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## The influence of preparation and pretreatment on the physicochemical properties and performance of plant-based biocoagulants in treating wastewater

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### ABSTRACT

Biocoagulants have gained attention as an alternative to chemical coagulants in water and wastewater treatment due to their ability to remove pollutant parameters such as turbidity, suspended solids, color, and organic compounds. Plant-based biocoagulants are currently the most promising due to their abundant availability and reliable performance. A proper preparation of plant-based biocoagulants is required to ensure the active compounds from the plants can be extracted. Plant raw materials are prepared into biocoagulants using drying and grinding, extraction, and purification. In mechanical preparation, drying and grinding exhibit great influence on the performance of plant-based biocoagulants due to the removal of moisture and higher contact with the carrier medium during dissolution. A smaller biocoagulant's size resulted in up to 78 % higher removal of turbidity. In chemical preparation, the oil needs to be removed before extracting the active ingredients to avoid low active compound extraction yields. The defatting of *Moringa oleifera* seeds showed an 18 % higher protein content. Salt and alcohol extractions were mentioned as superior extraction methods to obtain carbohydrate and protein from plant-based biocoagulants compared to water extraction, with up to a 5 % increment in turbidity removal. Preparation and pretreatment protocols have a great influence on the properties and performance of plant-based biocoagulants. Further extension of research on plant-based biocoagulants should address the problems in the initial characterization of plant material, standard pretreatment and preparation protocols, and real-scale application of plant-based biocoagulants.

### 1. Introduction

Biocoagulant has gained attention as an alternative compound to substitute the chemical coagulant in water and wastewater treatment (Ahmad et al., 2021a). Biocoagulant showed good capability in removing several common water pollutant parameters, including turbidity (Kurniawan et al., 2021b), suspended solids (Adnan et al.,

2017), color (Miyashiro et al., 2021), and organic compounds (Hoa and Hue, 2018). Biocoagulants may be sourced from several natural resources, including animals (Iber et al., 2021), plants (Ahmad et al., 2021a), and microorganisms (Al-Wasify et al., 2015). Among the various sources, plant-based coagulants are currently the most promising due to their abundant availability and reliable performance (Ahmad et al., 2021a; Kurniawan et al., 2021a).

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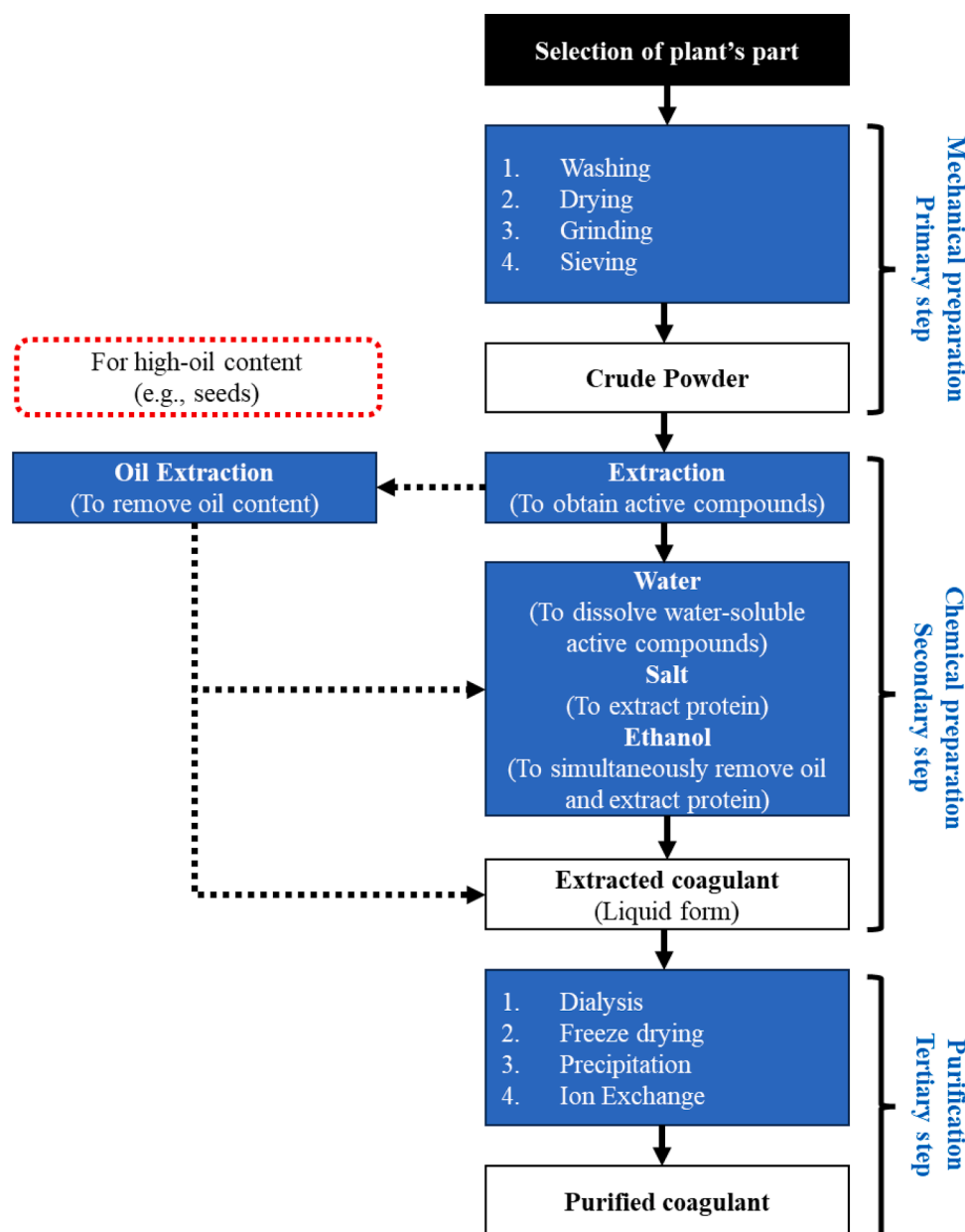


