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Evaluation of the coagulant property of carica papaya seeds in surface water treatment

Evaluación de la propiedad coagulante de las semillas de carica papaya en el tratamiento de aguas superficiales

A. P. Cadena-Álava*, R. E. Cevallos-Cedeño, S. A. García-Muentes
Departamento de Procesos Químicos, UTM, Av. Urbina y Che Guevara, Portoviejo, Manabí, Ecuador.
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Abstract

Surface water pollution has increased due to effluents discharged from various human activities, posing environmental and health concerns. Despite advancements, water scarcity issues persist, especially in rural areas of developing countries. The aim of this study was to evaluate the potential of Carica Papaya seeds as a coagulant in surface water treatment, focusing on the removal of turbidity, nitrates, and iron. The physicochemical properties (total protein content, soluble protein content, carbohydrate content, fat, ash) of the seeds were determined. The coagulant was obtained from the seeds using the salt extraction technique. Fourier transform infrared spectroscopy (FTIR) was performed on the biocoagulant to determine its morphology and functional groups present, respectively. The results indicate that papaya seed extract demonstrated high efficiency in turbidity removal up to 79.44% and nitrate reduction up to 92.32% with iron removal efficiency observed at 71.66%, surpassing in some cases aluminum sulfate, a conventional chemical coagulant. However, the presence of sodium chloride in the extract increased the levels of total dissolved solids (TDS) and electrical conductivity (EC). Nonetheless, the use of Carica Papaya seeds as a biocoagulant emerges as a promising, environmentally friendly, and economically viable alternative for surface water treatment, with significant implications for public health and the environment. Natural coagulants are biodegradable, produce fewer chemical residues, and reduce the risk of secondary pollution. Additionally, they often require less energy for production and processing compared to synthetic alternatives, contributing to a decrease in the overall ecological footprint of water purification.
Keywords: Carica papaya seed, biocoagulant, coagulation-flocculation, turbidity removal, nitrates, iron.

Resumen

La contaminación del agua superficial ha aumentado debido a los efluentes descargados por diversas actividades humanas, lo que plantea preocupaciones ambientales y de salud. A pesar de los avances, persisten problemas de escasez de agua, especialmente en zonas rurales de países en desarrollo. El objetivo de este estudio fue evaluar el potencial de las semillas de Carica Papaya como coagulante en el tratamiento de agua superficial, centrándose en la remoción de turbidez, nitratos y hierro. Las propiedades fisicoquímicas (contenido de proteínas totales, contenido de proteínas solubles, contenido de carbohidratos, grasas, ceniza) de las semillas. El coagulante se obtuvo de las semillas mediante la técnica de extracción con sal. Se realizó espectroscopía infrarroja por transformada de Fourier (FTIR) sobre el biocoagulante para determinar su morfología y grupos funcionales presentes, respectivamente. Los resultados indican que el extracto de semilla de papaya demostró alta eficiencia en la eliminación de turbidez (hasta 79,44%) y eliminación de nitratos (92,32%), superando en algunos casos al sulfato de aluminio, un coagulante químico convencional. Sin embargo, la presencia de cloruro de sodio en el extracto aumentó los niveles de sólidos disueltos totales (TDS) y conductividad eléctrica (CE). No obstante, el uso de semillas de Carica Papaya como biocoagulante surge como una alternativa prometedora, respetuosa con el medio ambiente y económicamente viable para el tratamiento de aguas superficiales, con importantes implicaciones para la salud pública y el medio ambiente.
Palabras clave: Semilla de Carica papaya, biocoagulante, coagulación-floculación, remoción de turbidez, nitratos, hierro.

* *Corresponding author. E-mail*: acadena3638@utm.edu.ec;
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1 Introduction

Over the years, surface water pollution has increased, which is attributed to the substantial of effluents discharged into water bodies as a result of various human activities (Nigussie & Gabbiye Habtu, 2023). This phenomenon is concerning and deserves attention due to its environmental and health consequences.

Population growth demands a constant supply of clean water, driving innovation in new technologies for efficient and cost-effective treatment of both water supplies and wastewater (Cazcarro, *et al*., 2016). Despite the progress that had been made in this field, serious problems persisted in several regions, where water scarcity remained a pressing reality. This challenge is particularly evident in developing countries, especially in rural areas, where access to safe drinking water remains a matter of great concern (Carrard, *et al*., 2019).

