms account for the high removal efficiency of activated natural coagulants.

Introduction

Lentic ecosystems, such as lakes and ponds, are vital for human and ecological well-being. They provide essential resources such as freshwater and food while also supporting biodiversity and offering crucial ecosystem services. However, these water bodies are under increasing pressure from anthropogenic activities, including urbanization, industrialization, and agriculture. These activities are the primary drivers of water quality degradation globally (Carpenter et al., 1998; Smith et al., 1999), with eutrophication being among the most critical consequences. A major issue is the inadequate treatment of domestic sewage, uncontrolled industrial effluents, and agricultural runoff (Cristoforetti et al., 2015). These factors accelerate the rate of eutrophication, a process that severely affects the quality of lentic water bodies. Eutrophication is defined as the uncontrolled growth of phytoplankton due to the excessive enrichment of nutrients, especially nitrogen and phosphorus, in lentic ecosystems (Akinnawo, 2023). This process can

lead to numerous problems, including decreased water quality, loss of aquatic biodiversity, increased microbial biomass, and the proliferation of cyanobacterial blooms (Akinnawo, 2023). Among these blooms, *Microcystis* is among the most common and dominant species (Phonmat et al., 2025; Prasertphon et al., 2020). Its presence not only reduces water transparency and dissolved oxygen levels but also releases microcystins when its cells are destroyed (Ma et al., 2024). [These toxins can cause acute and chronic health issues for humans and other organisms. Therefore, the development of effective methods to control and reduce algal growth is urgently needed.

Microcystis can be controlled through physical, biological, and chemical methods. Physical methods, such as water mixing, ultrasonic treatment, and pressure devices, can induce cell damage but are often limited by high costs and long operation times. Biological methods involve mainly predation and competition by other organisms and are generally low cost and safe. For instance, plant allelochemicals can inhibit the growth and colony formation of *Microcystis*, although they require a relatively long treatment period. Currently, chemical methods such as sedimentation, flotation, coagulation, and flocculation are preferred because they are simple and rapid and improve water treatment efficiency (Zhou et al., 2024). Among them, coagulation and flocculation are widely applied for the effective removal of *Microcystis.* Commercial coagulants, including ferric sulfate, ferrous sulfate, aluminum sulfate, and polyaluminum chloride (PAC), have shown strong pre-cipitation ability (Lucena-Silva et al., 2022; Tongman et al., 2023). However, the excessive use of these chemical coagulants, particularly metal salts, poses environmental concerns because of poor biodegradability (Shi et al., 2016). As a result, interest in natural coagulants has increased because of their low toxicity and biodegra-dability. Previous studies have demonstrated that soils modified with cationic starch can remove up to 86% of *Microcystis* (Shi et al., 2016), whereas *Vicia faba* and *Opuntia ficus-indica* can reduce the amount of chlorophyll-a by more than 85% (Bouaidi et al., 2020). Other natural coagulants, such as *Moringa oleifera* seed and *Aloe vera* mucilage, have also been successfully applied to remove turbidity or treat various wastewaters. Moreover, sonication has been used to synthesize magnetic *Moringa oleifera* coagulants, resulting in 83.8% removal of palm oil wastewater (Noor et al., 2022). Nevertheless, research comparing natural plant-based coagulants, activated natural coagulants, and commercial coagulants specifically for the removal of *Microcystis* from water bodies remains limited. On the basis of the literature review, a systematic comparison of the removal efficiencies of these coagulants for *Microcystis* control has not yet been comprehensively explored and is expected to provide novel insights into the selection of effective coagulants.

Thailand is among the world's leading agricultural producers, generating vast amounts of byproducts such as fruit peels and seed residues. Banana and orange peels are byproducts generated from various food-processing industries, such as banana chip production, dried banana processing, and orange juice manufac-turing. When effectively valorized, these residues can be converted into value-added resources, thereby reducing environmental burdens and contributing to sustainable waste management. Sweet basil and chia are grown in Africa, Central and South America and tropical regions of Asia, including Thailand. Sweet basil and Chia seeds contain mucilage that can serve as natural coagulants because of its high polysaccharide content and ability to interact with algal cells. The use of sweet basil leaves and chia seed-derived materials specifically emphasizes the utilization of low-grade, surplus, or market-rejected agricultural products, which are often unsuitable for human consumption. This approach highlights their potential to transform low-value or discarded biomass into environmentally beneficial applications. Converting both agricultural byproducts and seed-based materials into coagulants not only supports waste-to-resource management but also aligns with global efforts toward a circular economy and sustainable water treatment solutions. This study therefore aims to explore the novel possibility of synthesizing activated natural coagulants from agricultural byproducts (banana and orange peels) and seed mucilage basil (*Ocimum basilicum*) and chia (*Salvia hispanica*) combined with aluminum sulfate using a sonication method, which enhances the interaction between plant polymers and aluminum ions to improve coagulation performance. Fourier transform infrared spectroscopy (FTIR) was employed to charac-terize the functional groups of the phytoplankton and plant-derived materials, while coagulation–flocculation tests were used to compare the removal efficiency of natural coagulants, activated natural coagulants, and commercial coagulants (ferric sulfate, ferrous sulfate, and PAC) by phytoplankton (e.g., *M. aeruginosa*). In addition, the coagulation mechanism of activated natural coagulants was further investigated using oxidation–reduction potential (ORP) analysis. The outcomes of this study are expected to provide a sustainable, low-cost, and environmentally friendly approach for managing harmful algal blooms, particularly in regions with abun-dant agricultural waste such as Thailand and beyond, while also identifying which coagulants are most effective for phytoplankton removal.

Materials and methods

1) Study site and water sampling and water quality analysis

The study site was located within Phranakhon Rajabhat University (latitude 13.877699, longitude 100.591621), Bangkok, a freshwater system experiencing visible algal blooms (latitude 13.874617, longitude 100.588246). This water body receives domestic wastewater directly from nearby canals, and M. aeruginosa blooms were clearly observed at the sampling site. To assess eutrophication, 5 L water samples were collected in 2025 from three sampling points using the grab sampling method at a depth of approximately 15 cm below the water surface to avoid surface scum. The initial chlorophyll-a concentration, an indicator of nutrient enrichment and algal biomass, was determined using acetone extraction and a UV spectrophotometer (Evolution 201, Thermo Scientific). For the phytoplankton analysis, 10 L of water was filtered through a plankton net with a 20 µm mesh size. The collected samples were fixed with 4% formaldehyde and then examined under a microscope (DP27, Olympus) for species identification to the taxonomic level.

