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Revealing the potential of extracted *Tamarindus indica* seed as a biocoagulant for aquaculture wastewater treatment: Effect of solvent type, concentration, dose, and toxicity assessment

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ABSTRACT

Aquaculture wastewater contains high levels of organic matter and nutrients, which can be harmful to aquatic life if discharged improperly into surface water bodies. Coagulation-flocculation is currently the best practice for treating aquaculture effluent with biocoagulants, offering an alternative to metal-based coagulants. This study aims to investigate the potential of Tamarindus indica seeds as a biocoagulant for treating aquaculture wastewater, focusing on the optimal solvent extraction, concentration, and dose. This study also examines the toxicity of biocoagulants to aquatic organisms. Coagulation-flocculation study was conducted under jar test experiment with NaCl, NaOH, and HCl used as solvents; concentration of 0-10 g/L; and doses of 1-5 % v/v under 120 rpm (rotation per minute) rapid mixing for 1 min, 20 rpm slow mixing for 20 mins, and 60 mins sedimentation time. A characterization study showed that NaCl-treated T. indica has a positive zeta potential charge, attributed to the presence of hydroxyl, carbonyl, and amide functional groups. Under this optimum condition (NaCl-extract, 6 g/L, and 4 % v/v), the biocoagulant achieved high removal (>50 %) of turbidity, TSS (total suspended solid), and ammonia and considerably good removal of other parameters (TN [total nitrogen], BOD_5 [biological oxygen demand], COD [chemical oxygen demand]). The toxicity test revealed that no mortality was observed at a concentration of 1 g/L, whereas 10 g/L resulted in a 100 % mortality rate after 24 hours of exposure. Further toxicity analysis is suggested to be conducted using treated final effluent (not directly using biocoagulant substances) to observe the direct impact of the treated wastewater if discharged into the water bodies.

1. Introduction

Aquaculture has evolved as a significant contributor to the global food supply, particularly in Asia, which represents over 90 % of the world's aquaculture output [25]. The advancement of the aquaculture sector is anticipated to result in a heightened need for fish as a protein source for human consumption, reaching 21.2 tons in 2016 and progressively increasing every decade [26]. Increasing aquaculture production generates substantial volumes of aquaculture wastewater. The global aquaculture sector produces 356,590 m^3 of effluent annually [30, 47]. If not managed appropriately, it can adversely affect the ecosystem, including the deterioration of surface water quality ([4,5,14,70]).

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Aquaculture effluent is characterized by high concentrations of organic compounds and nutrients derived from fish feed waste and excreta [39, 40]. High introduction of organic compounds and nutrients can lead to eutrophication and anoxic conditions in aquatic environments, resulting in biodiversity loss and potentially long-term impacts on ecosystem functionality [1,18,63,68].

The current optimal methods in aquaculture wastewater treatment are coagulation, flocculation, and sedimentation [4,12], with alum shown to be the most prominent coagulant used for the treatment [7, 21]. Alum demonstrates exceptional efficacy in eliminating turbidity and suspended particles from aquaculture wastewater, achieving up to 98 % removal [22,35]. Despite their high effectiveness, the use of metal-based coagulants in wastewater treatment has raised significant concerns, including alterations in aquatic pH stability and the generation of non-biodegradable sludge [29,45].

The use of biocoagulants is a possible alternative to address the growing concerns over metal-based coagulants [20,50,51]. Biocoagulants consist of plant materials, bacteria, microalgae, and animal-derived substances [48], with plant-based components being the most frequently utilized [56]. A prior study demonstrated the efficacy of *Moringa oleifera* derivatives in eliminating suspended particles from aquaculture wastewater [24]. Furthermore, *M. oleifera* was also utilized as a bioflocculant for the recovery of algae from aquaculture effluent, with a recovery rate of up to 92 % [33]. As plant-based biocoagulants offer a promising future, the exploration of new biomass for current use awaits further investigation.

Tamarindus indica is a native tropical plant which naturalized in Asia [23]. *T. indica* is known as a traditional medicinal plant that is rich in bioactive compounds, including polysaccharides, carbohydrates, protein, and fibers [36]. Previous research has highlighted the performance of polysaccharides in *T. indica* in treating cheese processing wastewater, resulting in a 71 % removal of total dissolved solids (TDS) and a 75 % reduction in chemical oxygen demand (COD). Despite its potential, the use of *T. indica* seeds as a biocoagulant is currently limited, especially for treating aquaculture wastewater. This research aims to investigate the potential of *T. indica* seeds as a biocoagulant in treating aquaculture wastewater, exploring the effects of different solvent extraction methods, concentrations, and doses. This study also provides a toxicity assessment of *T. indica* as a biocoagulant to aquatic organisms, a topic that is currently rarely studied.

2. Materials and methods

2.1. Aquaculture wastewater collection

The aquaculture wastewater used in this study was sourced from catfish aquaculture ponds located in Ngaglik I, Sadenganmijen, Krian District, Sidoarjo City, East Java Province, Indonesia (7°25′47.86"S, 112°35′3.01"E). Samples were collected and preserved in a 40 L HDPE tank (Penguin, Indonesia). The preliminary attributes of the effluent were assessed immediately post-sampling, including TSS (total suspended solid), BOD_5 (biological oxygen demand), COD (chemical oxygen demand), TN (total nitrogen), ammonia, nitrate, nitrite, TP (total phosphate), pH, dissolved oxygen (DO), turbidity, color, and zeta potential. All parameters were assessed utilizing relevant standard methodologies [13].

