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REMEDIATION CAPACITY OF AQUATIC PLANTS FOR REDUCING ORGANIC LOAD AND NUTRIENTS IN NILE TILAPIA (*Oreochromis niloticus*) AQUACULTURE WASTEWATER

Kapasitas Remediasi Beberapa Tumbuhan Air dalam Menurunkan Beban Organik dan Nutrien pada Air Limbah Budidaya Ikan Nila (*Oreochromis niloticus*)

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ABSTRACT

Nile tilapia (*Oreochromis niloticus*) aquaculture wastewater contains substantial amounts of organic matter and nutrients. The accumulation of these pollutants may contribute to the deterioration of water quality. Phytoremediation has emerged as a safe and eco-friendly wastewater treatment technology. This study aimed to evaluate the remediation capacity of selected aquatic plants in reducing organic load and nutrients in Nile tilapia aquaculture wastewater. The aquatic plants used in this study were water hyacinth (*Eichhornia crassipes*), yellow velvetleaf (*Limnocharis flava*), water spinach (*Ipomoea aquatica*), and water lettuce (*Pistia stratiotes*). The control treatment consisted of wastewater without aquatic plants. Each treatment was performed in triplicate to obtain the mean and standard deviation values. The measured water quality parameters included total organic matter (TOM), nitrate, phosphate, bacterial abundance, dissolved oxygen (DO), pH, total dissolved solids (TDS), and turbidity. Leaf chlorophyll content of the aquatic plants was also measured. The results showed that water hyacinth reduced TOM by up to 37.5 ± 1.2%. Water spinach reduced nitrate by 81.0 ± 1.6%, while phosphate decreased by 79.4 ± 5.0%. The water spinach treatment showed the highest bacterial abundance on day 14, with an increase of (35.7 ± 2.0) × 10^3 CFU/mL from the initial value. This treatment also had the lowest reduction in leaf chlorophyll content, at 6.7 ± 2.9%. Water quality conditions after the remediation process met the water quality standards for aquaculture. These findings indicate that water spinach and water hyacinth were the most effective aquatic plants for reducing organic load and nutrients, particularly nitrate and phosphate. Water spinach also showed relatively high tolerance to Nile tilapia aquaculture wastewater and was able to maintain its photosynthetic pigments under wastewater stress conditions.

Key words: Remediation capacity, aquatic plants, organic load, nutrients.

ABSTRAK

Air Limbah budidaya ikan nila mengandung limbah organik dan nutrien yang berasal dari sisa pakan, feses dan metabolit ikan. Akumulasi bahan organik dan nutrien dari air limbah budidaya berpotensi untuk mencemari lingkungan perairan. Pengolahan air limbah dengan metode fitoremediasi merupakan salah satu metode yang aman dan ramah lingkungan. Penelitian ini bertujuan untuk mengetahui kapasitas remediasi beberapa jenis tumbuhan air untuk menurunkan beban organik dan nutrien pada air limbah budidaya ikan nila. Jenis tumbuhan air yang digunakan adalah: Eceng gondok (*Eichhornia crassipes*), Genjer (*Limnocharis flava*), Kangkung air (*Ipomoea aquatica*), dan Kayu apu (*Pistia stratiotes*). Kontrol yang digunakan adalah perlakuan tanpa menggunakan tumbuhan air. Setiap perlakuan diulang sebanyak 3 kali untuk mendapatkan nilai rata-rata dan standar deviasinya. Parameter kualitas air yang diukur adalah: Bahan Organik Total (BOT), nitrat, fosfat, kelimpahan bakteri, oksigen terlarut (DO), pH, jumlah padatan terlarut (TDS), dan kekeruhan. Kandungan klorofil pada daun tumbuhan air juga diukur. Hasil penelitian menunjukkan bahwa eceng gondok mampu menurunkan BOT hingga mencapai 37,5 ± 1,2 %. Kangkung air menurunkan nitrat hingga 81,0 ± 1,6%, sedangkan kandungan fosfatnya turun hingga 79,4 ± 5,0 %. Perlakuan kangkung air menunjukkan kelimpahan bakteri tertinggi pada hari ke-14, yaitu sebesar (38,9 ± 2,0) × 10^3 CFU/mL, dengan peningkatan tertinggi sebesar (35,7 ± 2,0) × 10^3 CFU/mL. Perlakuan ini juga menunjukkan penurunan kandungan klorofil daun yang paling rendah, yaitu sebesar 6,7 ± 2,9%. Kondisi kualitas air setelah proses remediasi telah memenuhi baku mutu kualitas air untuk budidaya ikan. Hasil ini mengindikasikan bahwa kangkung air dan eceng gondok paling efektif untuk menurunkan beban organik dan nutrien (nitrat dan fosfat). Kangkung air juga relatif tahan terhadap paparan air limbah budidaya ikan nila, dan mampu mempertahankan pigmen fotosintetik selama dalam tekanan air limbah tersebut.