2) Phytochemical analysis of plant extracts

Basil seed (*Ocimum basilicum*) and chia seed (*Salvia hispanica*) were purchased from a local market in Bangkok, while banana peel (Musa spp.) and orange peel (*Citrus sinensis*) were collected as fruit residues from the cafeteria at Kasetsart University, Bangkok. These materials were selected because they are readily available in Thailand and are rich in natural polysaccharides and phenolic compounds, which are known to enhance coagulation and flocculation processes. Before the activation process, the banana and orange peels were dried in an oven at 60 °C for 12 h. The dried samples were then ground and sieved to obtain particle sizes smaller than 1 mm and subsequently stored in a desiccator prior to use. For comparison, conventional chemical coagulants (ferric sulfate (Kemaus), ferrous sulfate (Kemaus), and commercial PAC (Q-Clean)) were also tested. All the reagents used in this study, including aluminum sulfate (Kemaus), were of analytical grade.

Banana peel, orange peel, chia seed, and basil seed contain phenolic compounds and flavonoids and exhibit DPPH (2,2-diphenyl-1-picrylhydrazyl) activity (Phong et al., 2021). In this study, we determined the total phenolic content (TPC, mg GAE (gallic acid equivalents) /g), total flavonoid content (TFC, mg QE (quercetin equivalents)/g), and antioxidant activity through a DPPH (%) scavenging assay, as described in the following methods. The TPC was determined using the Folin–Ciocalteu assay (Isiam et al., 2021). A 0.5 mL sample was mixed with 0.5 mL of Folin–Ciocalteu reagent and 1 mL of 7.5% Na_2HCO_3 solution. Distilled water was then added to adjust the final volume to 10 mL. The mixture was vortexed for 3 sec and left in the dark for 35 min before being centrifuged at 4,000 × g for 10 min. Absorbance was measured at 750 nm using a UV–Vis spectrophotometer (Evolution 201, Thermo Scientific).

The total flavonoid content (TFC) was determined following the method described by Isiam et al. (2021). One milliliter of plant extract was mixed in a Falcon tube with 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$ solution. The mixture was kept at room temperature for 5 min before 0.3 mL of 10% $AlCl_3$ solution was added. After incubation for 1 min at room temperature, 2 mL of 1 M NaOH and 2.4 mL of distilled water were added. The solution was then vortexed and centrifuged at 4,000 × g for 10 min. The mixture was incubated in the dark for 15 min, and the absorbance was measured at 510 nm using a UV–Vis spectrophotometer (Evolution 201, Thermo Scientific). The antioxidant activity was determined using the DPPH scavenging assay (Isiam et al., 2021). First, 0.1 mL of plant extract at various concentrations (62.5–500 mg mL^{-1}) was mixed with 0.2 mL of 0.1 mM DPPH solution in methanol. The mixture was incubated in the dark at room temperature (25 ± 5 °C) for 1 h. The absorbance was then measured at 515 nm using a UV–Vis spectrophotometer (Evolution 201, Thermo Scientific).

3) Preparation of activated natural coagulants and analytical methods

Activated natural coagulants were prepared from each plant material using a sonication method with a sonicator (Model: F5100b, Decon). Basil and chia seeds were initially soaked for 30 min to allow saturation swelling prior to use at a dosage of 1 g of seeds per 30 mL of reverse osmosis (RO) water. Afterward, 20 g of banana peels, orange peels, chia seeds, and basil seeds were activated with 1 M aluminum sulfate at 50 °C for 30 min. The synthesized activated natural coagulants were stored in a refrigerator at 4 °C until further use. The functional groups and chemical composition of the phytoplankton, natural coagulants and activated natural coagulants were characterized by FTIR (Nicolet iS5, Thermo Scientific). The samples were dried at 60 °C overnight prior to analysis. FTIR spectra were recorded using attenuated total reflectance (ATR) mode with a resolution of 4 cm^{-1} over the range of 400–4,000 cm^{-1}. The ORP of the phytoplankton before and after activation was measured using an ORP meter (Multi 3510 IDS, WTW) to evaluate redox changes.

4) Comparison of the coagulation–flocculation performance of natural, activated natural, and commercial coagulants

To simulate the coagulation–flocculation process, a six-beaker jar test apparatus (JR6D, MTEC) was used. Each beaker was filled with 500 mL of water sample collected from the hypereutrophic pond described in

the previous section, which exhibited harmful algal blooms. Coagulants of various types and doses, including four natural materials (banana peel, orange peel, basil seed mucilage, and chia seed mucilage) and their activated forms, were added to the beakers at 0.5, 1.0, 1.5, 2.0, and 2.5 g. Rapid mixing was conducted at 120 rpm for 2 minutes, followed by slow mixing at 20 rpm for 30 min. After an additional 60 min of stabilization, the supernatant was collected for chlorophyll-a determination, with *M. aeruginosa* as the predominant phytoplankton species, using acetone extraction and a UV spectrophotometer (Evolution 201, Thermo Scientific).

For comparison, conventional chemical coagulants, including ferric sulfate (Kemaus), ferrous sulfate (Kemaus), and commercial PAC (Q-Clean), were also tested. The coagulation–flocculation process was conducted with different commercial coagulants at 0.25, 0.5, 1, 2 and 3 g. The assays were conducted in a jar test apparatus (JR6D, MTEC) with 500 ml of water sample previously quickly agitated for 2 min (120 rpm), followed by 30 min of slow agitation (20 rpm). Following a 60-min stabilization period, the supernatant was collected for chlorophyll-a concentration analysis using acetone extraction and a UV spectrophotometer (Evolution 201, Thermo Scientific). A blank sample without coagulant was included as a control for comparison. All the solutions were prepared using RO water to ensure the consistency and reliability of the experimental results. All experiments were performed in triplicate, and the data presented in the figures represent the mean values of the three replicates.

5) Evaluation of coagulation efficiency and floc characteristics and data analysis

The chlorophyll-a concentrations were used to calculate the coagulation efficiency of phytoplankton (M. aeruginosa) removal (Eq. 1):

$$\text{Removal efficiency } (\%) = (C_0 - C_t/C_0) \times 100 \quad (Eq.1)$$

where C_0 is the chlorophyll-a concentration in the control ($\mu g\ L^{-1}$) and C^t is the chlorophyll-a concentration after treatment with each coagulant ($\mu g\ L^{-1}$).