The lack of adequate treatment of raw water has significant repercussions for public health, manifesting in diseases such as diarrhea, which can be fatal due to the consumption of nonpotable water. Furthermore, negative economic and social impacts are generated in affected communities (McClelland, *et al*., 2022; Fuente, *et al*., 2020). Water treatment is essential to prevent disease and promote the well-being of populations (Sharma & Bhattacharya, 2017).

Turbidity manifests as the physical form of water pollution, appearing in suspended particles that limit the penetration of sunlight (Rasheed, *et al*., 2023). The direct relationship between turbidity levels and the concentration of suspended particles results in lower transparency and increased turbidity as the particle concentration increases (Chik, et al., 2024). The type of treatment required depends on the contaminants present in the water. To decrease turbidity in surface waters, various methods are employed, such as precipitation, adsorption, ion exchange, membrane filtration, biological and electrolytic processes, as well as coagulation and flocculation (Rajoria, *et al*., 2022).

The physicochemical processes of coagulation-flocculation, commonly used in water treatment (Nhut, *et al*., 2021), demonstrated their effectiveness in removing organic matter, turbidity, and color (Ben Rebah, *et al*., 2018; Abebe, *et al*., 2016; Abidin, *et al*., 2013). Furthermore, these processes improve the efficiency of subsequent treatments, such as sedimentation, filtration, and sterilization (Nhut, *et al*., 2021).

In conventional water treatment processes, various flocculants are employed according to the chemical properties of the contaminants present in the water. These substances are classified into inorganic coagulants, such as iron and aluminum salts, and synthetic organic polymers, such as polyacrylamide and polyethylenimine derivatives (Ramavandi, 2014). Although aluminum sulfate stands out for its efficiency in turbidity removal and low cost, its use affects health and causes problems, especially in the sludge treatment stage (Pi, *et al*., 2019; Inan-Eroglu & Ayaz, 2018; Bodlund, *et al*., 2013).

In recent years, significant interest has arisen in the development of coagulants extracted from plant tissues, animals, or microorganisms (Ahmad, *et al*., 2022; Iber, *et al*., 2021), due to their positive impact on the environment and the absence of alterations in the pH of treated water. Biocoagulants have attracted attention as promising substitutes for inorganic coagulants and synthetic polymers, due to their low toxicity, biodegradability, local availability, high efficiency and low cost in managing generated sludge (Bouaouine, *et al*., 2018). In recent years, several studies were conducted on diferente plant-derived coagulants such as Cassia fistula (Nero *et al*, 2023), Moringa Oleifera (Bouchareb *et al*, 2021), Aloe Vera (Benalia *et al*, 202), Onion Seeds (Mahanna *et al*, 2023), oats (Mahanna *et al*, 2023), Musa peels (Daverey *et al*, 2019), Dolichos seeds (Daverey *et al*, 2019) and Jatropha curcas (Abidin *et al*, 2013).

Despite advances in this field, difficulties in the preparation and/or processing of bio-coagulants represent a significant obstacle that must be addressed before their successful commercialization. Therefore, it is essential to extrapolate simple methods for the preparation and extraction of natural coagulants(Daverey, *et al*., 2019).

Carica papaya, a fruit that grows in tropical and subtropical regions, generates waste constituting between 15% and 20% of its weight, mainly peels and seeds (Amran, *et al*., 2021). To minimize the amount of waste from papaya consumption, the use of seeds as a coagulant for turbidity removal in water treatment is proposed (Amran, *et al*., 2021). Therefore, this study aims to evaluate the efficiency of Carica Papaya seeds as a natural coagulant in surface water treatment, focusing on turbidity, nitrate, and iron removal. The extract obtained from the seeds will be characterized by determining soluble proteins, total proteins, and functional groups through Fourier transform infrared spectroscopy (FTIR).

2 Materials and methods

2.1 Surface water sampling

Surface water samples were collected from the Portoviejo River in Manabi province of Ecuador, the sampling procedure carried out in accordance with the Ecuadorian national standards NTE INEN 2176: 2013: Water, which specifically focuses on water quality and delineates sampling techniques.