Fig. 1. Flowchart of preparation process for plant-based biocoagulants

Preparation of plants' raw materials into biocoagulants is commonly done using drying and grinding as preparation methods (Zaidi; et al., 2019). The function of drying and grinding is to obtain powder from the plant materials. Different sizes of plant materials may give different performances during treatment. Finer biocoagulant particles exhibit greater removal performance, as stated by Putra and Airun (2021). The size of 150 mesh of dried *Carica papaya* seeds gave 89.3 % turbidity removal, while it increased to 92.2 % with the finer size of 250 mesh. Similarly, Siddique et al. (2016) also highlighted that the finer walnut shell of 50 mesh gave significantly higher removal as compared to 30 and 40 mesh. Due to the high variation of plant species used as biocoagulants, several pre-treatments are often also required to extract the active compounds from the raw materials. Kurniawan et al. (2022) mentioned that hydroxyl, amine, carboxyl groups, and protein contribute the most to the coagulation-flocculation process using biocoagulants.

Acid, base, solvent, salt, and water extractions are mentioned to be used as the pretreatment of plant-based biocoagulants to extract their

active compounds. Different extraction methods for the same plant material may exhibit different removal performances (Ahmad et al., 2022a). Benalia et al. (2018) mentioned the utilization of water, NaOH, NaCl, and HCl to extract acorn leaves, which showed turbidity removal of 84.77 %, 85.92 %, 91.07 %, and 92.92 %, respectively. Similarly, Abidin et al. (2013) utilized water, NaOH, and NaCl to extract *Jatropha curcas* seed, resulting in 99.4 %, 99 %, and 91.4 % turbidity removals, respectively.

There are many reviews discussing the utilization of plant-based biocoagulants for water and wastewater treatment, such as those by Iber et al. (2021), who reviewed the pros and cons of plant-based coagulants vs. chitosan focusing on aquaculture wastewater treatment, and Owodunni and Ismail (2021), who reviewed the application of plant-based biocoagulants for industrial wastewater treatment. In addition, Ahmad et al. (2022a) discussed several extraction methods, while Leiviskä and Santos (2023) reviewed purification techniques as pretreatments for plant-based biocoagulants. Currently, no review on the effect of the preparation and pretreatment of plant-based

biocoagulants on its physicochemical properties is available. Different performances obtained under different preparations and pretreatments clearly showed an indication that different interactions of biocoagulants with pollutants occurred. However, explanations of how the preparation and pretreatment of biocoagulants may affect their interactions with pollutants are currently also scarce. This review paper discusses the effect of preparation (to obtain powder form) and pretreatment (active compound extraction) methods on the physicochemical properties of plant-based biocoagulants and its relation to the removal performances. The presented review is expected to shed light on the determination of appropriate preparation and pretreatment processes for plant-based biocoagulants as an important step before the utilization in water and wastewater treatment to obtain the highest removal performance.

## 2. Plant-based biocoagulant production process

With the current growing concerns about the usage of chemical coagulants to treat wastewater, which affects the environment and human health, alternative approaches have been explored to resolve the issue. The plant-based source is one of the natural coagulants that is currently under research and exploration to investigate its full potential to replace chemical coagulants. Plant-based biocoagulants are the most frequent compared to animal-based and microorganisms-based biocoagulants due to their abundance and availability (Kurniawan et al., 2022; Saleem and Bachmann, 2019). Other advantages considered for its applicability are safety for humans, biodegradability, cost effectiveness, reduction in sludge formation, and sustainability (Ahmad et al., 2022b), these criteria have been good indicators for plants to be selected as alternative coagulants.

Plant-based biocoagulants consist of ionic or non-ionic polymers (Okoro et al., 2022), which can be obtained from different plants' parts (Saleem and Bachmann, 2019). Among the parts that are usable as coagulants are seeds, leaves, roots, fruits, mucilage, grains, gum, and sclerotium (Ahmad et al., 2021a; Saleem and Bachmann, 2019). In the ongoing research on water treatment, *Moringa oleifera* is the common plant used as a plant-based coagulant, with the seed being the most investigated part of the study. Other promising plant's parts that have been studied included leaves (*Manihot esculenta*, *Stenochlaena palustris*, *Melastoma malabathricum*, *Azadirachta indica*, *Piper sarmentosum*, *Syzygium polyanthum*, and *Dicranopteris linearis*) (Ahmad et al., 2021a), fruits from soy or sago (Muniz et al., 2020) and mucilage from okra and cactus (Ang and Mohammad, 2020; Owodunni and Ismail, 2021). This set of plant examples contains active compounds that possess potential for the coagulation-flocculation process by promoting specific working mechanisms such as polymer bridging, charge neutralization, and adsorption (Kurniawan et al., 2022).