The floc characteristics were observed under a microscope (DP27, Olympus) to examine the aggregation behavior after treatment. This setup allowed for the evaluation and comparison of the efficiency of the four natural coagulants in removing phytoplankton from eutrophic water. Statistical analyses were conducted using both descriptive and inferential approaches. The results are expressed as the mean ± standard deviation (SD). The error bars are displayed in the graphs to indicate variability. The phytochemical constituents of the plant extracts used in this study were analyzed by ANOVA using IBM SPSS Statistics (version 22, licensed software) to assess whether their compositions differed significantly among the extracts. In addition, statistical

comparisons of removal efficiency across treatments were conducted with one-way ANOVA. Differences were regarded as statistically significant when the p value was less than 0.05.

Results
1) Water quality analysis
The eutrophication status of the study area was evaluated using the Carlson Trophic State Index (TSI) (Carlson, 1977). According to the index, chlorophyll-a concentrations above 80 $\mu g\ L^{-1}$ indicate a hypertrophic state. The ammonium-nitrogen concentration was 770.45±0.43 mg L^{-1}, the total phosphorus concentration was 214.65±0.53 $\mu g\ L^{-1}$, and the nitrate-nitrogen concentration was 10.26 ±0.61 mg L^{-1}. In this study, the concentration of chlorophyll-a in the water sample was 341.25±0.76 $\mu g\ L^{-1}$, indicating that the study area was in a hypertrophic state. Moreover, *M. aeruginosa* was identified as the predominant phytoplankton species.

2) Phytochemical analysis of plant extracts
The phytochemical composition of the plant extracts is summarized in Table 1. Statistical analysis revealed that the phytochemical constituents varied significantly among the different plant extracts. The total phenolic contents of the banana and orange peels were 46.95 ±9.02 and 32.71±20.73 mg GAE g^{-1}, respectively, whereas those of the basil and chia seed mucilage were 4.43 ±0.29 and 3.24±0.08 mg GAE g^{-1}, respectively. Similarly, the flavonoid content was greater in banana peel (94.25±6.46 mg QE g^{-1}) and orange peel (53.63±2.99 mg QE g^{-1}) than in basil seed mucilage (7.88±1.48 mg QE g^{-1}) and chia seed mucilage (7.00±0.64 mg QE g^{-1}). The antioxidant potential of the plant extracts was evaluated using a DPPH assay, which revealed significant antioxidant activity. Notably, both seed mucilage extracts exhibited DPPH (%) values comparable to those of the peel extracts, suggesting that basil and chia seed mucilage possess active antioxidants primarily in the form of polysaccharides, whereas banana and orange peels contain phenolic and flavonoid-based antioxidants. These results demonstrate that all four plant materials contain bioactive compounds capable of contributing to coagulation and flocculation processes through chelation and hydrogen bonding mechanisms, which promote charge neutralization and the formation of bridging interactions between the coagulant molecules and suspended particles. The major phytochemical constituents of each extract are detailed in Table 1.

3) Characterization of phytoplankton, natural coagulants and activated natural coagulants
3.1) FTIR analysis of phytoplankton
The main functional groups present in the phytoplankton were identified using FTIR-ATR, as shown in Figure 1(a). For phytoplankton, a prominent broad peak

at 3,266 cm^{-1} was observed, corresponding to O–H and N–H stretching vibrations associated with hydroxyl groups in carbohydrates and amine groups in proteins. The peak at 2921 cm^{-1} is attributed to aliphatic C–H stretching vibrations, indicating the presence of lipid components within the cell membrane. Peaks at 1,627 cm^{-1} and 1,543 cm^{-1} are characteristic of the amide I and amide II bands (C=O and C–N stretching along with N–H bending), respectively, and represent the proteinaceous content of the cells. The peak at 1,399 cm^{-1} corresponds to C=O stretching vibrations of carbonyl groups, whereas the peak at 1,041 cm^{-1} is assigned to C–O and P–O (P=O) stretching vibrations found in polysaccharides and phospholipids. Finally, the peak at 477 cm^{-1} is attributed to the C≡C–H and C–H bending vibrations of alkynes (Pradhan et al., 2007). The possible functional groups of the phytoplankton are summarized in Figure 1(a). These functional groups, including amino, hydroxyl, carboxyl, and phosphate groups, are likely to play a key role in interactions with natural coagulants during the coagulation–flocculation process.

Amino, carboxyl, and phosphate groups identified in the FTIR spectrum influence the surface charge of phytoplankton cells, as shown in Figure 1(a). The relative composition of anionic and cationic groups imparts amphoteric properties to the cell surface. Under neutral and basic environmental conditions, most phytoplankton cell surfaces exhibit a net negative charge because the abundance of carboxyl and phosphate groups is greater than that of positively charged amino groups (Kim et al., 2006). Conversely, under acidic conditions, the net positive charge predominates because of the protonation of the carboxyl and phosphate groups, as well as the presence of amino groups (Kim et al., 2006). A schematic representation of the phytoplankton cell surface and its functional groups is shown in Figure 1(b).

Table 1 Phytochemical constituents of the plant extracts (n = 3) used in this study

Plant extract	Phenolic (mg GAE g^{-1})	Flavonoid (mg QE g^{-1})	DPPH (%)
Banana peel	46.95±9.02[a]	94.33±6.46[a]	54.49±4.54[a]
Orange peel	32.71±20.73[a]	53.16±2.99[b]	46.58±2.47[b]
Basil seed mucilage	4.43±0.29[b]	7.88±1.48[c]	43.02±1.77[b]
Chia seed mucilage	3.24±0.08[b]	7.00±0.64[c]	41.51±5.34[b]