The precise coordinates for the sampling location are 1º4'35.656 "S 80º25'55.146 "W. (2013).

2.2 *Preparation of carica papaya seeds*

The papayas were obtained from a local market in Sosote, located in Rocafuerte city, Manabi, Ecuador. Carica Papaya seeds were removed from the fruit and cleaned with distilled water to remove the mucilage covering them, then seeds were dried at 40°C for 24 hours. Subsequently, the seeds were ground to increase surface area and enhance extraction efficiency and stored at 4°C until use.

2.3 *Coagulant extraction*

Extraction with salt or sodium chloride is a method that allows isolating proteins that behave as polyelectrolytes during the coagulation process. Sodium chloride was used for its availability and economic advantages (Hadadi *et al*., 2023). For coagulant extraction, 5 g of Carica Papaya seed powder was taken and shaken with 100 mL of a0 1M sodium chloride solution to maximize protein extraction efficiency, as suggested by (Hadadi, *et al*., 2023) for 30 minutes at 200 rpm. Then, the resulting solution was centrifuged for 10 minutes. Afterwards, the supernatant was filtered with MN-615 filter paper, and then filtered through a 45 μm fiberglass filter.

2.4 *Characterization of seeds and coagulant*

The total protein content was determined by the Kjeldahl method, and the soluble protein content was determined following the methodology described by (Liu & Pan, 2017) using the Biuret method with bovine serum albumin (BSA) as the standard. The calibration curve was prepared by preparing solutions with different concentrations of bovine serum albumin (5, 10, 15, 20 mg/mL).

For the determination of functional groups on the surface of the samples in a range of 4000 to 500 cm^{-1}, a Fourier transform infrared spectroscopy spectrometer (Perkim Elmer Spectrum) was used.

2.5 *Coagulation-flocculation process*

The coagulation activity of Carica Papaya seed extract and aluminum sulfate was evaluated using the PB-900 jar test (Phipps & Bird, USA), which allows the coagulation-flocculation process to be carried out on a small scale. Six 1000 mL beakers were used, each containing 1000 mL of water. The concentrations of Carica Papaya extract were chosen based into effectivity of aluminum sulfate at concentration of (25-250 mg/L) (Amran, *et al*, 2021) to achieve a comparison at the same level. Agitation was carried out in two stages: rapid agitation at 200 rpm for 2 minutes, followed by slow agitation at 100 rpm for 20 minutes; then allowed to settle for 30 minutes, the supernatant from each beaker was taken and used to measure residual turbidity. The efficiency and activity of the coagulants were calculated according to equation 1 (Choy, Prasad, Wu, Raghunandan, & Ramanan, 2016):

$$\frac{Initial\ turbidity - Final\ turbidity}{Initial\ turbidity} \times 100\% \qquad (1)$$

2.6 *Determination of the physicochemical parameters of water samples*

Turbidity was measured in nephelometric turbidity units (NTU) using a Turbidimeter HANNA HI98713-01. Conductivity and total dissolved solids were determined using a conductivity meter WTW-Inolab Cond7110. Nitrates and iron in the water samples were determined using spectroquant kits with a photometer Photolab S6.

2.7 *Statistical analysis*

All data were expressed as mean ± standard deviation. Each treatment was performed in triplicate. Analysis of variance (ANOVA one way) followed by Tukey's test was conducted when p-values indicated statistically significant differences in the evaluated parameters. Minitab v20 was used for all statistical analyses.

3 Results and discussion

3.1 *Physicochemical characterization*

The physicochemical characteristics of Carica papaya seeds are shown in Table 1. The moisture content of the seeds, after the preparation process, was 5.2 ± 0.03, while the ash content was 7.34 ± 0.06. It was observed that the seeds had a fat content of 25.58 ± 0.99%, slightly lower than the 28.71 ± 0.43% determined by (Rosa, Evangelista, Machado, Sanches, & Telis-Romero, 2021); however, differences in species composition generally vary due to environmental factors (Hadadi, *et al*., 2023).

Table 1. Chemical composition of carica papaya seeds.

