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The Potential Application of Mulberry (*Morus alba* L.) Leaves as a Plant-based Coagulant to Remove Turbidity.

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ABSTRACT. In this study, the effectiveness of coagulant extracted from mulberry leaves (ML) was investigated. Using a conventional chemical coagulant, like aluminium sulphate and ferric chloride, in water treatment can result in an accumulation of aluminium and iron in the water supply. This led to a significant number of studies being carried out by researchers to create a novel method for treating turbidity in raw water with a natural coagulant. The synthetic turbid water was treated using a series of Jar Test experimental works with a range of operating conditions, including pH (4 - 9) and coagulant dosage (10 - 50 mg/L). The treatments were carried out with a fast-mixing speed of 150 rpm for 2 min, a slow-mixing speed of 35 rpm for 20 min, and a settling time of 30 min. Mulberry leaves coagulant (MLC) successfully removed 96.53 % of turbidity under the optimum dosage of 10 mg/L. pH 4 was the optimum pH for turbidity removal (99.67 %). According to the findings, turbidity reduction can be enhanced by employing ML as a novel natural coagulant in water treatment. Furthermore, the use of this natural coagulant can minimise the health risks associated with long-term use of chemical coagulants while somehow reducing the amount of chemical sludge product released into the environment.

Keywords: Jar test, Mulberry leaves, Natural coagulant, Turbidity.

INTRODUCTION

Water is a vital necessity of life when it comes to the survival of all living things. Clean water nourishes and may protect against disease. Water is used for many things, including cooking, drinking, irrigation, and manufacturing. This resource, however, is becoming depleted in its pure form because of the numerous anthropogenic sources of contamination. Water contains a variety of impurities, including suspended particles, such as silt, clay, sand, organic matter, or other fine debris (Malik, 2018; Muthuraman et al., 2017; Shahriari, T., NabiBidhendi, 2012). Turbid water, which can have a variety of causes and effects, is defined as having an opaque, hazy, or cloudy appearance. It is, therefore, used as an indicator of water quality (Karnena et al., 2022). Turbidity in water is caused by a variety of factors, such as waste discharge (Dorca et al., 2018), construction and land development, agricultural practices (Rayudu et al., 2018), industrial effluents (Vigneshwaran et al., 2020), and soil erosion, which poses a serious risk to the water cycle and public health.

Among the numerous existing water and wastewater treatment methods, the coagulation and flocculation process is

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the most preferred for turbidity issues (Mokhtar et al., 2019). This treatment is widely used because it is inexpensive, dependable, simple, and has a low energy consumption (Dorca et al., 2018). Eliminating suspended colloidal particles and decreasing turbidity in a body of water are its primary goals. Turbidity can be removed with the addition of coagulants, such as inorganic and organic coagulants. Inorganic coagulants include aluminium sulphate ($Al_2(SO_4)_3$) (Vigneshwaran et al., 2020), ferric chloride ($FeCl_3$), and calcium carbonate ($CaCO_3$), while synthetic organic polymer includes polyaluminium chloride (PAC) (Dollah et al., 2019; Mokhtar et al., 2019). Nevertheless, there were several drawbacks to using these chemical coagulants, including the production of hazardous amounts of sludge (Rayudu et al., 2018; Shamira Shaharom & Siti Quraisyah Abg Adenan, 2019). Excessive consumption of water containing alum residues after the coagulation process may result in aluminium accumulation inside the brain, leading to neurodegenerative diseases such as Parkinson's disease and Alzheimer's dementia (Amruta & Munavalli, 2017; Menkiti et al., 2018; Nidheesh et al., 2017; Rayudu et al., 2018; Vigneshwaran et al., 2020; Yan et al., 2016). These shortcomings have recently sparked investigations into possible natural coagulants. In comparison to inorganic coagulants, natural coagulants are generally highly biodegradable and pose little health risk to living things (Dorca et al., 2018). Additionally, natural coagulants are usually considered to be more environmentally friendly in terms of production and use, but the associated disadvantages have prompted the search for them despite the proven treatment efficacy of inorganic and synthetic organic coagulants. However, the primary appealing features of natural coagulants are their biodegradability, nontoxicity, renewability (feedstock can be readily obtained), and relative cost-effectiveness (cost of coagulant and handling sludge) (Amran et al., 2018; Lun & Wahab, 2020).

Plant-based coagulants are organic coagulants that are natural, water-soluble, and derived from various plant parts and species (Ahmad, Kurniawan, et al., 2022). Natural coagulants have several advantages in wastewater purification, including being inexpensive, biodegradable, renewable, non-toxic, capable of effectively eliminating turbidity, and safe for human health (Dollah et al., 2019; Koul et al., 2022; Menkiti et al., 2018) as presented in Figure 1.