Remark: Values within the same column followed by different superscript letters (a, b, c) indicate statistically significant differences ($p < 0.05$) among the plant extracts, as determined by one-way ANOVA.
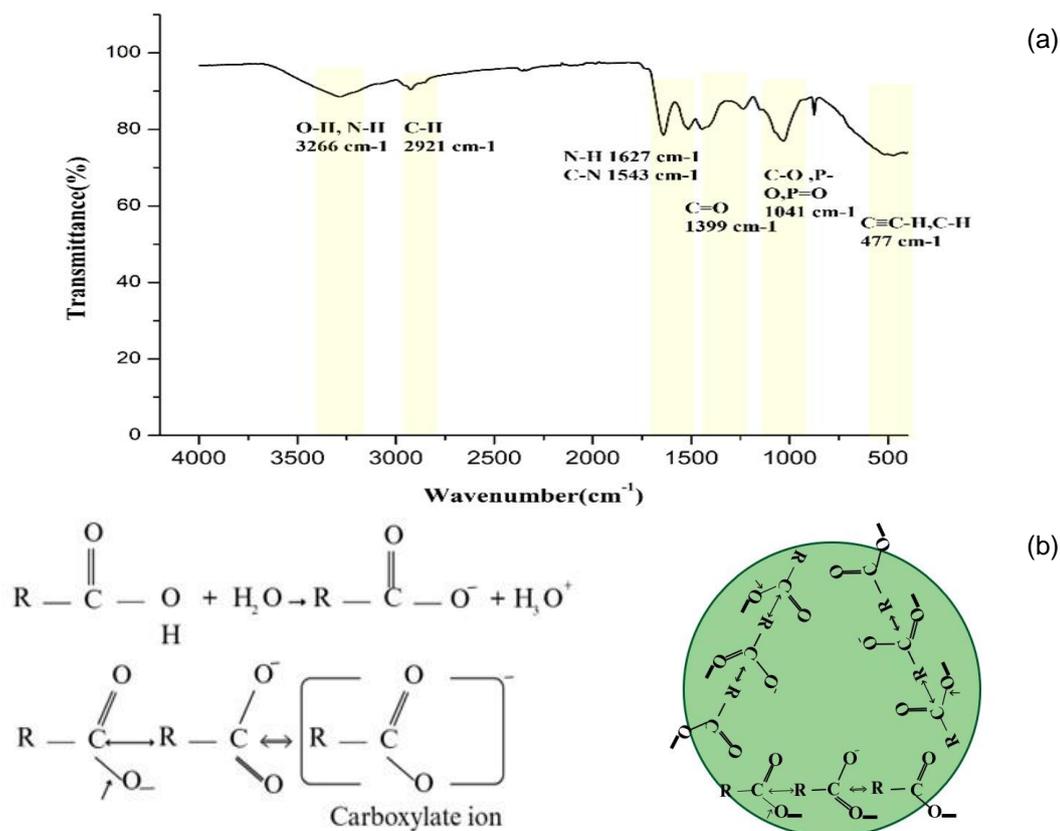
Figure 1 FTIR-ATR spectrum of phytoplankton showing the main functional groups, including hydroxyl (O–H), amine (N–H), aliphatic C–H, amide I and II, carbonyl (C=O), phosphate (P–O), and alkyne (C≡C–H) groups (a), and a schematic representation of the possible functional groups (amino, carboxyl, and phosphate groups) on phytoplankton cell surfaces, adapted from Pradhan et al. (2007) (b).

3.2) FTIR analysis of natural coagulants and activated natural coagulants

The characteristic functional groups of the natural coagulants were identified by FTIR-ATR spectra: (A) banana peel, (B) orange peel, (C) basil seed mucilage, and (D) chia seed mucilage (as illustrated in Figure 2a). Lines A and B represent the FTIR spectra of banana and orange peel powders, respectively. A broad absorption band at 3,350–3,200 cm^{-1} corresponds to O–H and N–H stretching vibrations of water and amines, indicating the presence of alcohol and phenolic groups (Bouaidi et al., 2020). Furthermore, the peaks observed at 1,790–1,740 cm^{-1} and 1680–1620 cm^{-1} are attributed to C=O and C=C stretching vibrations, respectively, confirming the presence of additional alcohol and phenolic groups (Kalibbala et al., 2023). As shown in lines C and D of Figure 2(a), the FTIR spectra of the chia and basil seed mucilage powders exhibit a broad absorption band at 3,350–3,200 cm^{-1}, corresponding to abundant O–H groups in the carbohydrates. An intense band at 1,089 cm^{-1} is attributed to C–O–H or C–O–R linkages (alcohols or esters) (Fard et al., 2021), whereas the band at 3,100–3,000 cm^{-1} corresponds to C–H stretching vibrations. Additionally, bending vibrations in the 1,700–1,400 cm^{-1} region are associated with carbonyl (C=O) stretching from esters, carboxylate groups (COO$^-$), and aliphatic chains (–CH$_2$– and –CH$_3$–), which form the basic structure of lignocellulosic materials (Figueiredo et al., 2022). Bands observed between 1,084–1,041 cm^{-1} are attributed primarily to polysaccharides containing galactose and glucan units (Venegas-Garcia et al, 2024). After activation with aluminum sulfate, the FTIR spectrum (Figure 2b) shows a distinct peak at 907 cm^{-1}, corresponding to Al–OH vibrations, which partially overlaps with the broad C–O stretching band at 1,089 cm^{-1}. These spectral features indicate that aluminum sulfate interacts with the anionic phenolic functional groups present in banana and orange peels. Similarly, the polygalacturonic acid present in the mucilage of basil and chia seeds was successfully complexed with aluminum sulfate via a sonication-assisted method. The phenolic compound structure and polygalacturonic acid structure are illustrated in Figure 2(c) and (d), respectively.

4) Comparison of the coagulation–flocculation performance of natural, activated natural, and commercial coagulants

The effects of natural and activated natural coagulants on the removal of chlorophyll-a containing *M. aeruginosa* are shown in Figure 3(a). The flocculation efficiency of chlorophyll-a increased when activated natural coagulants were applied, resulting in more than 90% cell removal. In comparison, the nonactivated natural coagulants removed approximately 50–60% of the phytoplankton cells, resulting in a residual chlorophyll-a concentration of 136 µg L^{-1}, which remains relatively high for aquatic systems. These findings indicate that when natural coagulants are applied in freshwater management, phytoplankton density should be carefully considered to ensure compliance with ecological safety criteria. Conversely, the activated natural coagulants achieved a removal efficiency of approximately 90%, reducing the residual chlorophyll-a concentration to 34 µg L^{-1}. At this level, phytoplankton density is less likely to adversely affect water quality and is therefore more suitable for practical applications, particularly in eutrophic water sources. Statistical analysis confirmed that the effects of activated natural coagulants differed significantly from those of natural coagulants on the removal of chlorophyll-a. The effect of the coagulant dosage was also investigated. Increasing the dosage from 0.5 to 2.5 g initially increased the removal efficiency to an optimal value; however, further increases beyond the optimum did not improve the removal efficiency and, in some cases, led to a decrease. At very low dosages, the removal efficiency was limited because the coagulant was insufficient to neutralize the cell surface charge.