Composition	**Percentage**
Moisture content	5.20±0.03
Ash	7.34±0.06
Total protein	32.63±1.25
Soluble proteins	5.76±0.27
Fats	25.58±0.67
Carbohydrates	29.25±1.13

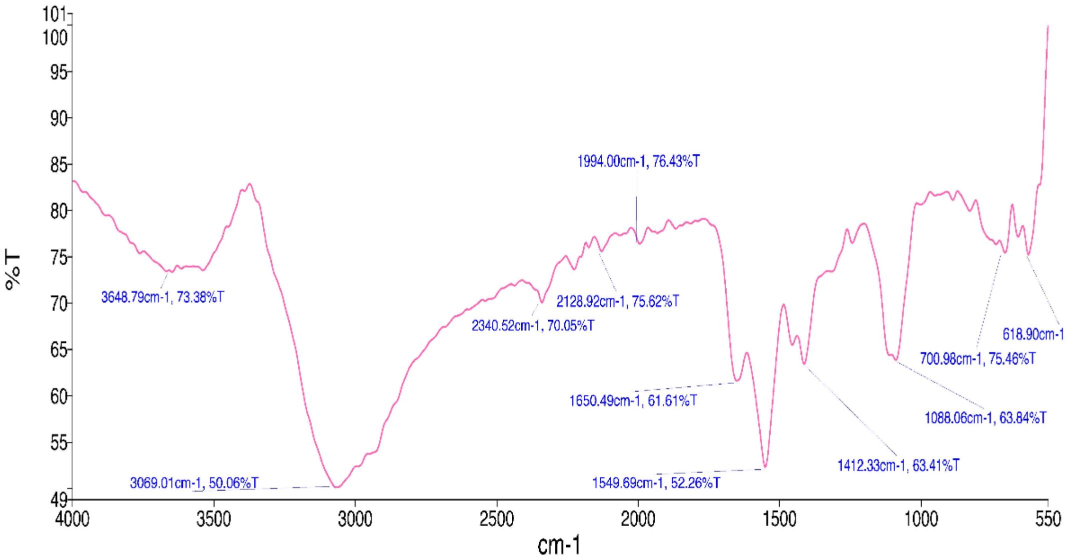


Figure 1. Carica Papaya biocoagulant of the FTIR spectrum.

Table 2. Effect of coagulant dosage on the physicochemical properties of water.

Treatment	Concentrations (mg/L)	TRI	EC	TDS	NRI	IRI
Carica papaya extract	0	16.38±2.63^{j}	156.33±0.58^{h}	87.33±1.15^{hi}	0^{f}	0^{f}
	25	64.693±0.61^{g}	180.67±1.155^{efg}	89.67±0.58^{gh}	36.07±6.44^{e}	10.35±1.36^{f}
	50	62.66±2.01^{g}	198.67±3.21^{e}	99.67±0.58^{e}	89.66±1.51^{a}	53.76±0.72^{e}
	100	74.49±1.66^{de}	249.33±1.15^{d}	123±1.73^{d}	89.66±1.51^{a}	71.66±1.21^{cd}
	150	76.621±0.76^{cde}	303±2^{c}	149.33±1.15^{c}	92.32±2.39^{a}	54.63±4.02^{e}
	200	77.08±0.87^{cd}	340.67±0.58^{b}	170±1^{b}	60.69±1.06^{bc}	50.93±0.80^{e}
	250	79.44±0.61^{c}	376.67±23.12^{a}	196.67±1.15^{a}	53.28±3.97^{cd}	51.83±2.06^{e}
Aluminum sulfate	0	24.15±1.292^{i}	156.33±0.589^{h}	83.33±0.58^{j}	0^{f}	0^{f}
	25	69.12±1.02^{f}	166.67±0.58^{gh}	84.67±0.58^{ij}	35.34±4.26^{e}	80.24±2.0^{bc}
	50	93.11±0.49^{a}	178.33±0.58^{fg}	87.67±0.58^{hi}	48.10±4.46^{d}	97.17±0.12^{a}
	100	86.96±0.29^{b}	181.67±0.58^{efg}	89.67±0.58^{gh}	55.97±3.62^{bcd}	90.52±1.99^{ab}
	150	72.75±2.054^{ef}	184.33±0.58^{efg}	92.67±1.15^{fg}	53.79±3.83^{cd}	79.62±15.05^{bc}
	200	47.71±0.50^{h}	193.33±3.06^{ef}	93.33 ±0.58^{f}	62.44±4.37^{bc}	71.66±1.21^{cd}
	250	26.44±0.85^{i}	199.33±3.06^{e}	99.33±2.52^{e}	65.41±4.69^{b}	64.17±3.82^{de}