2.2. Preparation and characterization of biocoagulant

T. indica was obtained from Sukorejo Village, Kebonsari District, Madiun Regency, Indonesia. *T. indica* seeds were taken as much as 1 kg. The preparation of *T. indica* seeds as a biocoagulant was carried out in several stages. The first stage was drying the seeds using an oven (Memert, Germany) at a temperature of 60°C for 24 h [8]. The dried seeds were then crushed using a pestle and crushed again into powder using a blender (Maspion, Indonesia). The *T. indica* seed powder was then sieved using a 100-mesh sieve [69]. The seed powder that did not pass through the mesh was crushed again with a blender until it did pass through the mesh. Dried *T. indica* seeds with a size of 100 mesh were used as raw biocoagulants in the following stages. The raw biocoagulant characteristic tests consisted of performing SEM-EDX (scanning electron microscopy-energy dispersive X-ray) tests (HITACHI FLEXSEM 100, Japan) to examine its surface morphology and elemental composition.

2.3. Determination of optimum condition of biocoagulant

2.3.1. Effect of solvent extraction types

The raw biocoagulant was weighed in amounts of up to 1 g; then, the powder was dissolved in 100 mL of 0.5 M NaCl, 100 mL of 0.1 M HCl, and 100 mL of 0.01 M NaOH. The solution was then mixed using a magnetic stirrer on a magnetic stirrer hot plate (Sigma Aldrich, Netherlands) for 10 mins with a stirring speed of 150 rpm [62]. The mixed solution was then filtered using a 0.45 μm pore filter paper. The filtered solution was used as a biocoagulant [55], which was then characterized by the FTIR [60] and zeta potential [49]. A biocoagulant was also used to test the preliminary turbidity removal performance, which will be used to determine the solvent to be used in the following steps. The preliminary turbidity removal test was conducted using the jar test method [3,9], in which 100 mL of aquaculture wastewater was used as a pollutant to be treated in a 500 mL glass beaker. A total of 10 % volume/volume (v/v) of the biocoagulant was introduced into the jar. Rapid mixing was set at 120 rpm for 1 min, slow mixing was set at 20 rpm for 20 min, and sedimentation was set for 60 min. The final effluent was collected at approximately 1 cm from the water surface and tested using a turbidimeter (Sigma Aldrich, Netherlands).

2.3.2. Effect of biocoagulant concentrations

After obtaining the best solvent from the previous stage, a total of 0, 0.2, 0.4, 0.6, 0.8, and 1 g of raw biocoagulant were diluted into 100 mL of 0.5 M NaCl solution (to produce 0, 2, 4, 6, 8, and 10 g/L biocoagulant), which then undergo the same extraction procedure as described in Section 2.3.1. As much as 3 % (v/v) was used as biocoagulant doses in this stage [57]. A similar coagulation-flocculation operational parameter, as described in Section 2.3.1, was then applied. The results of this stage were used in the following stage to determine the optimum biocoagulant doses.

2.3.3. Effect of biocoagulant doses

In this stage, several biocoagulant doses of 1 %, 2 %, 3 %, 4 %, and 5 % were tested [54]. A biocoagulant extract was added to the aquaculture wastewater sample, and the coagulation-flocculation process was conducted according to the previously established operational parameters (Section 2.3.1). Optimum biocoagulant doses were selected based on the turbidity removal value and will be used to test the overall performance.

2.4. Performance of biocoagulant in treating aquaculture wastewater under optimum condition

Based on the results of the previous stage, the overall biocoagulant performance in treating aquaculture was tested. In this stage, several wastewater parameters were tested to reveal the overall biocoagulant performance, including BOD_5, COD, TN, ammonia, TSS, and turbidity.

2.5. Toxicity assessment

The toxicity assessment of the biocoagulant was conducted on freshwater crustaceans, namely the Cladocera species, *Daphnia pulex* Leydig, 1860, and *Daphnia magna* Straus, 1820. This step was conducted in the Hydrobiology Laboratory at the Institute of Biology, University of Szczecin, Poland, following the previously established methodology [64]. Four concentrations of bioflocculant, specifically 0.01, 0.01, 1, and 10 g/L, were chosen to evaluate the toxicity of this substance. *D. pulex*

Table 1
Characteristics of aquaculture wastewater.

Parameters	Unit	Values	Standard*
TSS	mg/L	180	200
BOD_5	mg/L O_2	104	50
COD	mg/L O_2	194	100
TN	mg/L NH_3-N	61.17	30
Ammonia	mg/L NH_3-N	49.40	5
Nitrate	mg/L NO_3-N	0.53	20
Nitrite	mg/L NO_2-N	0	1
TP	mg/L PO_4-P	13.27	-
pH	-	7.2	6-9
DO	mg/L O_2	0	-
Turbidity	NTU	60.2	-
Color	Pt-Co	1834.7	-
Zeta potential	mV	-22.1	-

*Indonesian national standard for unclassified effluent No. 5, 2014

and *D. magna* were sourced from freshwater ponds in north-west Poland (53°27′31.4″N 14°32′45.6″E for *D. pulex* and 53°44′47.6″N 17°31′51.6″E for *D. magna*), three months before the experiments. Daphnids were sustained under the same environmental conditions during both cultivation and experimentation (nonsparkling mineral water, Lewiatan, Poland) [32,57]. Three groups of five randomly selected daphnids were evaluated in 100 mL plastic reactors for each species and tested concentration. The daphnids received 1 µg of feed (*Chlorella* sp. powder) per day for each reactor. The toxicity assessment was conducted over 7 days, consisting of an illumination phase of 8 h (using a white 6 W light-emitting diode) and a dark phase of 16 h. The mortality rate was illustrated in Kaplan–Meier survival curves using R-studio.