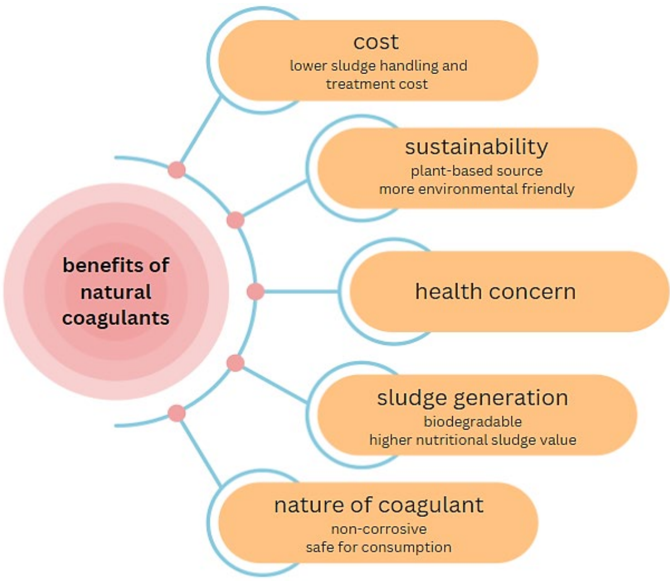

Figure 1. Advantages of natural coagulants over chemical-based coagulants.
Adopted from (Koul et al., 2022)

Plant-based coagulants have been shown to remove pollutants from effluent, improving turbidity, total suspended solids (TSS), chemical oxygen demand (COD), and other parameters (Benalia et al., 2018). There are several types of plant-based coagulants, such as fruit peels (Dollah et al., 2019; Mokhtar et al., 2019; Rayudu et al., 2018; Shamira Shaharom & Siti Quraisyah Abg Adenan, 2019), seeds (Kristianto et al., 2019; Unnisa & Bi, 2018; Vigneshwaran et al., 2020), plant leaves (Benalia et al., 2018; Fahmi et al., 2014), tannins (Chemistry, 2010), legumes (Yin, 2010) and starch (Yan et al., 2016). Previous studies have shown that plant-based coagulants can outperform chemical coagulants in terms of performance (Amran et al., 2018; Amruta & Munavalli, 2017; Koul et al., 2022; Kristianto et al., 2019). In addition, active compounds can be found in plant-based coagulants. Thus, plant-based coagulants containing active compounds can neutralise charges and form bridges between pollutant particles, resulting in flocs that settle quickly for subsequent segregation (Ahmad, Kurniawan, et al., 2022). Therefore, in recent years, scientists have become interested in natural coagulants, particularly those derived from plants. The effectiveness of natural plant-based coagulants in generating potable water has been studied and published in reports (Figure 2).

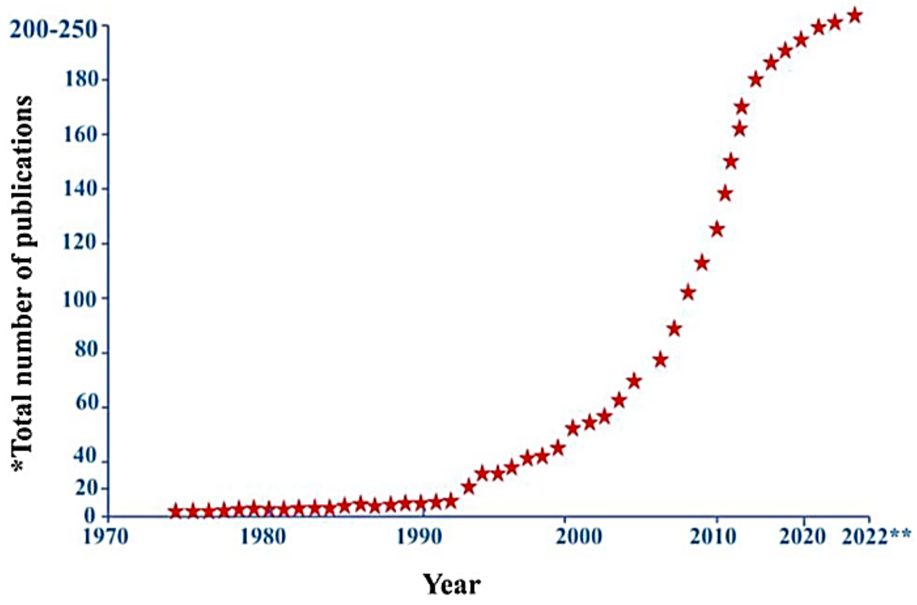

Figure 2. Publication statistics about coagulants made from plants used in water treatment. (Data obtained from the Web of Science and Scopus databases). Source: (Karnena & Saritha, 2022)