Kata Kunci: Kapasitas remediasi, tumbuhan air, beban organik, nutrien.

INTRODUCTION

Tilapia farming is among the most widely developed freshwater aquaculture activities due to its high economic value, ease of cultivation, and broad market acceptance. With increasing demand, tilapia farming systems have gradually shifted from traditional practices toward semi-intensive and intensive farming systems (Gustiano et al., 2023). However, this intensification may potentially increase organic and nutrient waste loads released into aquatic environments. Tilapia aquaculture wastewater contains high levels of organic matter derived from uneaten feed, feces, fish metabolic excretions, and other organic particles (Dauda et al., 2019). Without appropriate treatment, effluents generated from tilapia farming may lead to water quality deterioration and changes in the physicochemical characteristics of aquatic environments (Husni et al., 2026). Over the long term, the continuous discharge of organic matter and nutrients may promote eutrophication and excessive phytoplankton growth, leading to reduced dissolved oxygen levels and negative impacts on aquatic biota (Malone et al., 2020).

Wastewater management in tilapia farming requires effective, low-cost, practical, and environmentally sustainable approaches. One promising alternative is the use of aquatic plants for remediation, commonly known as phytoremediation. Aquatic plants can absorb nutrients from water and reduce their concentrations in the aquatic medium (Raza et al., 2023). The mechanisms involved in this process include nutrient uptake by plant tissues, sedimentation of suspended particles, absorption or adsorption of certain compounds on root surfaces, and the provision of habitat for microorganisms that decompose organic matter (Fang et al., 2024; Feng et al., 2020). In addition, the roots of aquatic plants can provide attachment sites for microbial biofilms that play an important role in organic matter decomposition and nutrient transformation (Shahid et al., 2020). Thus, wastewater remediation is driven by interactions among aquatic plants, microorganisms, and the physicochemical components of the water. Several aquatic plant species may exhibit different remediation capacities due to differences in their morphological and physiological characteristics, such as root length and density, root surface area, biomass growth rate, adaptability to wastewater conditions, and nutrient uptake efficiency (Manolaki et al., 2020).

Although remediation using aquatic plants has been widely applied (Manolaki et al., 2020; Endut et al., 2016; Gomes et al., 2024; Maranho et al., 2024), studies specifically comparing the remediation capacities of different aquatic plant species in reducing organic and nutrient loads in tilapia farming wastewater remain limited and require further investigation, particularly with regard to the presence and role of microorganisms within the system and the physiological responses of aquatic plants to wastewater exposure. This information is important because the characteristics of aquaculture wastewater differ from those of domestic or industrial wastewater, particularly because its organic matter and nutrient contents are strongly influenced by feed input, fish metabolism, stocking density, and pond management. Total organic matter (TOM), nitrate, and phosphate were used as key parameters to evaluate remediation effectiveness, as they reflect the major environmental pressures caused by aquaculture wastewater, particularly organic matter accumulation and nutrient enrichment. Changes in these three parameters can be used to evaluate how effectively aquatic plants reduce pollutant loads and to determine which species shows the best remediation performance. Therefore, this study is important as a scientific basis for developing simple and easily applicable wastewater treatment technology for aquaculture. The findings of this study are expected to provide recommendations for selecting effective aquatic plant species for tilapia farming wastewater treatment systems, ranging from laboratory-scale and experimental pond-scale applications to field implementation.