The removal efficiency (%) of chlorophyll-a with different commercial coagulants, highlighting the influence of coagulant type on flocculation performance, is shown in Figure 3(b). The removal efficiency of chlorophyll-an increased when all commercial coagulants were applied, with more than 90% of the cells successfully flocculeting. Increasing the dosage of commercial coagulants from 0.25 to 3 g initially increased the removal efficiency, reaching an optimal value. Further increases beyond this optimum resulted in a decrease in removal efficiency. At excessively high dosages, the removal efficiency was limited because of steric effects and surface charge repulsion, which hinder effective particle aggregation. Statistical analysis indicated that the removal efficiency of different commercial coagulant types for chlorophyll-a significantly differed at each dosage tested. Among the tested coagulants, ferrous sulfate exhibited lower removal efficiency than ferric sulfate, aluminum sulfate, and PAC, likely because the bivalent nature of ferrous ions limits their ability to effectively interact with negatively charged *M. aeruginosa* cell surfaces.

5) ORP analysis and proposed mechanisms of coagulation–flocculation

The ORP is a substance's ability to either oxidize or reduce another substance. Substances with higher ORP values tend to acquire electrons and are referred to as oxidizing agents, whereas substances with lower ORP values have excess electrons and act as reducing agents. All the natural coagulants functioned as oxidizing agents, resulting in higher ORP values than those of the phytoplankton (74.57 mV). After activation by sonication with aluminum sulfate, the ORP values significantly increased for all the activated natural coagulants, as

illustrated in Figure 4, indicating that aluminum cations were incorporated into the structure of the coagulants. When natural and activated natural coagulants were applied to eutrophic water, the ORP decreased for all the treatments. This decrease is attributed to the interaction of positively charged species (e.g., aluminum ions) with the negatively charged surfaces of the phytoplankton cells, leading to charge neutralization. Phytoplankton aggregates subsequently formed and precipitated, resulting in a reduction in the chlorophyll-a concentration. However, this interpretation is based on indirect evidence, as surface charge was not directly measured in this study. Additional analyses, such as zeta potential measurements, are therefore recommended to confirm the proposed charge neutralization mechanism.
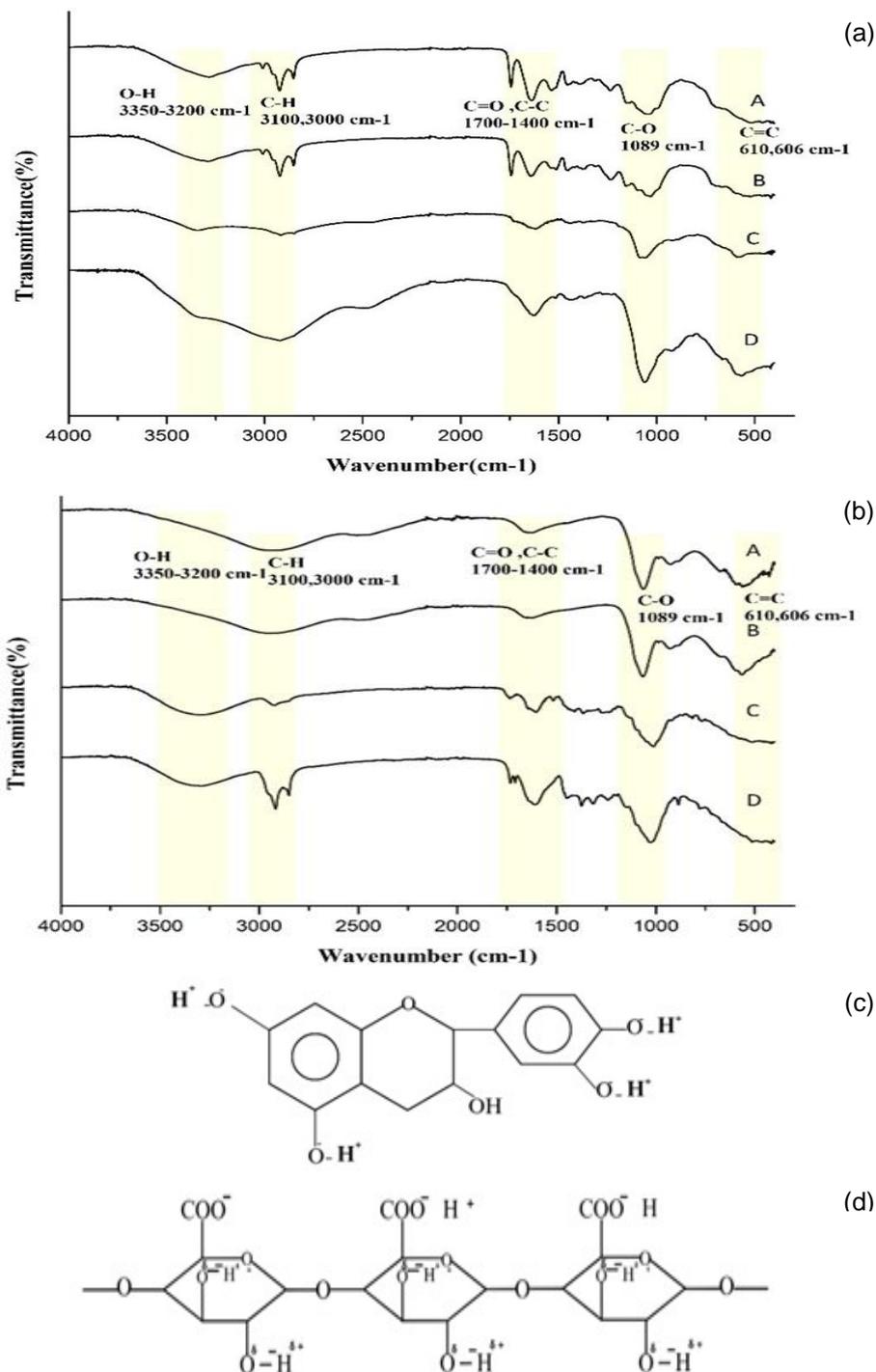