Generally, these active compounds can be categorized into proteins and carbohydrates, which are responsible for the performance of plant-based coagulants (Ahmad et al., 2021a). Thus, a proper preparation of plant-based coagulants is required to ensure the active compounds from the plants can be extracted for a possible higher amount for the efficiency of wastewater treatment. A flowchart is presented in Fig. 1 showing a process of plant-based coagulants, which is required for the raw material of plants' parts into three stages of primary, secondary, and tertiary for the production process to produce coagulant form in crude powder, extracted solution, and purified form. Further discussion on the relevant stages will be elaborated on in the next section.

## 3. Mechanical preparation

### 3.1. Washing

Washing is commonly the initial step before further processing for biocoagulant. Washing is performed to remove contaminants, such as grain impurities and sand or soil, in order to prevent the presence of fungi and yeast and enhance the pretreatment phase of biocoagulant

extraction (Ang and Mohammad, 2020; Sowmeyan et al., 2011). Washing can be conducted using tap water or more specific solutions, such as distilled water (Ahmad et al., 2021a; Kurniawan et al., 2020).

### 3.2. Drying

Drying is one of the primary steps in the preparation of biocoagulant. Drying is used mostly to remove water content and moisture inside the raw materials (Mahmood and Zaki, 2019). Drying can be carried out naturally using sunlight (Ahmad et al., 2021a; Kristianto, 2017) or assisted by an oven/microwave (Ibrahim et al., 2021). Drying has a critical effect on the characteristics of biocoagulants. Appropriate drying temperature and time need to be analyzed before performing this step to assure that no active compounds, such as proteins, be defected by this process (Kurniawan et al., 2022). In addition to the destruction of active compounds, too high temperatures of drying may cause the phenomenon of "case hardening" in which the outer part of the materials is dried too fast and hardens, preventing the inner part of moisture from escaping, and lowering the efficiencies of moisture removal (Ibrahim et al., 2021).

### 3.3. Grinding and sieving

Grinding and sieving are two unseparated steps to obtain a uniform size of the biocoagulant powder (Ahmad et al., 2022a; Anju and Mophin-Kani, 2016). Powder biocoagulant is preferable for selling and storage purposes over liquid form. In addition, the smaller powder size of the biocoagulant allows the materials to have more contact with the carrier solution later during usage (Beyene et al., 2016; Mohamed et al., 2015). Putra and Airun (2021) reported that the smaller particle size of 250 mesh of biocoagulant produced from *Carica papaya* seeds showed higher efficiency in removing turbidity from wastewater as compared to the 150 mesh. This result was obtained due to the higher contact of active ingredients in the biocoagulant with the carrier solution during dissolution. Overall, the mechanical preparation was carried out to obtain crude powder (focusing on the changing of physical form) with minimum alteration in chemical properties.

## 4. Chemical preparation

Plant-sourced natural coagulant can be used after a simple powdering preparation (as discussed in Section 3) or modified chemically to extract its active component and thus improve its coagulant activity. As widely highlighted, protein is a crucial component in the natural coagulation mechanism (Kurniawan et al., 2023, 2021b). Research on extracting and purifying active components started to be relevant when studies found that employing fresh coagulant in its original form cause an increment in organic load in the water after treatment (Baptista et al., 2015). Focusing on chemical preparation, chemical solvents are used to extract active compounds from plants with different outcomes depending on plant species and part, besides the solution and methods used (Ahmad et al., 2021a; Saleem and Bachmann, 2019). Water extraction is a very common methods to obtain liquid form of biocoagulants and activating some water-soluble active ingredients (e.g., some functional groups) (Ndabigengesere et al., 1995). Salt and alcohol solutions as substitutes for water for coagulant preparation had been taking place, as believed to produce coagulant with greater performance (Ahmad et al., 2021b). Despite its excellent performance, the disadvantages of liquified chemically extracted coagulant are its short shelf life and need to be stored at  $-18^{\circ}\text{C}$  to maintain its characteristics (Ueda Yamaguchi et al., 2021). While mechanically prepared coagulant can last up to 12 months without deterioration, but it could increase turbidity in treated water (Lim et al., 2018). One alternative to lengthen the shelf life of extracted coagulant is to store it in freeze-dried solid precipitate form, which can last more than 19 months in room temperature (Ueda Yamaguchi et al., 2021).