RESEARCH METHODS

Time and location

This study was conducted in January 2025 at the Experimental Pond of the Faculty of Marine Science and Fisheries, Universitas Udayana. Wastewater quality analysis was carried out at the Fisheries Science Laboratory, Faculty of Marine Science and Fisheries, Universitas Udayana.

Sample collection

Wastewater from tilapia farming was collected from a harvested grow-out pond at the UPTD Balai Benih Ikan (BBI) Pesiapan, Bali. The aquatic plants used in this study were collected from several locations around UPTD BBI Pesiapan.

Experimental design

This experimental study consisted of five treatments: four treatments using different aquatic plant species and one control without aquatic plants. The aquatic plants used were water hyacinth (*Eichhornia crassipes*), yellow velvetleaf (*Limnocharis flava*), water spinach (*Ipomoea aquatica*), and water lettuce (*Pistia stratiotes*). Each treatment was performed in triplicate to obtain the mean and standard deviation values. Each experimental unit consisted of a 5-L plastic container, resulting in a total of 15 containers used in this study. Each container was filled with 4 L of tilapia farming wastewater, after which 250 g of aquatic plants were added. To accelerate the decomposition of organic compounds, 4 mL of EM4 Septic Tank solution was added to each container. Aeration was also provided to optimize the decomposition process. Remediation was conducted for 14 days, with 200 mL water samples collected on days 1 and 14.

Measurement of total organic matter (TOM) in wastewater

Total organic matter (TOM) in water was determined using the titrimetric permanganate method (SNI 06-6989.22-2004). Briefly, 100 mL of the water sample was transferred into a 300 mL Erlenmeyer flask and mixed with 5 mL of 8 N H_2SO_4 and 10 mL of 0.01 N $KMnO_4$. The mixture was then heated at 105°C for 20 min. After cooling, 10 mL of 0.01 N $C_2H_2O_4$ (oxalic acid) was added to the Erlenmeyer flask, and the mixture was shaken until clear. The solution was then titrated with 0.01 N $KMnO_4$ until a persistent pink color appeared. The volume of $KMnO_4$ solution used was then recorded as data. The TOM value was calculated using the formula provided in the corresponding SNI method.

Measurement of nitrate and phosphate in water

Nitrate concentration in water was measured using the cadmium reduction method (SNI 6989.79:2011). The pH of the water sample was first adjusted to 7–9 using HCl or NaOH. A 25 mL aliquot of the water sample was transferred into a 100 mL volumetric flask, followed by the addition of 75 mL of concentrated NH_4Cl-EDTA solution, and the mixture was shaken until homogeneous. The solution was then passed through a cadmium reduction column, and the first 25 mL of the effluent was discarded. The subsequent effluent was collected in a clean Erlenmeyer flask. A 50 mL aliquot of the reduced solution was quantitatively transferred and mixed with 2 mL of color reagent. After shaking, the solution was allowed to stand for 10 min at room temperature. The absorbance was measured using a spectrophotometer at 543 nm. The total nitrate concentration was determined from a calibration curve prepared using nitrate standard solutions.

Phosphate concentration was measured using the ascorbic acid method (SNI 06-6989.31-2005). A 50 mL aliquot of the water sample was pipetted in duplicate, and each aliquot was transferred into a 100 mL Erlenmeyer flask. One drop of phenolphthalein indicator was then added. If the solution turned pink, 5N H_2SO_4 was added dropwise until the color disappeared. Subsequently, 8 mL of mixed reagent was added to the sample and homogenized. The mixed reagent consisted of 5N H_2SO_4, potassium antimonyl tartrate, ammonium molybdate, and ascorbic acid solutions. The solution was transferred into a spectrophotometer cuvette, and the absorbance was measured at 880 nm within 10–30 min after the addition of the mixed reagent. Phosphate concentration was determined from a calibration curve prepared using phosphate standard solutions.