Figure 2 FTIR-ATR spectra of natural coagulants: (A) banana peel, (B) orange peel, (C) basil seed mucilage, and (D) chia seed mucilage, showing characteristic functional groups (a) and FTIR spectra of activated natural coagulants: (A) banana peel, (B) orange peel, (C) basil seed mucilage, and (D) chia seed mucilage after activation with aluminum sulfate (b) and schematic representation of the main bioactive compounds in the studied natural coagulants: phenolic compounds in (c) banana peel and orange peel and (d) polygalacturonic acid in basil seed and chia seed mucilage.
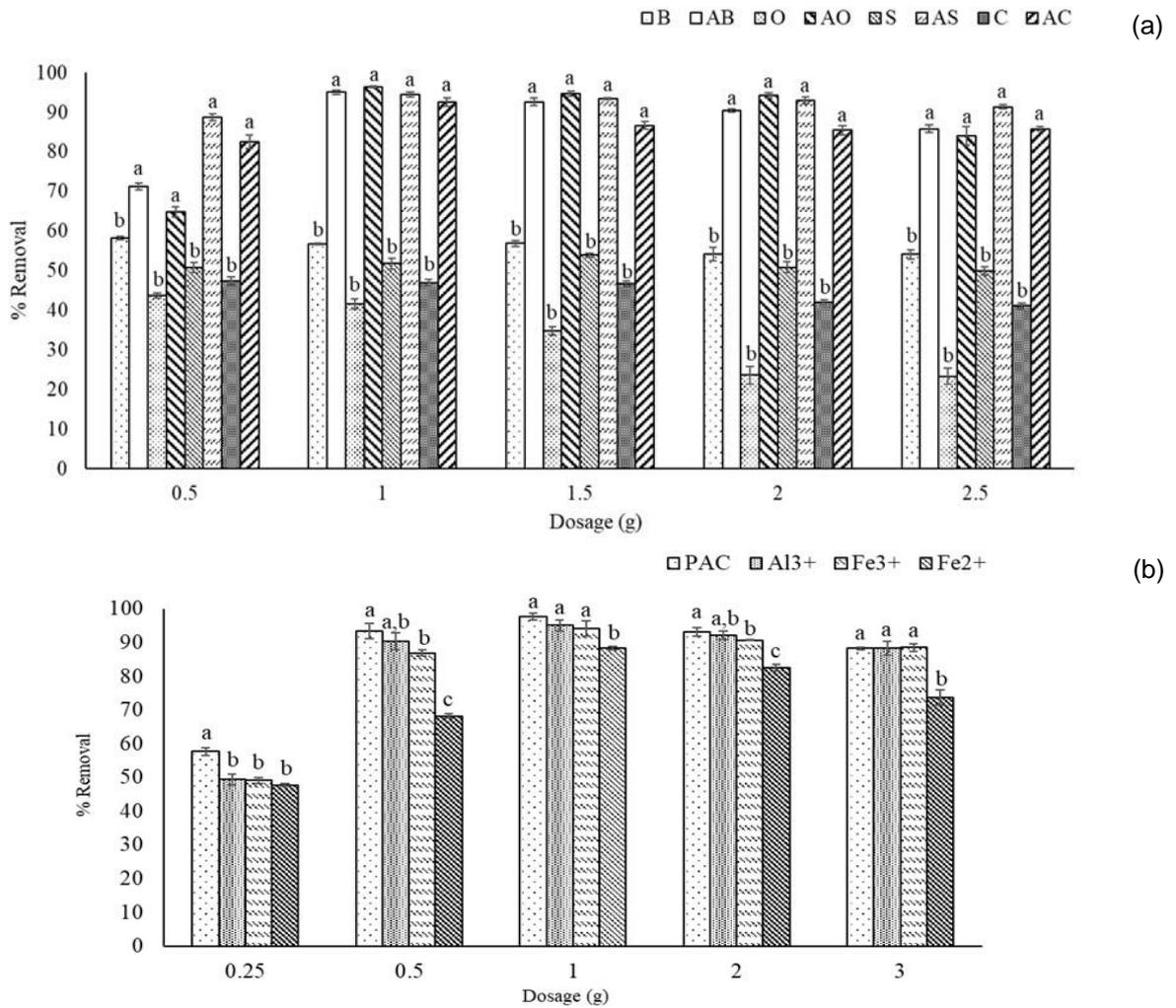
Figure 3 Removal efficiency (%) of chlorophyll-a using different natural coagulants (n = 3), showing the effect of coagulant type and dosage on flocculation (a) and using different commercial coagulants, illustrating the effect of coagulant type (b).

Remarks: (B) banana peel, (O) orange peel, (S) basil seed mucilage, (C) activated banana peel, (AO) activated orange peel, (AS) activated basil seed mucilage, (AC) activated chia seed mucilage and different superscript letters (a, b, c) on the bars indicate that activated natural coagulants differed significantly ($p < 0.05$) from natural coagulants in their removal efficiency of chlorophyll-a, as determined by one-way ANOVA.
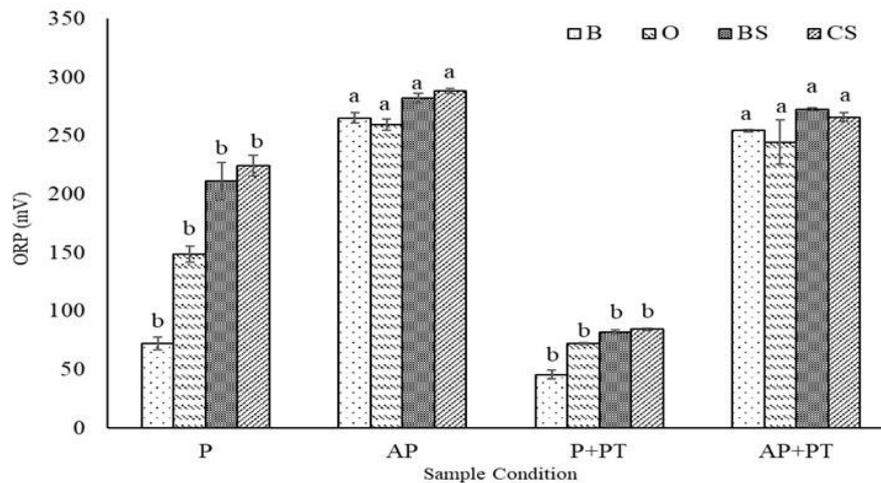
Figure 4 Oxidation–reduction potential (ORP) of natural coagulants (n = 3) under different conditions, showing their redox behavior before and after activation.