Even though much study has been done on natural coagulants, primarily using plant seeds, there has not been much research done on local plant leaves (Ahmad, Abdullah, et al., 2022; Karnena & Saritha, 2022). The presence of active compounds like proteins, polysaccharides, carbohydrates and certain functional groups (Yin, 2010), which have been linked to the effect of purification due to coagulation, makes leaves a good choice for treating water and wastewater. When it comes to treating water, they typically work well on medium turbidity levels between 50 and 500 NTU (Koul et al., 2022). However, by improving the coagulant extraction and purification procedures, the effectiveness of natural coagulants can be increased. The Mulberry (*Morus alba* L.) plant, which belongs to the Moraceae family, is one of the most valuable and abundant in natural ingredients (Gryn-rynko et al., 2016). The Mulberry plant is highly adaptable to a variety of soil and climate conditions, making it possible to grow it in many places (Duran, 2023). Asian regions with warm climates are home to most mulberry species. It is a small to medium-sized mulberry tree that grows quickly, reaching a height of 10 - 20 m. Because mulberry trees are grown all over the world, they offer a readily available, local source of coagulant material. This can be beneficial for communities that have easy access to mulberry leaves (ML). ML contains a high amount of protein (Gryn-rynko et al., 2016; Kadam & Dhumal, 2019). A multitude of bioactive compounds, such as rutin, ferulic acid, catechin, isoquercitrin, polysaccharide, 1-deoxynojirimycin (DNJ), γ-aminobutyric acid (GABA), and many others, are abundant in ML and have been linked to a number of health benefits, including the prevention of obesity, atherosclerosis, inflammation, and diabetes (Tao et al., 2016). Because ML contains a comparatively high amount of crude protein, they can act as coagulants by encouraging the aggregation of suspended particles in water. This helps in the formation of larger and heavier flocs that are easier to remove (Lun & Wahab, 2020).

To ascertain whether ML is a suitable substitute coagulant for eliminating turbidity from synthetic turbid water was the goal of the present study. It is also discussed that pH, coagulant dose, and coagulation time are the three primary independent variables that effectively contribute to the removal of turbidity (Kumar et al., 2021).

METHODOLOGY

Plant-based Coagulant Preparation

ML is one of the most precious and abundant natural ingredient plants. Compared to other green leafy vegetables, ML have a noticeably higher protein content (Thaipitakwong et al., 2018), and this can contribute to their effectiveness as a coagulant in water treatment (Gryn-rynko et al., 2016). The proteins in mulberry leaves can play a role in the coagulation process by promoting the aggregation of suspended particles in water. Fresh ML with an oval shape was gathered from the nearby mulberry farm in Perlis and Pulau Pinang (Figure 3). Then, it was thoroughly washed with distilled water to remove dust and other impurities. After washing, the leaves were dried in the oven at 50 °C for 24 h to reduce their moisture content. The ML was ground into a fine powder using a grinder and stored at room temperature in tightly sealed containers. The extraction of coagulation was carried out by mixing 1 g of ML fine powder with 1000 mL of distilled water and blended for 2 min. To encourage the water extraction of the active coagulating component, which is proteins (Fakhruddin & Hossain, 2011), the suspension was vigorously shaken for 20 min with a magnetic stirrer (Fahmi et al., 2014). To preserve crude extracts and eliminate any microscopic residue, 125 mm filter paper (Whatman no. 42) was used to filter the mixture solution. The filtrate portions were used for the required dose of mulberry leaf coagulant (MLC). Fresh solutions were prepared daily and kept refrigerated to prevent any ageing effects (such as changes in pH, viscosity, and coagulation activity). Solutions were shaken vigorously before use. Stock solutions were made for the coagulant dosage to ensure uniformity and replication across the treatment procedures. Using the following equation (1), the stock solution was diluted:

$$M_1V_1 = M_2V_2 \tag{1}$$

Where:

M_1 = initial stock solution concentration (mg/L)

V_1 = volume of stock solution required (L)

M_2 = concentration of solution to be produced (mg/L)

V_2 = volume of solution to be produced (L)

Figure 3. Mulberry (*Morus alba* L.) leaves

Preparation of Synthetic Turbid Water

To achieve the desired turbidity ranges, synthetic turbid water was used rather than raw water samples from a nearby river (Dollah et al., 2019). In this study, the high turbid synthetic water (>100 NTU) was made by adding 10 g of clay powder in 1 L of distilled water. To achieve uniform clay powder dispersion, the clay suspension was then vigorously agitated for an hour at 200 rpm (El Gaayda et al., 2022). To ensure that the clay particles were evenly distributed, the suspension was stirred for approximately 60 min. After that, the clay materials were left to settle for at least 24 h to ensure full hydration (Fakhruddin & Hossain, 2011).