3.2 Fourier transform infrared spectroscopy (FTIR)

The spectrum shown in Figure 1 displays two faint peaks at 3650-3500 cm^{-1} indicating the presence of -OH and N-H radicals; however, the vibration of these peaks overlaps. The sharp peak at 3069 cm^{-1} was associated with the stretching of C-H in alkanes or carboxylic acids (Ghodke, *et al*, 2021). The peak at 1650.49 cm^{-1} is linked to the stretching of the C=O bond, the carbonyl group present in proteins and fatty acids. The bending vibration of the amine group (NH2) is attributed to the peak at 1549.69 cm^{-1}; this band indicates that the coagulant from Carica Papaya seeds has a protein structure containing amino groups(Putra, Amri, & Ayu, 2020). The bending vibrations of methylene and methyl are observed in the band at 1412.33 cm^{-1} (Irnawati, Riyanto, & Sudibyo Martono, 2021). The presence of acetate esters is observed in the peak at 1088.06 cm^{-1} (Gatew & Worku, 2023). The presence of functional groups such as amides and carboxylates, identified via FTIR, suggests strong binding capacities. Amide groups are known for their ability to form hydrogen bonds and electrostatic interactions with negatively charged particles, enhancing the aggregation and subsequent removal of suspended solids. Carboxylate groups, on the other hand, can interact with metal ions and organic molecules, contributing to the coagulant's effectiveness in reducing turbidity and removing contaminants such as nitrates and iron. These interactions highlight the multifunctional nature of the biocoagulant, providing a robust mechanism for water purification. The diverse array of functional groups in the papaya seed extract thus underlines its potential as a versatile and efficient coagulant for treating surface water. The main functional groups present in the biocoagulant from Carica papaya seeds are -OH, NH-, compounds of amide groups, and amino acids which were observed in the FTIR region, indicating the potential of the coagulant to absorb a wide range of contaminants, capable of facilitating the removal of suspended and dissolved particles in water.

3.3 Effect of the coagulant dose on the physicochemical properties of water

The values pertaining to the evaluation of the parameters established as response variables are depicted in Table 2, allowing for the observation of the influence of papaya extract at different doses in comparison to the reference coagulant (aluminum sulfate).

Means that do not share a letter are significantly different p<0.005. TRI: Turbidity reduction index; EC: Electrical conductivity; TDS: Total dissolved solids; NRI: Nitrate reduction index; IRI: Iron reduction index. Mean ± Standard deviation, %.

3.3.1 Turbidity

The effect of coagulant agent dosing in the range of 25 – 250 mg/L at a pH of 7.013±0.006 on the evolution of turbidity is shown in Figure 2. The results indicate significant differences between the treatments and the dosage ($p<0.05$). Tukey's test reveals significant differences between the groups, with a maximum turbidity removal of 79.44±0.61% at a dose of 250 mg/L of biocoagulant and 93.11±0.49% at a dose of 50 mg/L for the chemical coagulant**.** (Amran, *et al*, 2021), found that when the initial turbidity is >100 NTU, the efficiency of the biocoagulant increases; for instance, at a dose of 50 mg/L and the initial turbidity of 500 NTU, they obtained 92.6% turbidity removal, in contrast to 2.7% when the turbidity is <100 NTU. Similar findings were confirmed by Mahanna, Fouad, & Zedan (2023), who used oat and onion seeds with initial turbidities of 130, 100, 70, 40, and 10 NTU. They found that at 130 NTU, removal efficiency was 97.6% for oats and 98.53% for onions, compared to 82.9% and 77.8%, respectively, at 10 NTU. Considering the initial turbidity in this study was <40 NTU, it demonstrates that Carica Papaya extract has the capacity to efficiently remove turbidity even under low turbidity conditions.