2.6. *Statistical analysis*

Statistical analysis was performed using the Statistical Product and Service Solutions (SPSS) software [52,53]. The obtained data were analyzed using One-Way Analysis of Variance (ANOVA). If the results of the ANOVA tests were significant, analysis was then continued with the Duncan test at $\alpha = 0.05$. Data tested with ANOVA were previously assessed for normality using the Kolmogorov-Smirnov test. If the data were not normally distributed, the Kruskal-Wallis test was used instead.

3. Results and discussions

3.1. *Characteristics of aquaculture wastewater*

Aquaculture wastewater is categorized as a pollutant due to its high concentration of organic and nutrient contents. Based on Table 1, it can be observed that the aquaculture wastewater used in this research was rich in organic substances (high BOD_5 and COD) and contained high amounts of nutrients (TN, ammonia, and TP). The characteristics of aquaculture wastewater depend significantly on the cultured species [37,66,71]. High organic content in aquaculture wastewater originates from fish excreta [31] and feed residue [34,58], while high nutrient content might originate from the use of fertilizer to regulate the growth of algae in the pond (acting as additional feed for grown species) [39, 65]. In previous research, aquaculture wastewater from Pasuruan Regency, Indonesia, was recorded to have content of BOD_5, COD, ammonia, TN, and TP higher than permissible standards [43]. Febrianto et al. [27] also mentioned BOD_5 values of 75.75 mg/L, COD of 124.67 mg/L, and ammonia of 4.304 mg/L in tilapia wastewater. If discharged into surface water bodies without proper treatment, high organic content may cause a reduction in dissolved oxygen, which is detrimental to aquatic life. High nutrient contents (N and P) may also contribute to eutrophication, which can exacerbate the reduction of dissolved oxygen.

3.2. *Characteristics of biocoagulant*

Based on microscopic analysis (Fig. 1), the biocoagulant used had an uneven and irregular grain structure with a slightly coarse surface texture. The compositional analysis revealed that *T. indica* seed powder contained minerals such as K (11 %), Mg (0.3 %), and Ca (0.1 %). Similarly, the waste from palm petioles comprises Ca, Fe, Al, and Si as the primary metallic constituents, along with carbon and oxygen [78]. Biocoagulants produced from walnut husks also contain Ca, Fe, Al, Si, K, and Mg [77].

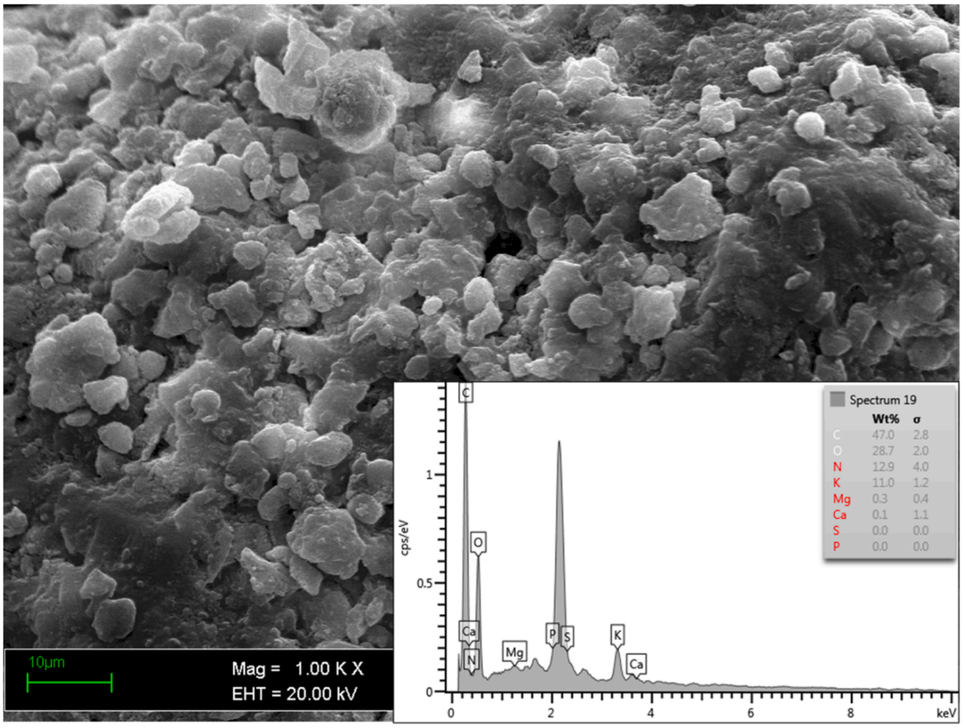

Fig. 1. Morphology and elemental analysis of *T. indica* powder by SEM-EDX with 1000× magnification.