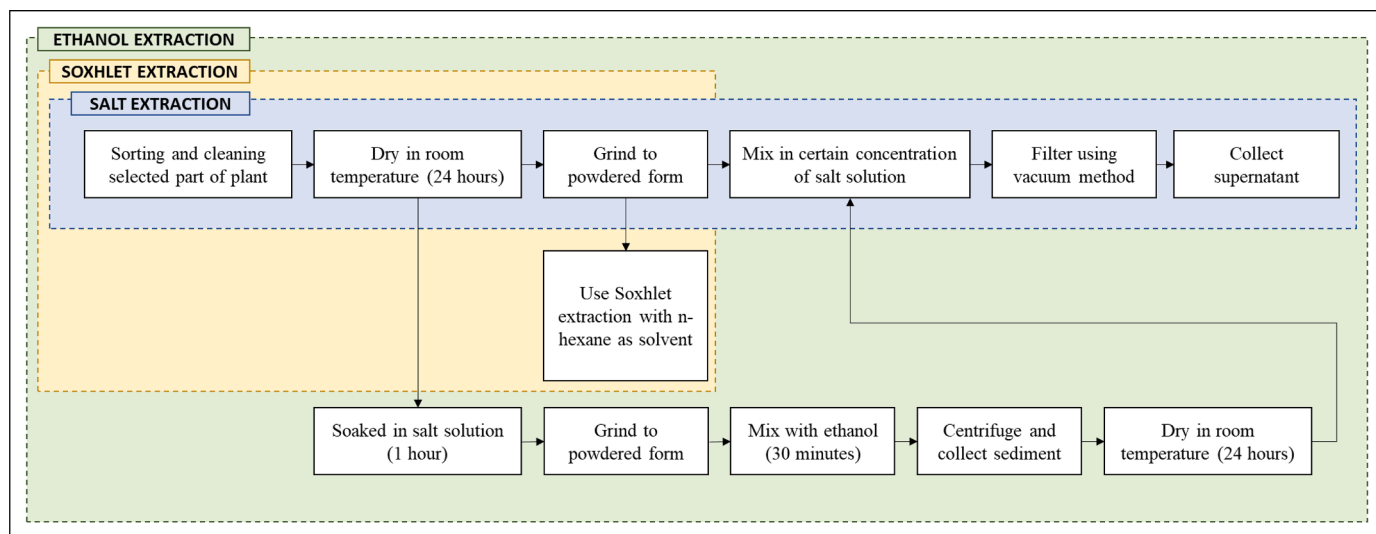


Fig. 2. General process flow for chemical preparation of plant-based biocoagulant

#### 4.1. Oil extraction

Oil extraction is usually done to separate the oil content from a selected plant coagulant to add its sustainability values (Magalhães et al., 2021). It can be reutilized for various purposes such as industrial, food, and renewable energy. The advantages of oil extraction are that it reduces the organic load from coagulants while producing valuable oil as a by-product (Camacho et al., 2017; Ueda Yamaguchi et al., 2021). Oil content in plants was reported to cause a reduction in coagulation binding sites that affect its effectiveness by re-stabilizing the destabilized particles, creating the need of oil extraction (Ahmad et al., 2021b).

Through oil extraction, only the contents of fatty acids, phenolic, and aromatic compounds were reduced while retaining the value of protein and polysaccharides in the extracted coagulant. Significant performance differences with oil and grease removal of approximately 82 % for both extracted and unextracted *M. oleifera* coagulant in treating synthetic oilfield-produced water proved that protein content was not altered after the oil extraction process (Camacho et al., 2017; Magalhães et al., 2021). Besides, oil-extracted seed of *M. oleifera* also widens the operational condition variety since it is allowed and proven to be compatible with a wider pH range of water, such as drinking water, produced water, and wastewater. The SEM images also showed a reduction in agglomerate volume with an increment of surface area on the coagulant after oil extraction (Magalhães et al., 2021). Oil extraction commonly carried out by Soxhlet extraction method using n-hexane as solvent (Magalhães et al., 2021).

#### 4.2. Salt extraction

Salt extraction was used to extract protein from plant coagulant, which helps to increase coagulation activity through its ionic strength and protein solubility compared to non-extracted plant coagulant (Ueda Yamaguchi et al., 2021). The existence of salt also helps in particle aggregation due to its ionic strength, which enables double layer compression to happen (Benalia et al., 2018). Typical salts used for protein extraction are NaOH, NaCl, NaNO<sub>3</sub>, KCl, KNO<sub>3</sub>, and KCl (Madrona et al., 2010; Ueda Yamaguchi et al., 2021). Salt extraction is usually done by blending and agitating cleaned, grinded selected plant parts with a specified concentration of salt solution for 30 min using a magnetic stirrer. Mixed solution then filtered through vacuum method to collect the supernatant as extracted coagulant (Baptista et al., 2015; Madrona et al., 2010). Increment in concentration of salt solution used in the extraction process practically produced liquified coagulant with

linearly better coagulant performance. As studied by Madrona et al. (2012), *M. oleifera* seed extracted using KCl to treat superficial water exhibited different turbidity removals of 60, 80 and 99.7 % using 0.01, 0.1, and 1 M of KCl, respectively. Protein content increased from 9750 to 23,400 mg/L for 0.01 M and 1 M KCl salt solutions, respectively. But there is also a study found that coagulation activity for NaOH-extracted coagulant only increases with an increment in salt concentration until it reaches its maximum point. This is due to the possibility of denaturing proteins at high concentrations of NaOH, causing protein insolubility in the extracting solution (Benalia et al., 2018).

#### 4.3. Ethanol extraction

Ethanol extraction basically combines salt extraction and oil extraction using ethanol instead of the Soxhlet method (Fig. 2) to simultaneously remove oil and extracting protein contents. Camacho et al. (2017) first washed *M. oleifera* with 1 M NaCl before oil extraction using 95 % ethanol was executed. Dried powder from the previous procedure were then mixed with 1 M NaCl and filtered to get coagulant in the form of supernatant. This extraction did not cause a significant difference in protein percentage in *M. oleifera* seed but obviously reduced the oil content to 7 % compared to 30 % of the unextracted seed. After the ethanol extraction, plant-based biocoagulants will be available in liquid form with retained protein and some polysaccharide content (Plaskova and Mlcek, 2023).