Remarks: (B) banana peel, (O) orange peel, (BS) basil seed mucilage, (CS) chia seed mucilage, (P) plant extracts, (AP) activated plant extracts, (P+PT) plant extracts+phytoplankton, (AP+PT) activated plant extracts+phytoplankton and different superscript letters (a, b, c) on the bars indicate significant differences ($p < 0.05$) in oxidation–reduction potential (ORP) before and after activation (P vs AP, P+PT vs AP+PT), as determined by one-way ANOVA.

6) Floc characteristics after flocculation

To further investigate the coagulation mechanism, the floc characteristics were examined using a microscope, as shown in Figure 6. In the top panel of Figure 5(a), (A) shows *M. aeruginosa* colonies, while (B), (C), and (D) illustrate cell destabilization and sweep flocculation induced by ferrous sulfate, ferric sulfate, and aluminum sulfate, respectively. Panel (E) shows *M. aeruginosa* flocs formed by PAC, which are spherical in shape because of polymer bridging. In the bottom panel of Figure 5(b), (A) and (B) show M. aeruginosa colonies treated with basil seed mucilage and chia seed mucilage, respectively, while (C) and (D) illustrate destabilization and sweep flocculation induced by banana peel and orange peel, respectively.

Discussion
1) Characterization of natural coagulants and activated natural coagulants

The phytochemical composition of the plant extracts was evaluated in terms of total phenolic content, total flavonoid content, and DPPH radical scavenging activity. The results revealed that all the plant extracts had a phytochemical composition that included phenolic compounds, flavonoids and DPPH. However, the phytochemical composition of banana peel extract is the greatest. Previous studies have reported that banana peels contain 53.80 ± 2.88 mg GAE g^{-1} of total phenolics, 16.44 ± 1.45 mg QE g^{-1} of total flavonoids, and $0.29 \pm 0.09\%$ DPPH (Isiam et al., 2021). Orange peels contain $9.61–31.62$ mg GAE g^{-1} of total phenolics and 35.50 ± 1.04 mg QE g^{-1} of total flavonoids (Lagha-Benamrouche et al., 2013). Basil seed mucilage contained 18.24 mg GAE g^{-1} of total phenolics, whereas chia seed mucilage contained 14.22 mg GAE g^{-1} (Nazir and Wani, 2021). However, it is challenging to compare our results with previous findings, as the extraction of phenolic compounds from natural matrices is complicated by their structural diversity and their susceptibility to oxidation and hydrolysis. Previous studies have shown that organic extracts containing phenolic compounds, such as fava bean (*Vicia faba*) (Bouaidi et al., 2020), *Moringa oleifera* (Ng and Elshikh, 2021), and sorghum seed (Shahzadi et al., 2024), can effectively remove turbidity when used as natural coagulants. In addition, mucilage from okra (Freitas et al., 2015), cactus (Kalibbala et al., 2023), and *Aloe vera* (Venegas-Garcia et al., 2024) has been successfully applied to remove metals and turbidity from wastewater. In addition, several factors can influence quantification, including fruit variety, environmental conditions, storage methods, ripening stage, extraction substrates, and genetic background. Moreover, parameters related to the extraction process itself, such as temperature, contact time, solvent-to-solid ratio, and solvent type, can further affect the outcomes.

2) FTIR analysis of natural coagulants

To further understand the coagulation mechanism, it is essential to characterize the functional groups of each plant extract. The FTIR provides a reliable method for identifying these functional groups and elucidating the chemical interactions involved in the coagulation process. The results revealed that banana and orange peels contain alcohol and phenolic functional groups, whereas the mucilage of chia and basil seeds contains ester groups, carboxylate groups (COO^-), and aliphatic chains ($–CH_2–$ and $–CH_3–$), which form the basic structure of lignocellulosic materials (Figueiredo et al., 2022). These findings indicate that compared with okra and *Aloe vera* mucilage, chia and basil seed mucilage is primarily composed of polysaccharides dominated by galactose, rhamnose, and galacturonic acid. The presence of galacturonic acid indicates that these mucilage extracts function as anionic electrolytes because of the deprotonation of their carboxyl groups (Freitas et al., 2015).