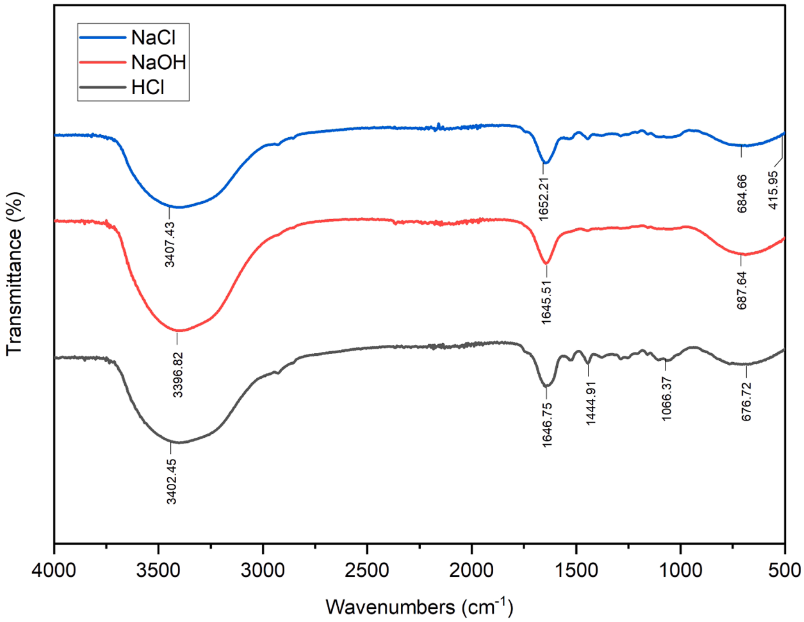

Fig. 2. Comparison of functional groups of *T. indica* as biocoagulants after extraction with NaCl, NaOH, and HCl.

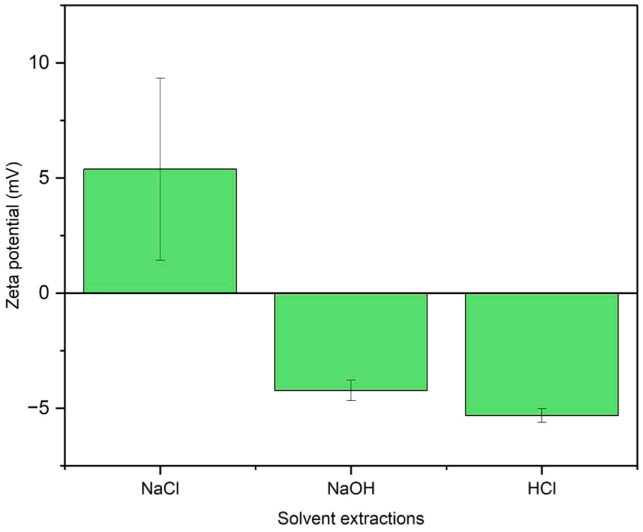

Fig. 3. Comparison of the zeta potential value of *T. indica* as biocoagulants after extraction with NaCl, NaOH, and HCl.

3.3. *Performance of biocoagulant to treat aquaculture wastewater*

3.3.1. *Effect of solvent extraction types*

3.3.1.1. Functional group analysis. Based on the FTIR analysis (Fig. 2), all three solvents used for the extraction of T. indica yielded similar spectra. Broad peaks were observed around 3400 cm^{-1}, with strong absorption bands at 3407.43 cm^{-1} for NaCl, 3398.82 cm^{-1} for NaOH, and 3402.45 cm^{-1} for HCl. Broad peaks around this region typically correspond to O-H stretching, suggesting the presence of hydroxyl (-OH) groups, possibly from water molecules or hydroxyl-containing functional groups. All three extractions also showed peaks around 1650 cm^{-1} (1652.21 for NaCl, 1645.51 for NaOH, and 1646.75 for HCl); this vibration typically corresponds to the C=O stretching (indicating the presence of carbonyl groups) or N–H bending (indicating the presence of amide groups). In the lower wavenumber region, all three solvents showed vibration around 690 cm^{-1}, which is typically associated with the C-H structure from the aromatic band. Differently, HCl-treated powder showed additional vibrations at 1444.91 and 1066.37 cm^{-1}, while NaCl-treated powder showed additional vibrations at 415.95 cm^{-1}. Observed additional vibrations in HCl treated sample typically correspond to C–O stretching and C–H bending, while additional vibration in NaCl treated sample might be associated with a metal-oxygen bond. Based on this result, HCl seems to alter the structure of *T. indica,* indicated by the formation of more functional groups [6,10,74], while NaOH showed a pronounced O-H band, which may correspond to the increased hydroxylation [2,76].