### 5. Influence of preparation and pretreatment to physicochemical properties and performance

Mechanical preparation is usually carried out to obtain biocoagulants with small particle sizes and dry conditions. By obtaining particles of small and fine size, the surface area will be wider, so the reaction rate will increase. The particle size of the biocoagulant affects the efficiency of pollutant removal. This is proven by research conducted by Novita et al. (2019) that treated coffee wastewater with biocoagulant from *Moringa oleifera* seeds with variations in biocoagulant size at 60, 70, and 80 mesh. The results of the experiment showed that the size of 80 mesh has greater turbidity removal efficiency than 70 and 60 mesh, namely 91.35 %, 88.93 %, and 88.15 %, respectively. The same proof was also carried out by Putra and Airun (2021) and Siddique et al. (2016) as described above.

The water content in the biocoagulant material should be removed so as not to interfere with the extraction process. Removal of water content

**Table 1**  
Performance of plant-based biocoagulants in treating wastewater

Name of Plant	Part of Plant	Type of Wastewater	Operational Condition	Optimum Condition	Removal Performances	Refs.
<i>Carica papaya</i>	Seeds	Textile	100 rpm for 5 min then 40 rpm for 4 min followed by 60 min sedimentation	1000 mg/250 mL	Turbidity: 92.2 %	Putra and Airun (2021)
<i>Cassia obtusifolia</i>	Seeds	Palm oil mill effluent	Rapid mixing 150 rpm for 5 min and slow mixing 50 rpm for 15 min followed by 60 min of sedimentation	2.5 g/L	TSS: 87 %	Shak and Wu (2015)
<i>Moringa oleifera</i>	Seeds	Textile	150 rpm for 2 min then 50 rpm for 25 min followed by 60 min sedimentation	5 g/100 mL	Turbidity: 70.4 % Color: 44.5 %	Ibrahim et al. (2021)
<i>Moringa oleifera</i>	Seeds	Synthetic wastewater	High stirring 100 rpm for 2 min, low stirring 30 rpm for 15 min, settling time 20 min	5 g/100 mL	Turbidity: 91.63 %	Zaid et al. (2019)
<i>Moringa oleifera</i>	Seeds	Market wastewater	Agitated 40 rpm for 20 min	5.0 g/L dose, pH 7.73	Turbidity: 82.86 %	Magaji et al. (2015)
<i>Moringa oleifera</i>	Seeds	Market wastewater	Agitated 40 rpm for 20 min	100 mL/L, pH 7.40	Turbidity: 88.03 %	Magaji et al. (2015)
<i>Moringa oleifera</i>	Seeds	Synthetic wastewater	1 min for 100 rpm and 30 min for 30 rpm	0.4–0.6 mL/L	Turbidity: 88 %	Skaf et al. (2021)
<i>Moringa oleifera</i>	Seeds	Coal beneficiation plant effluent for	Mixing 15 min, settling 30 min	8 mL/L	TSS: 97.40 % Turbidity: 97.48 %	Kapse and Samadder (2021)
<i>Moringa oleifera</i>	Seeds	Synthetic turbid water	-	40 wt% fraction of biocoagulant	Turbidity: 93 %	Saleem et al. (2020)
<i>Moringa oleifera</i>	Seeds	Domestic wastewater tertiary treatment	Rapid mixing 1200/s for 30 s and slow mixing 50/s for 15 min	600 mg/L	Turbidity: 94 % Color: 73 % Bacterial load: 99 %	Vega Andrade et al. (2021)

needs to be done to maximize protein extraction (Ibrahim et al., 2021). A similar study has been reported by Olabode et al. (2015) that water content can affect water-soluble proteins, so it needs to be removed from the biocoagulant raw material. An optimum drying condition is required to maintain the active compounds inside the biocoagulant. Ibrahim et al. (2021) reported that hot air drying at 50 °C for 6.5 h removed up to 67.8 % of the moisture content of *M. oleifera* seeds as compared to lower removal at 40 and 60 °C. In addition to drying, Putra and Airun (2021) also reported that the biocoagulant size of 250 mesh showed better performance in removing TDS as compared to 150 and 200 mesh, indicating that the smaller particle size of biocoagulant gives better efficiency.

In addition to mechanical preparation to obtain dry and fine particles of biocoagulant, another preparation that is often used is extraction. The extraction that is often carried out after the biocoagulant powder is obtained is the extraction of oil or defat. The presence of oil will increase the amount of organic matter added to the water, and oil extraction is recommended as a method for purifying biocoagulant extracts (Zaid et al., 2019). Oil extraction can also increase the activity of the *Moringa oleifera* biocoagulant (Garcia-Fayos et al., 2016). This was proven by an experiment comparing the efficiency of turbidity removal using the biocoagulant *Moringa oleifera*, which was previously carried out with oil extract and without oil extract. Biocoagulants treated with oil extraction were able to remove turbidity up to 88 %, while those without oil extraction were 30 % lower. The solvents used in the experiment were varied to obtain the most effective type of solvent, and from three solvents (hexane, acetone, and ethanol), the ethanol solvent was proven to be more effective (Garcia-Fayos et al., 2016). Similar experiments were also carried out by Magaji et al. (2015) which prove that the *M. oleifera* biocoagulant that was previously defatted has a turbidity removal efficiency that is 1.46 times higher than that without defatting. Although the difference tends to be small, it has been proven to affect the efficiency of turbidity removal. Kapse and Samadder (2021) also defatted the *M. oleifera* press cake biocoagulant and were able to remove 97.4 % of TSS and 97.48 % of turbidity from coal beneficiation plant effluent.