The ability of Carica Papaya extract to reduce turbidity in water samples can be explained by sorption/adsorption, destabilized particle binding, neutralization, and charge binding. Neutralization of charges is caused by the positive charges of the proteolytic protein. An increase in biocoagulant dosage results in improved collision, neutralization, and effective binding of colloids (Nhut, Hung, & Lap, 2021). However, if the concentration of coagulant exceeds the optimal dosage, it causes an increase in

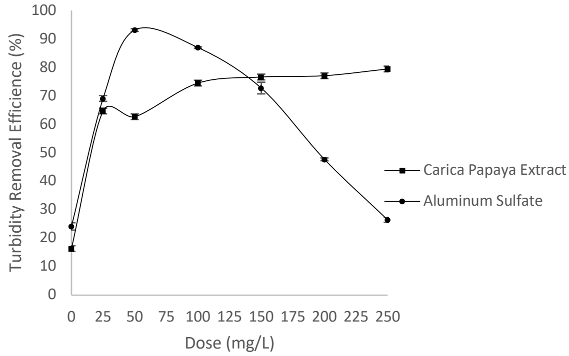


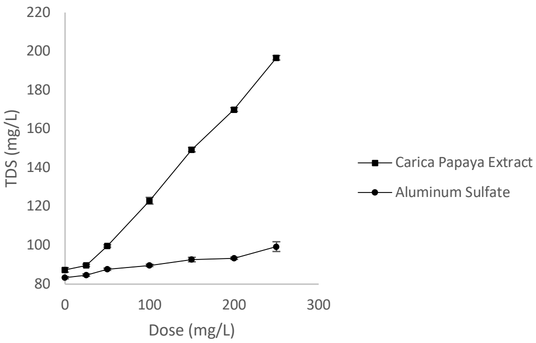
Figure 2. Effect of biocoagulant and chemical coagulant doses on turbidity removal (%) at pH 7.013 ± 0.006 and an initial turbidity of 30 NTU. Error bars represent the standard deviation of triplicate experiments.

water turbidity because the excess does not interact with colloidal particles with opposite charge.

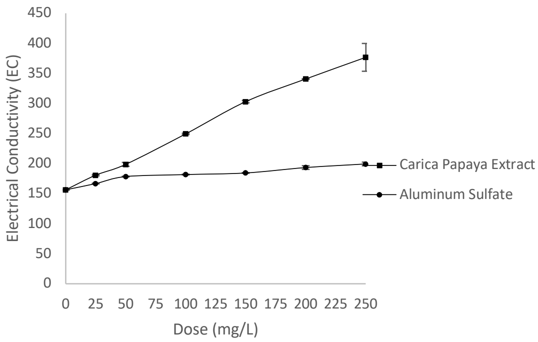
Therefore, Carica Papaya extract can be used as an alternative to chemical coagulants to reduce water turbidity and ensure human health. This leads to greater efficiency in the clarification of low- and high-turbidity water, especially in rural areas.

3.3.2 TDS and electrical conductivity (EC)

Total dissolved solids (TDS) and electrical conductivity (EC) indicate the presence of minerals, charged macromolecules, and other ionic compounds that can dissolve or dissociate in solutions (Putra, Amri, & Ayu, 2020). Figure 3 shows the effect of biocoagulant dosage on TDS and EC measurements, revealing an increase in both parameters for both the biocoagulant and aluminum sulfate. According to the analysis of variance, there were significant differences between the treatments and doses used ($p<0.05$), and according to Tukey's test for group differences, Carica Papaya extract at doses of 100-250 mg/L considerably increased the evaluated parameters. The increase in TDS and EC due to the biocoagulant is attributed to the presence of dissolved sodium chloride ($NaCl$) in the extract. (Putra, Nasriyanti, & Sarkawi, 2022), Similar results were obtained when the biocoagulant dose was increased, leading to a higher TDS and, consequently, a higher electrical conductivity (EC). However, according to the World Health Organization (WHO) regulations (1998), the maximum concentration limit for TDS is 1000 mg/L, and thus the parameters are within this limit. An increase in TDS could indicate a higher concentration of dissolved salts, which could affect the palatability of the water and potentially limit its use for certain purposes, such as agricultural irrigation, due to the sensitivity of some crops to salinity. Higher EC levels suggest an increased potential for corrosion in water distribution infrastructure, such as pipes and equipment, which



a)



b)