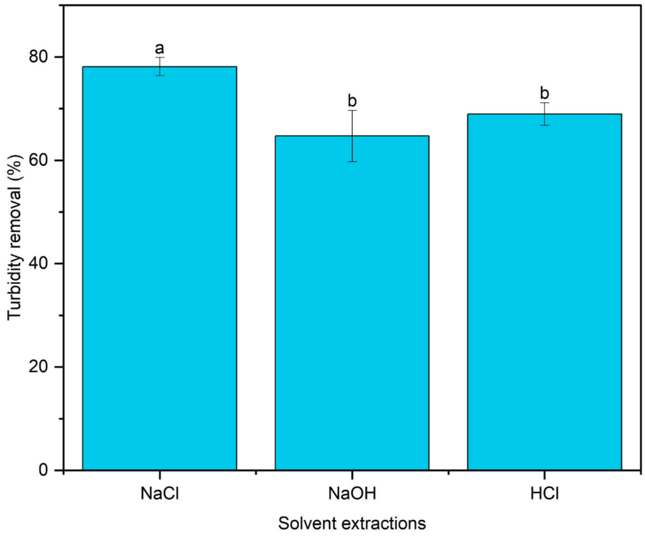

Fig. 4. Effect of solvent extraction on 1 gram of biocoagulant to remove turbidity in aquaculture wastewater. Annotations (a, b, and c) represent significant differences in turbidity removal between different solvent extractions (p<0.05).

3.3.1.2. Zeta potential analysis. Zeta potential analysis (Fig. 3) showed that NaCl-treated biocoagulant has a positive charge, while NaOH and HCl-treated biocoagulants showed a negative charge. The shift of the zeta potential indicates that acidic and basic extraction on *T. indica*

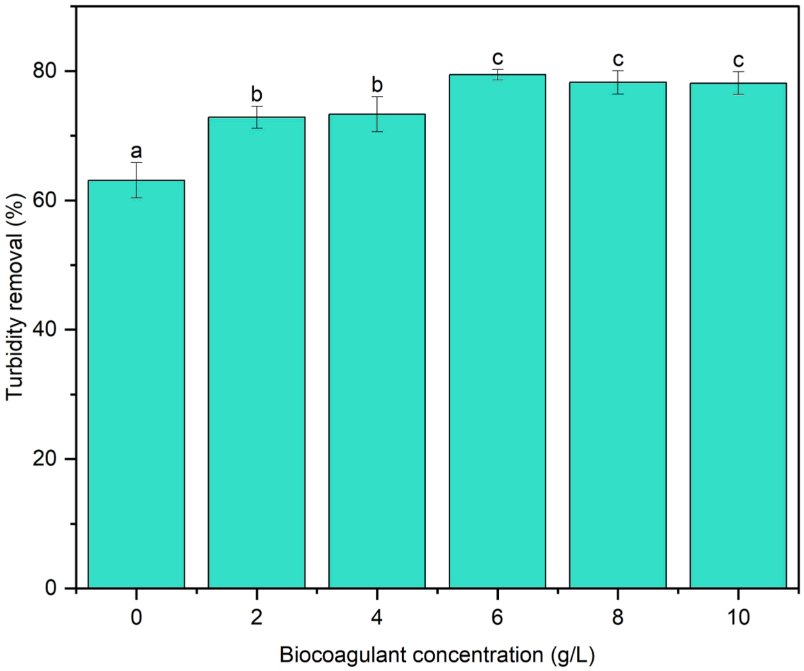

Fig. 5. Effect of biocoagulant concentrations after extraction with NaCl to remove turbidity in aquaculture wastewater. Annotations (a, b, and c) represent significant differences in turbidity removal between different biocoagulant concentrations ($p<0.05$).

significantly alters the surface charge properties [6,10,74]. Acid extraction may lead to protonation, while basic extraction may cause deprotonation and the introduction of hydroxyl groups, which increase the net negative charge [75]. In previous research, *M. oleifera* was recorded to also have a positive charge of 12.27 mV at pH 8 [46], while salt-treated *M. oleifera* showed a negative charge of -3.53 mV [61]. This suggests that different treatments may lead to a significant charge reversal of the plant-based biocoagulant.

3.3.1.3. Turbidity Removal. Fig. 4 shows that the NaCl-extracted biocoagulant performed significantly higher compared to the NaOH- and HCl-treated biocoagulants. The higher turbidity removal by NaCl-extracted biocoagulant (77.39±2.11 %) can be correlated with the positive charge of the extract [24]. The NaCl-extracted biocoagulant exhibits some degree of electrostatic repulsion, which may facilitate a charge neutralization mechanism to coagulate the suspension in aquaculture wastewater [44,67]. The lower performance of turbidity removal by NaOH- and HCl-extracted biocoagulants might be attributed to the negative charge of the extracts, which leads to increased repulsion between particles in aquaculture wastewater, causing a reduction in