Another extraction besides the removal of fat or oil is the extraction of the active ingredients in biocoagulants. There are several types of solvents used in the extraction process, according to the type of biocoagulant raw material. Commonly used solvents are distilled water, alcohol/organic solvents, salt solvents, ammonium acetate, etc. (Ang and Mohammad, 2020; Magaji et al., 2015; Zaidi et al., 2019). The

concentration of the added solvent affects the removal efficiency of the pollutant. Bouchareb et al. (2021) varied the concentration of NaCl in the extraction of biocoagulant *M. oleifera* at 0.5, 1.0, and 1.5 M. The results of experiments with a dose of 140 mg/L biocoagulant showed that the removal of turbidity and BOD<sub>5</sub> was the least at 1 M NaCl concentrations, namely, 97.14 % and 96.67 %, while at concentrations of 1.5 M, performance decreased. According to Abidin et al. (2013), this phenomenon is called salting-out, in which protein solubility decreases with salt concentrations above the optimum. Bouchareb et al. (2021) also compared the performance of the biocoagulant with the extraction of active ingredients using distilled water and NaCl. The experiment yielded information that extraction with NaCl reduced the optimum dose of biocoagulant from 170 mg/L to 140 mg/L. This shows that the amount of active biocoagulant ingredients extracted using a sodium chloride solution is greater than that extracted using distillate water. Protein solubility will increase in the presence of salt at an optimum concentration (salting-in phenomenon) (Abidin et al., 2013). Thus, a lower coagulant concentration is required to achieve greater pollutant removal efficiency because more biocoagulant active agents mean more biocoagulation activity is possible (Khalid Salem et al., 2023). Magaji et al. (2015) also extracted the biocoagulant protein *M. oleifera*, which had previously been defatted. The results of the coagulation experiments showed that protein extract from the biocoagulant *Moringa oleifera* was able to provide higher turbidity removal efficiency than without extraction, namely 88.03 % and 82.86 %, respectively.

After the extraction process, the last step to obtain a pure biocoagulant is purification. The purification methods commonly used are ion exchange, dialysis, lyophilization, and precipitation (Ahmad et al., 2022a). Purification of biocoagulants is rare, and few studies have used this process. Some of them purify their experiments, namely Ghebremichael et al. (2005) who purified *M. oleifera* biocoagulant using ion exchange with cation exchange resin. From the results obtained, it can be concluded that purification can reduce the organic matter content in the wastewater treatment system. Another study by Taiwo et al. (2020) also purified *Moringa oleifera* with ion exchange and gel filtration chromatography. Through this purification, biocoagulants can reduce total coliform by up to 58 %. Information regarding the effect of pretreatment of plant-based biocoagulants on their physicochemical properties and performance from several studies can be seen in Table 1.

It is clear that preparation and pretreatment give significant improvements to the plant-based biocoagulant's properties and

**Table 2**  
Influence of preparation and pretreatment to properties and performance of plant-based biocoagulants

Biocoagulant	Preparation and Pretreatment		Highlights	Refs.
	Mechanical Preparation	Chemical Preparation		
<i>Carica papaya</i> seeds	Grinding, sieving	-	Size 200 mesh: turbidity removal 86.2 % Size 250 mesh: turbidity removal 92.2 %	Putra and Airun (2021)
<i>Moringa oleifera</i> seeds	Grinding	-	Size 2 mm: turbidity removal 98 % Size 250 µm: turbidity removal 91 %	Zaid et al. (2019)
<i>Moringa oleifera</i> seeds	Grinding	-	Size 60 mesh: addition of turbidity up to 199 NTU Size 80 mesh: removal of turbidity 78 %	Novita et al. (2019)
Acorn leaves	-	Extraction	Water: turbidity removal 84.77 % NaOH: turbidity removal 85.92 % NaCl: turbidity removal 91.07 % HCl: turbidity removal 92.92 %	Benalia et al. (2018)
<i>Jatropha curcas</i> seeds	-	Extraction	Water: turbidity removal 99.4 % NaOH: turbidity removal 99 % NaCl: turbidity removal 91.4 %	Abidin et al. (2013)
<i>Moringa oleifera</i> seeds	-	Defatting	Seeds powder: carbohydrate 31.64 % Defatted powder: carbohydrate 53.4 % Seeds powder: turbidity removal 78 % Defatted powder: turbidity removal 85.5 %	Magaji et al. (2015)
<i>Moringa oleifera</i> seeds	-	Extraction	Water: turbidity removal 90 % NaNO <sub>3</sub> : turbidity removal 87 %	Vega Andrade et al. (2021)
<i>Moringa oleifera</i> seeds	-	Extraction	Ultrapure water extraction: protein 12.8 % 0.1 M NaCl extraction: protein 7.3 %	Skaf et al. (2021)