Jar Test Experiment

A jar test apparatus with six agitators with variable speed, six beakers, and a light source was used to assess the coagulation performance of MLC. Different dosages of MLC (10, 20, 30, 40, and 50 mg/L) were added to 500 mL of synthetic turbid water in each beaker. This batch's control was prepared in a single beaker with no coagulant dosage. The mixture was then stirred for 2 min at 150 rpm and then for 20 min at 35 rpm. To achieve the optimal pH, the MLC concentration in each beaker was set at 10 mg/L, and the pH range was varied (4 – 9) by adjusting using a solution of 1 M HCl or 1 M NaOH. After mixing, the solution in the beaker was left for 30 min to allow the flocs to settle in the beakers, as shown in Figure 4. Cleared samples were removed from the top of the beaker by using a pipette after 30 min of sedimentation to conduct analysis.

Figure 4. The settling floc after the experiment

Turbidity Test

To determine the initial and final turbidity of the water sample tested, laboratory testing was done. The turbidity was measured in Nephelometric Turbidity Units (NTU) using a turbidity metre (2100Q Portable Turbidimeter). Water samples were taken at a depth of 2 cm below the surface after the settling period was complete (Asharuddin et al., 2018). The following equation (2) was used to determine the percentage of removal:

$$removal\ (\%) = \left(\frac{C_i - C_f}{C_i}\right) \times 100 \tag{2}$$

Where:
C_i = initial turbidity of the sample
C_f = final turbidity of the sample

RESULTS AND DISCUSSION

Effect of pH on Natural Coagulants

An essential component of coagulation efficiency is pH. The pH may have an impact on the coagulants' surface charge during the coagulation process. Low surface charge coagulants may slow down the growth of flocs, which would result in poor removal efficacy in water treatment (Muda et al., 2020). As a result, jar testing is essential to figuring out the ideal pH for treating turbid water. Based on the findings shown in Figure 5, the MLC was effective in enhancing the quality of the water by eliminating turbidity. The pH values varied from 4, 5, 6, 7, 8, to 9, at a constant dosage of MLC (10 mg/L). Each test was conducted with turbidity >100 NTU and at ambient temperatures between 26 and 32°C. The effectiveness of turbidity removal across different pH ranges is 99.67 %, 98.55 %, 74.27 %, 65.29 %, 39 % and 39 %, respectively. Based on the figures, the best pH value to remove turbidity was at pH 4, while the lowest percentage removal of turbidity was at pH 8 and 9. According to the findings, the highest turbidity removal occurred in acidic conditions, not in alkalic conditions. This is due to the possibility that high pH levels with hydroxyl ions could prevent wastewater constituents from adhering to the coagulant in any way (Amran et al., 2018). At higher pH, the surface chemistry of the mulberry coagulant may change in a way that hinders its ability to adsorb onto suspended particles effectively. It was also discovered that this natural coagulant reacts ineffectively as a coagulant and is incapable of removing colloidal in water in the alkali group (Nidheesh et al., 2017). According to (Muda et al., 2020), removal trends were observed in their study on natural coagulants, with the percentage of removal rising from pH 2 (acidic condition) and rapidly decreasing at pH 7.5 (alkaline condition).