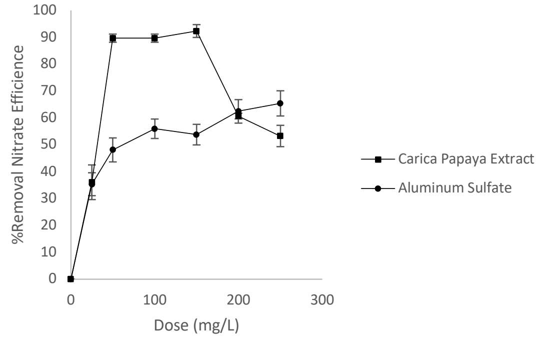
Figure 3. Effect of increase coagulant doses on (a) total dissolved solids and (b) electrical conductivity. Error bars represent the standard deviation of triplicate experiments.

could raise maintenance costs and reduce the lifespan of these systems. Therefore, the use of papaya seed extract coagulant obtained with sodium chloride has its limitations in waters with elevated TDS and EC levels, necessitating the addition of a unit operation for their reduction.

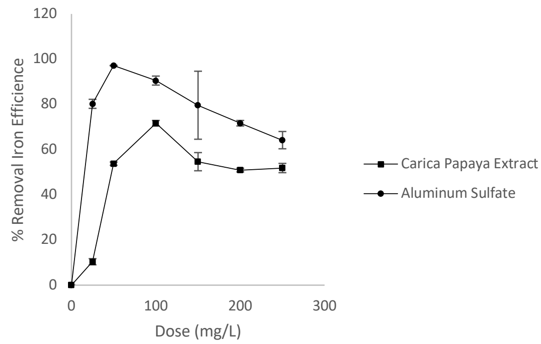
3.3.3 Nitrates and iron

Figure 4 illustrates the impact of biocoagulant dosage on nitrate (NO3-) and iron (Fe) removal. A notable nitrate removal of 92.32±2.39% was achieved with Carica Papaya extract at a dosage of 150 mg/L. Furthermore, no significant alteration ($p<0.05$) in nitrate removal was discerned within the biocoagulant dosage range of 50-150 mg/L, as depicted in Figure 4-a. These findings resonate with those reported by (Getahun, Asaithambi, Befekadu, & Alemayehu, 2023), who employed a composite biocoagulant (acanthus tribe, aloe vera, and moringa stenopetala), yielding a 99.12% reduction in nitrates. Likewise, in this study, escalating the coagulant dose led to a decline in nitrate removal efficiency.

In Figure 4-b, increasing coagulant doses facilitated the destabilization of colloidal suspension destabilization and floc formation, resulting in heightened removal. Aluminum sulfate exhibited an iron removal efficiency of 97.16% at a dose of 50 mg / L, while the Carica Papaya extract achieved an efficiency of 71.66% at a dose of 100 mg/L. Significant discrepancies ($p<0.05$) were observed, which could



a)



b)

Figure 4. Effect of coagulant doses on a) Nitrates and b) Iron. Error bars represent the standard deviation of triplicate experiments.

be attributed to a lesser increase in floc area by the biocoagulant (Pramanik, Pramanik, & Suja, 2016).

Conclusion

The biocoagulant extracted from the Carica Papaya seeds proved to be a promising agent for the treatment of surface water. FTIR and physicochemical analyzes confirmed the presence of functional groups -OH, CN, NH2, and C=O, rendering the extract a polyelectrolyte. These functional groups facilitate the coagulation process. Turbidity removal reached 79.44±0.61%; however, TDS and EC levels increased as a consequence of using sodium chloride for solubilizing the proteins present in Carica Papaya seeds. Nitrate removal was better compared to aluminum sulfate, reaching 92.32%; on the other hand, iron removal was higher with aluminum sulfate. The use of Carica papaya seeds as a biocoagulant in water treatment proved to be an efficient, environmentally friendly and feasible replacement compared to the use of chemicals. Future research should focus on developing scalable extraction processes and assessing long-term impacts on treated water quality.

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