**Table 2 (continued)**

Biocoagulant	Preparation and Pretreatment		Highlights	Refs.
	Mechanical Preparation	Chemical Preparation		
<i>Moringa oleifera</i> seeds	-	Extraction	NaCl: turbidity removal 99 % NaOH: turbidity removal 95 %	Muthuraman and Sasikala (2014)
<i>Phaseolus vulgaris</i> kernels	-	Extraction	NaOH: turbidity removal 85 % NaCl: turbidity removal 85 %	Muthuraman and Sasikala (2014)
<i>Strychnos potatorum</i> seeds	-	Extraction	NaOH: turbidity removal 93 % NaCl: turbidity removal 95 %	Muthuraman and Sasikala (2014)

performance. The influences of preparation and pretreatment are juxtaposed side-by-side in Table 2. The particle size of the biocoagulants affects the turbidity removal from wastewater. Smaller particle sizes allow for better solubility of compounds inside biocoagulants. However, too small a particle size may also cause an increase in turbidity due to the contribution of the biocoagulant's powder itself (Novita et al., 2019). Optimum particle size is specific to the type of the plant-based biocoagulant used; thus, the selection of optimum particle size needs to be conducted before the application of the biocoagulants. In addition to particle size, extraction methods affect the physicochemical properties and performance of biocoagulants in treating wastewater. For example, defatting *M. oleifera* seeds resulted in higher carbohydrate content (Magaji et al., 2015). Carbohydrate showed coagulation and flocculation activity by acting as a bridging agent with a high molecular weight (Liu et al., 2020). In addition to carbohydrate, protein also showed coagulation and flocculation activity via bridging or patch flocculation mechanisms due to its charge and molecular weight (Alju-boori et al., 2016; Czemińska et al., 2015; Kurniawan et al., 2022). As reported by Skaf et al. (2021), the use of ultrapure water extraction resulted in a higher protein content as compared to NaCl. Referring to the turbidity removal performances, salt extraction of NaCl showed higher turbidity removal as compared to other solutions for acorn leaves (Benalia et al., 2018) and *M. oleifera* seeds (Muthuraman and Sasikala, 2014). On the other hand, NaCl and NaOH extraction showed lower turbidity removal values for *Jatropha curcas* seeds as compared to water extraction (Abidin et al., 2013). From these results, it can be seen that the extraction solution was also specific to the type of plant-based biocoagulants used. The optimum extraction solution and method need to be conducted depending on what compounds inside plant-based biocoagulants are functionalized in coagulating and flocculating activities (Ahmad et al., 2022a; Kurniawan et al., 2022).

## 6. Current challenges and future approaches

Understanding that preparation and pretreatment play an important role in the plant-based biocoagulant's performance, several challenges are currently faced to simplify the preparation and pretreatment processes and to show the real performance. The challenges and future approaches as research directions are summarized in Fig. 3.

### 6.1. Initial characterization

Screening is a very crucial part in relation to removal performance (Ahmad et al., 2021a; Kristianto, 2017). Each plant species has different characteristics, and different plants' parts also have different chemical structure (Balestri et al., 2017). The challenge is to select the best