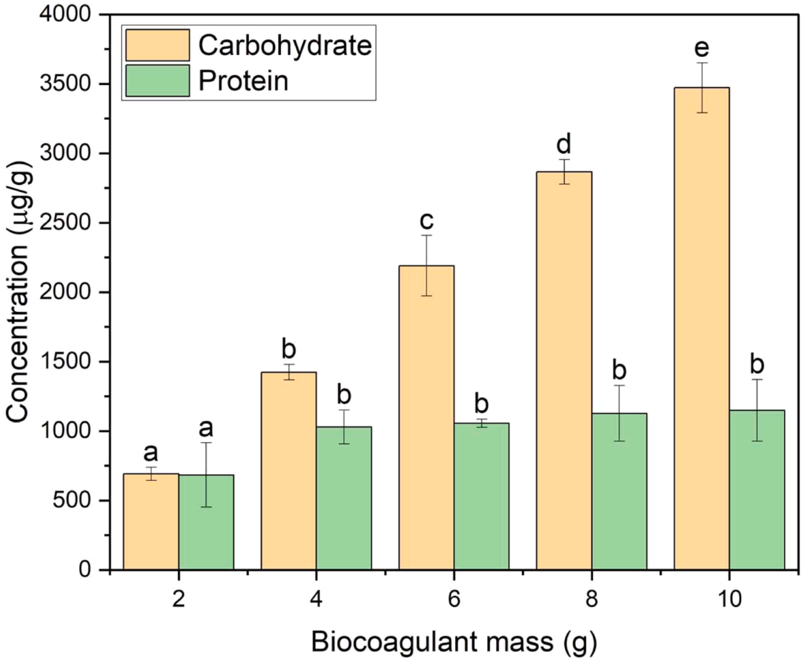

Fig. 6. Carbohydrate and protein concentration in various biocoagulant concentrations after extraction with NaCl. Annotations (a, b, c, d, and e) represent significant differences in carbohydrate and protein concentrations among biocoagulant masses ($p<0.05$).

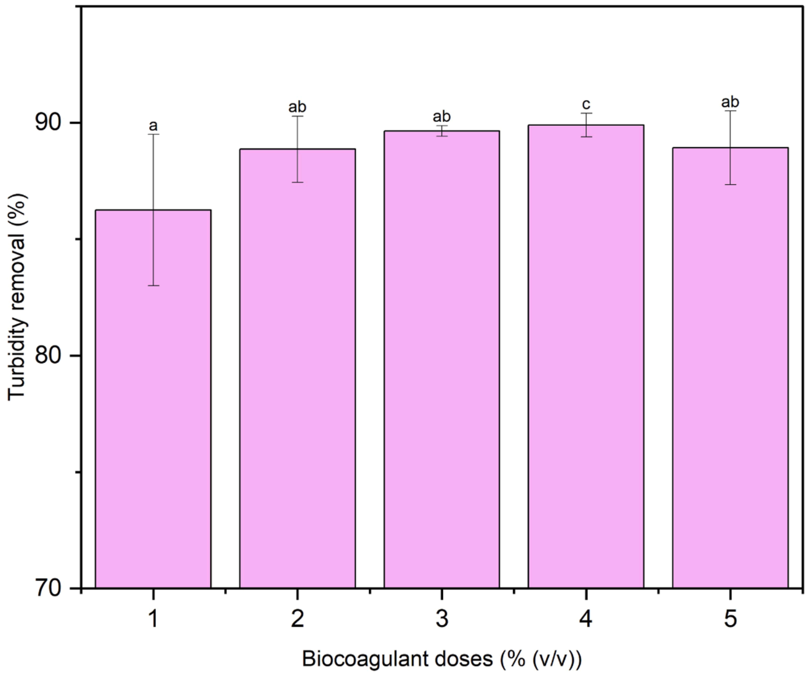

Fig. 7. Effect of biocoagulant doses to remove turbidity in aquaculture wastewater. Annotations (a, b, and c) indicate significant differences in turbidity removal between different biocoagulant doses ($p < 0.05$).

coagulation efficiency ([11,55], 2020). Based on these results, NaCl was selected as the extraction solvent and used in subsequent steps to evaluate its overall performance.

3.3.2. *Effect of biocoagulant concentrations*

The increasing biocoagulant concentration used to treat aquaculture wastewater improved turbidity removal; however, at a certain level, it did not yield any significant improvement [61,69]. A significant increment of turbidity removal was observed from 0 to 2 g/L and 4 to 6 g/L of biocoagulant. These suggested that an increasing concentration from 6 to 10 g/L did not yield any significant improvement in turbidity removal efficiencies, and may have even slightly reduced them. Excess biocoagulant may provide additional turbidity to the wastewater, which needs to be avoided [28,73]. Based on these results, 6 g/L of NaCl-extracted *T. indica* was selected as the optimum concentration for turbidity removal.

Fig. 5

Previous literature has suggested that carbohydrates and proteins are responsible for the coagulation mechanisms of biocoagulants ([41,42, 51], 2022b). To observe this, carbohydrate and protein content were analyzed in this stage (Fig. 6). Referring to Fig. 6, it can be clearly seen that the increase in biocoagulant concentration was in accordance with

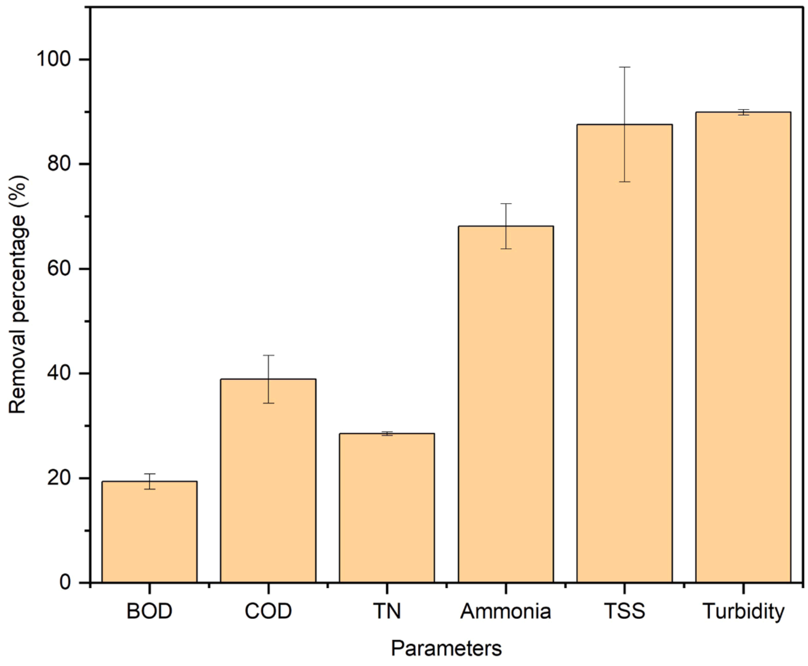

Fig. 8. Performance of *T. indica* as a biocoagulant to remove various parameters in aquaculture wastewater under optimum conditions.