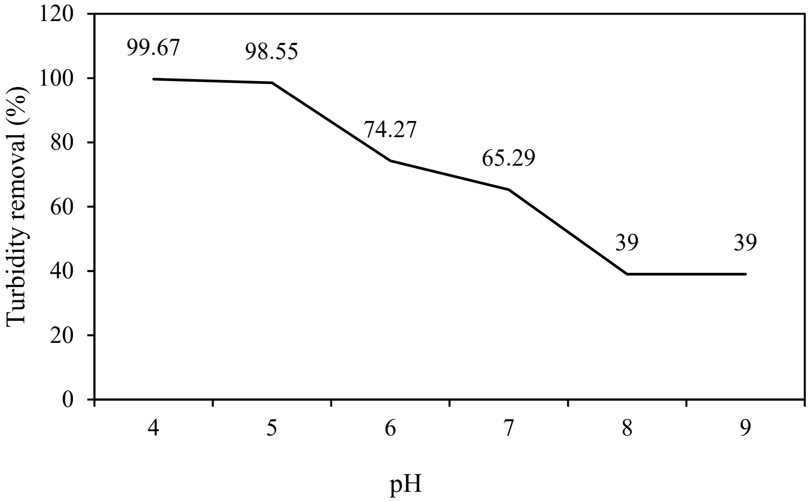

Figure 5. Effect of various pH on turbidity removal of MLC

Effect of Dosage on Natural Coagulants

It is critical to identify the ideal coagulation process conditions to achieve greater water treatment outcomes. The tiny colloidal particles present in the raw water become trapped when coagulants are added. Figure 6 shows the performance of MLC under various dosages. Based on the previous optimum result, the pH was maintained at 4.

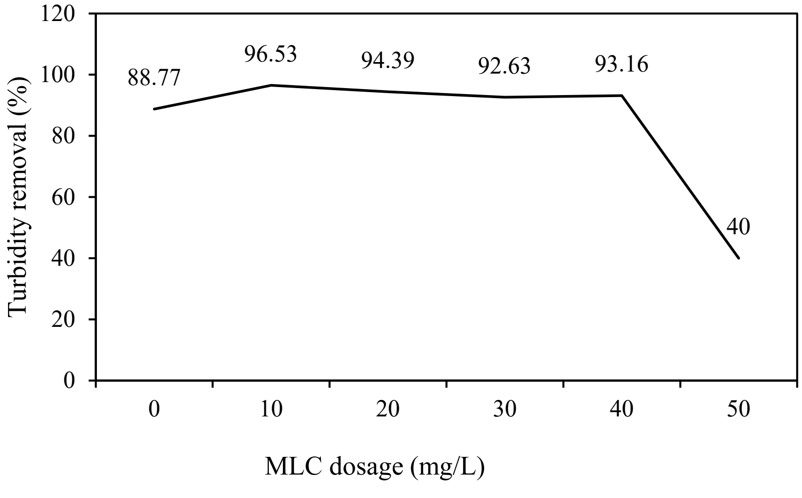

Figure 6. Effect of various MLC dosages on turbidity removal

The highest percentage of turbidity removal for MLC was 96.53 % at a dosage of 10 mg/L, as can be seen in Figure 6. There was a clear similarity in the percentage of turbidity removal (above 90 %) between 10 and 40 mg/L of MLC dosage. It was found that in the acidic condition, a small dosage of MLC was needed to remove the colloidal clay solution. The lowest turbidity removal was 40 % at a dosage of 50 mg/L. The performance was reduced as the natural coagulant dosage was increased. This phenomenon is related to particle steric stabilisation caused by an overdose of the natural coagulant (Benalia et al., 2018).

As a further discussion, ML contains naturally occurring coagulation-active compounds such as alkaloids (Kadam & Dhumal, 2019), proteins (Gryn-rynko et al., 2016), polysaccharides and flavonoids (Bai et al., 2023). Because of their inherent sustainability and low toxicity, these compounds can interact with suspended particles in water, promoting their aggregation and aiding in the formation of larger flocs. This coagulation process helps in the removal of turbidity. As an example, polysaccharides are long chains of sugar molecules. ML contains various polysaccharides, including acidic and neutral polysaccharides, which can help in coagulation by adsorbing onto suspended particles and creating bridges between them, leading to the formation of larger flocs (Belbahloul et al., 2015). Flavonoids are a class of polyphenolic compounds found in many plants, including ML. The ability of flavonoids to both eliminate and inhibit the formation of free radicals makes them a promising class of natural antioxidants and can help in the removal of suspended particles (Rodr et al., 2020).

CONCLUSION

The aim of this study was to evaluate the turbidity-reducing of a natural coagulant that was extracted or derived from mulberry leaves. With turbidity removal efficiencies of over 90 %, this natural coagulant showed interesting results in both powder and solution extracted by distilled water. Future studies should concentrate on enhancing the performance by mixing starch and the natural coagulant. To achieve maximum turbidity removal, other factors that can affect the coagulation-flocculation process when using ML as a coagulant can be thoroughly investigated. These factors include mixing speed and duration.

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AUTHOR CONTRIBUTIONS

Nur Shafieza Aizzan: conceptualisation, investigation, methodology, data analysis and writing; **Nurul Najihah Ghazali**: methodology and data analysis. All authors have read and agreed to the published version of the manuscript.

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COMPETING INTEREST

The authors declare that there are no competing interests.

COMPLIANCE WITH ETHICAL STANDARDS

Not applicable

SUPPLEMENTARY MATERIAL

Not applicable.

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