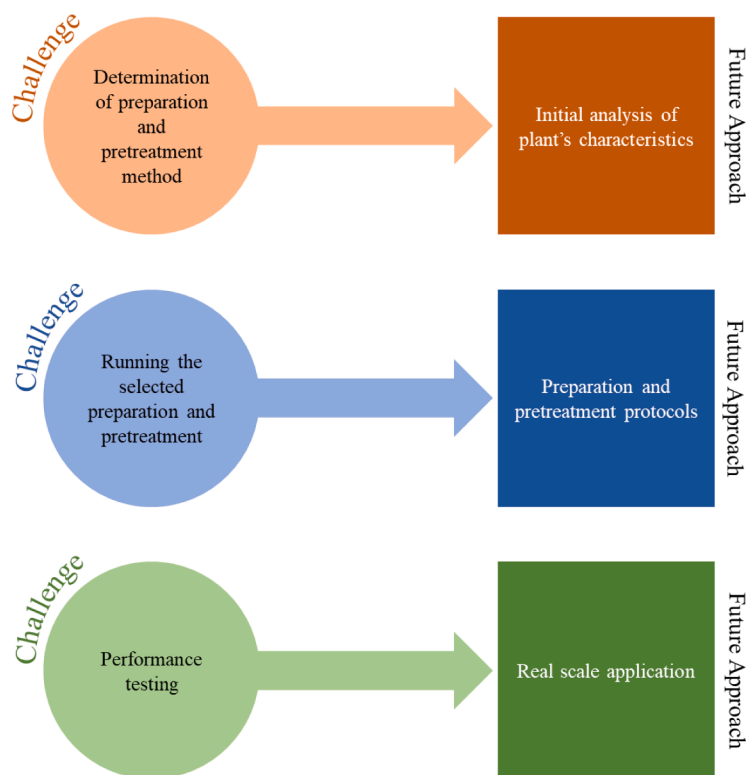


Fig. 3. Challenges and approaches for future plant-based biocoagulant research

preparation and pretreatment to prepare biocoagulant with the highest removal performance. This challenge can be overcome by providing an initial characterization of plant species and parts. Each genus/family of plants has specific characteristics that make them belong to the same group. The specific characteristics are more likely related to its physical features and their living mechanisms. Within the genus, morphological and genetical similarities are found (Seeland et al., 2019). By this fact, there is a possibility to perform initial screening of plant species based on their genus before performing the preparation and pretreatment to produce biocoagulant. For example, the flower of *Musa* sp. is identified as having a tannin-rich content which contributed to the coagulation and flocculation of heavy metal-containing wastewater (Braga et al., 2018). Similarly, *Musa paradisiaca* peel also showed a high protein content that contributed to the coagulation process (Daverey et al., 2019). This evidence showed that *Musa* sp.'s part has a high protein content; thus, pretreatment related to protein extraction may become the appropriate selection. In addition, Feihrmann et al. (2017) reported the high lipid content in the seed of *M. oleifera*, in accordance with Chethana et al. (2016) which also stated that *M. oleifera* and *Cicer arietinum* seeds presented high lipid content. From these literatures, *M. oleifera* seed is categorized as a high-lipid content material, while there is also the possibility that every seed part of the plant contains high lipid; thus, delipidation may become the best pretreatment since lipid will interfere with the coagulation and flocculation processes (Ang and Mohammad, 2020).

### 6.2. Preparation and pretreatment protocols

As of now, most of the preparation and pretreatment of biocoagulants refers to previous works (Benalia et al., 2018; Benavente et al., 2022; Yellapu et al., 2019). An optimized and standardized preparation and pretreatment method for biocoagulants is currently becoming a challenge (Ahmad et al., 2022a). Preparing protocols for the preparations and pretreatments of biocoagulants may be the solution to this particular matter. Protocols should include a flowchart on how to

select the most appropriate method based on the selected plant's characteristics. In addition, the protocol should also include the optimum condition to obtain the highest yield of the biocoagulants based on the selected pretreatment. The protocol may serve as a shortcut and reference for the preparation and pretreatment of biocoagulants before it can be used to treat water or wastewater.

### 6.3. Real scale application

Most plant-based biocoagulant applications are tested under laboratory conditions, in which pollutants characteristics are previously known and parameters are run under controlled conditions (Aziz et al., 2018; Benalia et al., 2018; Bouaouine et al., 2018). The real challenges of biocoagulants are to clearly portray that the utilization of plant-based coagulants exhibits similar or even greater performance as compared to chemical coagulants and control treatments (without the addition of any compounds). Igwegbe et al. (2021) showed a great performance of *Garcinia kola* seed as a coagulant in treating aquaculture wastewater in laboratory scale, but comparison with chemical coagulant (alum) was only done by economic analysis, and comparison with the control reactor is yet to be found. Similarly, Lim et al. (2022) focused on the optimization of treatment conditions for coagulation-flocculation using fenugreek seed, which showed similar removal efficiencies of turbidity, suspended solids, and COD as compared to alum, but the control reactor was still missing from presentation. Ahmad et al. (2021) showed the control reactor after the jar test as compared to the several plant-based biocoagulants, but it showed slight differences with the treated reactors. To clearly portray the biocoagulant performance, a clear comparison between the control reactor and the chemical coagulant needs to be included in one frame. Moreover, a real-scale application of the plant-based coagulant is suggested to enhance the knowledge of coagulation and flocculation using biocoagulant, as it is still mostly carried out using jar tests (Bello et al., 2020; Miyashiro et al., 2021; Priyatharishini and Mokhtar, 2020).

This presented review highlights the importance of the preparation



and pretreatment of plant-based biocoagulants, which are contributing towards sustainable development goals number 6 (clean water and sanitation) and number 14 (life below water). The use of biocoagulants can be a solution to provide clean water while later also preventing the harmful effects caused by the utilization of chemical coagulants. In addition, treatment of wastewater using plant-based biocoagulants may avoid pollution caused by untreated wastewater discharge. On the other hand, there are still many challenges that need to be considered in order to fully use this technology in real-scale applications. The extraction and purification of active ingredients functioning in the coagulation-flocculation process are very important steps; however, the protocols are somehow still requiring many chemicals, which may increase the production cost. The price competition with currently commonly available chemical coagulants needs to be considered for commercialization. Although the utilization of raw plant-based biocoagulant powder showed good performance in removing some pollutants, it is not suggested since it may elevate the organic content in the wastewater.

## 7. Conclusion

Biocoagulants are an alternative to chemical coagulants commonly used in water and wastewater treatment. Plant-based biocoagulants are promising due to their availability and comparable performance. Pretreatment and preparation of plant-based biocoagulants have a great influence on their properties and performances. Drying and grinding were mentioned as the most influential mechanical preparations for plant-based biocoagulants. The optimum drying temperature needs to be optimized based on the plant's characteristics, and smaller particle sizes show better performance of biocoagulants. Extraction using salt and alcohol showed better performances as compared to water, especially to obtain and utilize protein content inside the biocoagulants. The current limitations in the initial characterization of plant properties in relation to determining the appropriate preparation protocols are a barrier to further large-scale applications for water and wastewater treatment.

## CRedit authorship contribution statement

**Setyo Budi Kurniawan:** Writing – original draft, Writing – review & editing, Visualization, Conceptualization. **Azmi Ahmad:** Writing – original draft, Visualization. **Nor Sakinah Mohd Said:** Writing – original draft. **Kiki Gustinasari:** Writing – original draft. **Siti Rozaimah Sheikh Abdullah:** Supervision, Funding acquisition. **Muhammad Fauzul Imron:** Writing – original draft.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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