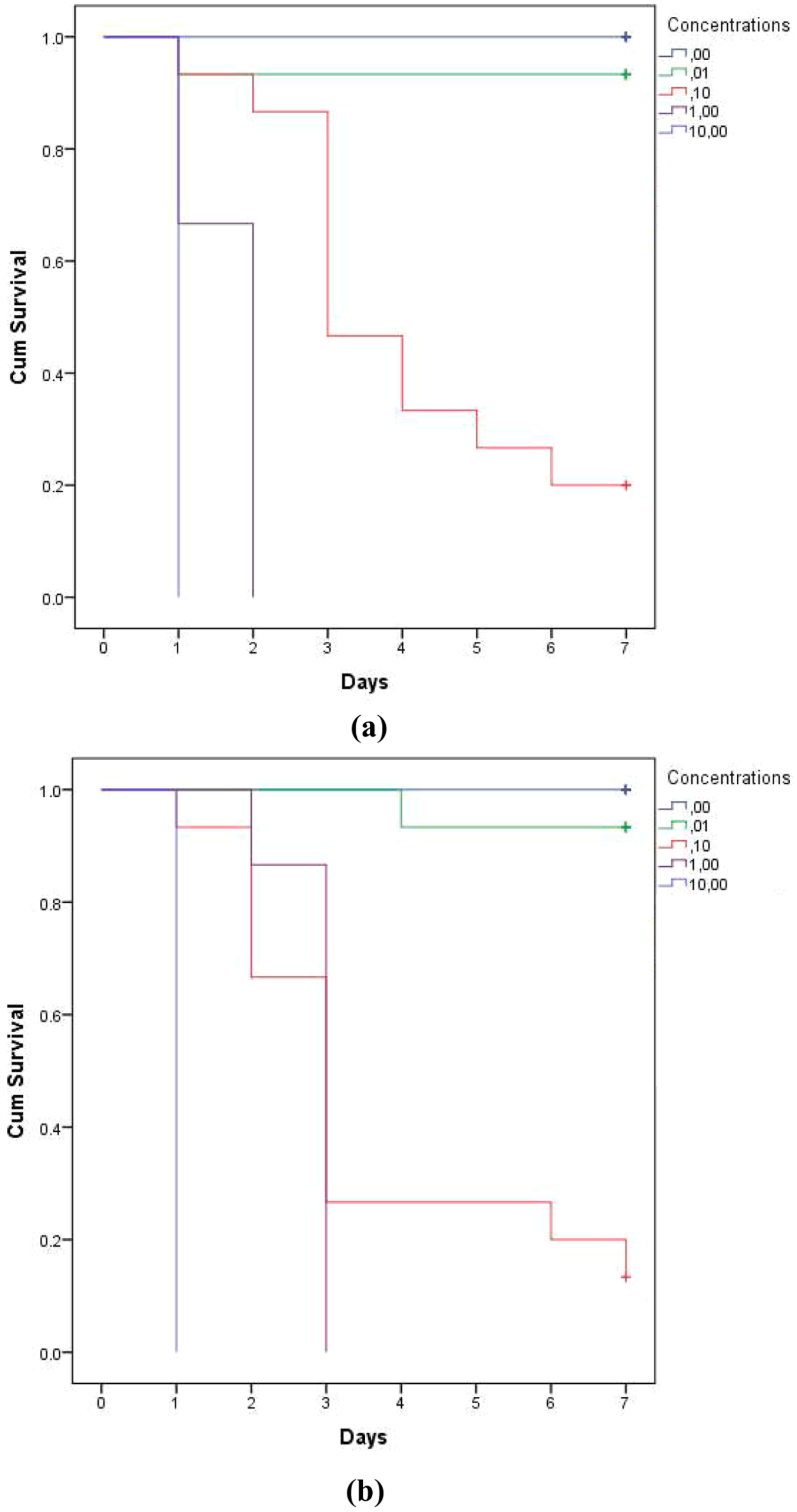

Fig. 9. Kaplan–Meier survival curve for (a) *D. pulex* and (b) *D. magna* exposed to various concentrations of biocoagulant.

the increase in carbohydrate content in the extract. In contrast, the protein content in the extract did not increase significantly with increasing biocoagulant concentration. It was mentioned that salt extraction cannot efficiently extract protein from plant biomass; however, it is necessary to reduce the oil content, which can interfere with the biocoagulant performance [51]. Alcohol extraction is suggested to be used in order to obtain a high yield of protein from plant biomass [19]. Correlating the observed carbohydrate and protein contents with turbidity removal at various biocoagulant concentrations, the results indicate that turbidity removal in aquaculture wastewater is mainly facilitated by protein rather than carbohydrate.