3) Coagulation–flocculation test and ORP analysis

This study demonstrated the effective removal of Microcystis by activated natural coagulants via coagulation–flocculation processes. The removal efficiency of chlorophyll-a increased when all the activated coagulants were applied, with more than 90% cell removal. However, increasing the dosage beyond the optimum did not further increase removal efficiency and, in some cases, resulted in a decrease. This trend is consistent with previous studies showing that overdosing natural coagulants can impair coagulation performance by restabilizing colloidal particles (Freitas et al., 2015; Shahzadi et al., 2024). Four main mechanisms contribute to coagulation and flocculation, including double-layer compression, sweep flocculation, adsorption and charge neutralization, and interparticle bridging (Fard et al., 2021). The proposed mechanism of action of the activated coagulants is described in Figure 6.
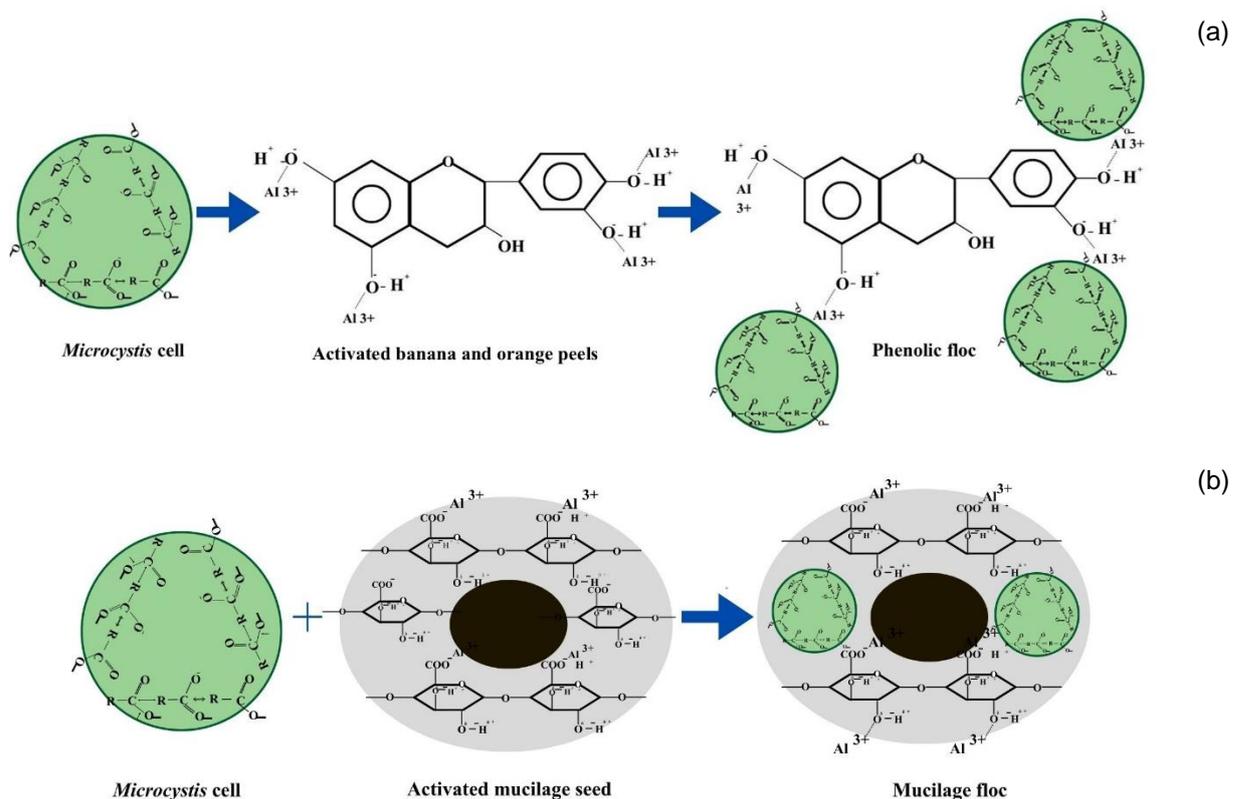
Figure 6(a) and (b) present the proposed mechanisms of coagulation–flocculation of *M. aeruginosa* by activated natural coagulants. Phytoplankton acted as reducing agents because of their electron-donating properties, whereas banana and orange peels functioned as oxidizing agents because the phenolic compounds in their structures bound aluminum ions from aluminum sulfate via sonication. Consequently, the activated natural coagulants destabilized the phytoplankton, leading to flocculation and effective removal of *M. aeruginosa*, the predominant species in the water samples. A similar mechanism was observed in basil and chia seed mucilage, where aluminum ions attached to the galacturonic acid structures in the mucilage through sonication. Compared with natural coagulants, all commercial coagulants resulted in high chlorophyll-a removal efficiency, resulting in more than 90% cell flocculation. Ferric and aluminum ions destabilize *M. aeruginosa* cells, leading to floc formation,

whereas sweep flocculation occurs at higher coagulant dosages. The higher removal efficiency of aluminum than of ferric sulfate can be attributed to its lower molecular weight (23 g mol⁻¹ vs. 55.8 g mol⁻¹), which facilitates more efficient attachment to cell surfaces. PAC has the highest removal efficiency because of its dual-structure: a cationic aluminum "head" and a long polymer "tail," enabling polymer bridging between cells.

An ORP analysis was further conducted to provide insights into the electrochemical interactions that occur during the coagulation process. Activated natural coagulants act as strong oxidizing agents and are capable of accepting electrons from reducing agents such as phytoplankton. When activated coagulants were added to the phytoplankton samples, the ORP values slightly decreased because redox reactions occurred during the coagulation–flocculation processes. This phenomenon confirmed that activated coagulants acted as oxidizing agents, while phytoplankton acted as reducing agents.

The results of floc characterization demonstrated that all the activated coagulants effectively precipitated

Microcystis through coagulation–flocculation processes, leading to a substantial reduction in phytoplankton biomass. This reduction is particularly important from a public health perspective. According to the WHO recreational water quality guidelines, chlorophyll-a concentrations < 10 µg L⁻¹ are associated with a low acute health risk, concentrations between 10–50 µg L⁻¹ indicate a moderate acute health risk, and concentrations > 50 µg L⁻¹ correspond to a high acute health risk (U.S. Environmental Protection Agency, 2019). Therefore, decreasing chlorophyll-a levels through effective coagulation not only improves water clarity but also contributes to lowering potential health risks for recreational water users. Similar outcomes have also been reported in previous studies conducted in natural water bodies. For example, Tongman et al. (2024) demonstrated that the application of PAC as a coagulant effectively reduced phytoplankton in lentic waters. In addition, the amount of accumulated sludge gradually decreased over a two-week period, likely because of ongoing biodegradation processes, suggesting that posttreatment residues may be naturally attenuated over time.

Figure 6 Proposed mechanisms of coagulation–flocculation of *M. aeruginosa* by activated natural coagulants: (a) phenolic compounds in banana and orange peels activated with aluminum sulfate, and (b) polygalacturonic acid in basil and chia seed mucilage activated with aluminum sulfate.

Conclusions

In this study, banana peel, orange peel, basil seed mucilage, and chia seed mucilage were successfully activated with aluminum sulfate using a sonication method to serve as eco-friendly natural coagulants. These plant-derived materials, obtained from fruit wastes

and seed mucilage, effectively removed chlorophyll-a (*M. aeruginosa* as the predominant phytoplankton species) with efficiencies exceeding 90%, comparable to those of conventional chemical coagulants. FTIR analysis revealed phenolic compounds in banana and orange peels and polygalacturonic acid in basil and

chia seed mucilage, which facilitated aluminum ion incorporation and increased the oxidizing potential, as supported by the ORP measurements. The coagulation–flocculation mechanisms were clarified through FTIR, ORP, and microscopic observations, which revealed that peel-based coagulants acted mainly via charge neutralization and sweep flocculation, whereas mucilage-based coagulants promoted polymer bridging through their long-chain polygalacturonic structures. To ensure the development of more sustainable strategies for *Microcystis* control in lentic water systems, further investigations are recommended, particularly regarding residual aluminum levels, microcystin toxicity, cost-effectiveness, and the feasibility of scaling these materials for use in municipal wastewater treatment plants or natural water bodies. Overall, this study highlights the potential of integrating agricultural byproducts and seed-derived mucilage as sustainable, low-cost, and environmentally friendly coagulants, offering a promising alternative for managing harmful algal blooms and mitigating eutrophication in freshwater systems in Thailand and beyond.

Acknowledgements

Data availability statement

Information and data used in the study will be disclosed upon request.

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Conflicts of interest

The authors declare that there are no conflicts of interest in competing financial or personal relationships that could have appeared to influence the work reported in this work.

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