3.3.3. *Effect of biocoagulant doses*

According to Fig. 7, doses 1, 2, 3, and 5 (% v/v) shared the same significance annotation, indicating no significant turbidity removal obtained by those doses. Only doses of 4 % (v/v) showed a significantly higher turbidity removal. Similar to the effect of biocoagulant concentration, the increase of doses may contribute to a higher turbidity removal, and at a certain point, it did not give any significant addition and even worsened the overall removal efficiency due to the addition of turbidity by the extracts [61,69]. Based on these results, 4 % (v/v) was selected as the optimum condition to observe the overall removal performance.

3.3.4. Performance of biocoagulant under optimum condition

From the previous steps, the optimum condition for aquaculture wastewater coagulation-flocculation using T. indica as a biocoagulant was determined to be NaCl-extracted, with a concentration of 6 g/L and a dose of 4 % (v/v). Under these optimum conditions, the biocoagulant demonstrated high removal of turbidity, TSS, and ammonia (>50 %), as well as considerable removal of other parameters (TN, BOD_5, COD). The treated aquaculture wastewater has the following characteristics: BOD_5 83.89 mg/L, COD 118.55 mg/L, TN 43.74 mg/L, ammonia 15.74 mg/L, TSS 22.42 mg/L, and turbidity 6.08 NTU. Some of the tested parameters still exceed the national permissible standard for discharge into water bodies; thus, combining this technique with further polishing treatments, such as filtration (Fitriani et al., 2023) or constructed wetlands (Al-Ajalin et al., 2020; Kurniawan et al., 2021b), is highly recommended.

By using biocoagulant, the removed solids from aquaculture effluent (sludge as treatment by-product) can be directly used as a soil conditioner [15] and fertilizer [59,72]. To avoid odor problems, further processing of the resultant sludge can be conducted via drying and anaerobic digestion [16,17]. Anaerobic digestion has been proven to enhance the quality of fertilizer derived from aquaculture sludge. In addition to that, further utilization of the resultant biomass can be conducted via vermicomposting to produce compost (for soil conditioner) and worm biomass (for fish feeds) [38].

Fig. 8

3.4. Toxicity assessment of biocoagulant to Daphnia pulex and Daphnia magna

The toxicity of NaCl-extracted *T. indica* as a biocoagulant is presented in the Kaplan-Meier survival curve, as shown in Fig. 9. Fig. 9 illustrates that the elevated concentration of biocoagulant markedly influenced the survival rates of both species. The duration of exposure also influenced the survivability. The results demonstrated that higher concentrations of biocoagulant reduced the survivability of *D. pulex* and *D. magna*. In coherence with concentration, prolonged exposure duration diminished the survivability [32,64]. Both species exhibited no mortality during the initial hour of exposure; however, the first instances of mortality were recorded at a biocoagulant concentration of 10 g/L after 24 hours of exposure for both species. The results indicate that *D. magna* exhibits greater survivability to biocoagulant exposure, as evidenced by their higher survival at elevated bioflocculant concentrations throughout the testing period (a 100 % mortality rate at 1 g/L on day 3 vs. day 2 for *D. pulex*).

Referring to these results, the use of 6 g/L as biocoagulant concentration in this research seems to negatively affect the survivability of *D. pulex* and *D. magna*, which represents the biocogulant's toxicity in the aquatic environment. However, it is worth noting that the used biocoagulant will be flocculated alongside the particles in the aquaculture wastewater, resulting in a lower biocoagulant concentration in the treated effluent. The toxicity test was performed directly with the initial concentration used for treatment without considering the final effluent concentration of the biocoagulant after treatment, which would certainly be lower. Further analysis of the biocoagulant remaining concentration in the treated effluent can be conducted to enrich the results obtained in this study. Additionally, a toxicity test can be conducted using the final treated effluent to assess its direct impact.

4. Conclusion

Aquaculture effluent, rich in organic matter and nutrients, endangers aquatic life if inadequately released into surface water bodies. *Tamarindus indica* seeds were utilized as a biocoagulant for treating aquaculture wastewater. The NaCl-treated *T. indica* exhibited a positive zeta potential charge alongside hydroxyl, carbonyl, and amide functional groups. Under these optimal conditions, the biocoagulant achieved significant removal of turbidity, total suspended solids (TSS), and ammonia, along with effective removal of other parameters. Removal of pollutants was subjected to the following charge neutralization mechanisms. Toxicity assessments indicated no fatalities at a concentration of 1 g/L, but a concentration of 10 g/L resulted in a 100 % fatality rate after 24 hours of exposure. It is recommended to do further toxicity studies utilizing treated final effluent to assess the direct effects of treated wastewater on aquatic species.

CRediT authorship contribution statement

Setyo Budi Kurniawan: Writing – review & editing, Writing – original draft, Visualization, Data curation, Conceptualization, Validation. **Azzahra Aulia Haya:** Investigation, Formal analysis, Data curation. **Thin Soedarti:** Supervision. **Eko Prasetyo Kuncoro:** Supervision. **Łukasz Sługocki:** Resources, Methodology, Investigation. **Kacper Nowakowski:** Methodology, Investigation. **Peer Mohamed Abdul:** Resources. **Muhammad Fauzul Imron:** Writing – original draft, Visualization, Supervision, Funding acquisition, Data curation, Conceptualization.

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.

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Data availability

Data will be made available on request.

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