Determination of bacterial abundance in water samples

Bacterial abundance in water was measured to assess the role of microorganisms in the remediation of Nile tilapia aquaculture wastewater. Total bacterial abundance in water was determined using the Total Plate Count method, referring to SNI 2332.3:2015. Water samples were collected on day 1 and day 14 and placed into sterile bottles for further analysis. The samples were serially diluted using 0.85% physiological NaCl solution. Bacterial inoculation was performed using the pour plate method. A 1 mL aliquot of the selected dilution was transferred into a sterile Petri dish, followed by the addition of sterile Plate Count Agar (PCA) medium at approximately 45 °C. The Petri dish was gently swirled to ensure even mixing of the sample with the medium, then allowed to solidify and incubated in an inverted position at 37 °C for 24 h. After incubation, colonies were counted on plates containing 25–250 colonies. The results were expressed as CFU/mL.

Measurement of chlorophyll content in aquatic plant leaves

Chlorophyll content in aquatic plant leaves was measured to evaluate the physiological response of plants to exposure to Nile tilapia aquaculture wastewater. Chlorophyll content was determined using the Arnon method with 80% acetone extraction (Nalini et al., 2024). Fresh leaves from each aquatic plant species were rinsed with distilled water and air-dried to remove residual water from the leaf surface. A 1 g leaf sample was then weighed and ground using a mortar and pestle until a fine paste was formed. Subsequently, 10 mL of 80% acetone was added, and the mixture was homogenized. The mixture was incubated in the dark for 10–15 min to optimize chlorophyll pigment extraction. It was then centrifuged at 5,000–10,000 rpm for 5–10 min, and the supernatant was collected. This process was repeated several times until the supernatant became colorless. All extracted supernatants were combined, homogenized, and the final volume was recorded as the total extract volume. The absorbance of the chlorophyll extract was measured using a spectrophotometer at 663 nm for chlorophyll a and 645 nm for chlorophyll b. Total chlorophyll content was calculated as the sum of chlorophyll a and chlorophyll b.

Measurement of other water quality parameters

Other water quality parameters measured in this study included dissolved oxygen (DO), pH, total dissolved solids (TDS), and turbidity. DO was measured using a DO meter equipped with a galvanic sensor. The pH of the water samples was measured using a pH meter based on the potentiometric method (SNI 6989.11:2019). TDS was measured using a TDS meter based on the conductometric method (SNI 06-6989.27:2019). Turbidity was measured using a turbidimeter based on the nephelometric method (SNI 06-6989.25-2005).

Data analysis

The reduction rates of TOM, nitrate, and phosphate concentrations, as well as the increase in bacterial abundance, were statistically analyzed using one-way ANOVA followed by Duncan's multiple range test (DMRT) at a 95% confidence level to determine significant differences among treatments.

RESULTS

Initial Characteristics of Wastewater Before Remediation

The results showed that the total organic matter (TOM) concentration in the wastewater was 25.28 mg/L (Table 1). The initial nitrate concentration was 0.35 mg/L, which was substantially below the Class III water quality standard of 20 mg/L. This result indicates that nitrogen accumulation as nitrate in Nile tilapia aquaculture wastewater was relatively low. In contrast to nitrate, the phosphate concentration was 1.29 mg/L, exceeding the water quality standard of 1.0 mg/L. This finding indicates that phosphate is one of the key nutrient parameters that requires particular attention in Nile tilapia aquaculture wastewater. The dissolved oxygen (DO) concentration was 5.4 mg/L, exceeding the minimum water quality standard of 3.0 mg/L. This indicates that the wastewater still contained sufficient dissolved oxygen to support biological processes. The total dissolved solids (TDS) concentration was 110 mg/L, substantially below the water quality standard of 1,000 mg/L, indicating that dissolved solids in the wastewater had not reached a level associated with severe pollution. However, the turbidity value of 186 NTU indicated a relatively high turbidity level in the wastewater.

Table 1. Initial characteristics of Nile tilapia aquaculture wastewater before remediation

Water quality parameter	Measurement Result	Water Quality Standard
TOM (mg/L)	25.28	-
Nitrate (mg/L)	0.35	20
Phosphate (mg/L)	1.29	1.0
DO (mg/L)	5.4	3.0
pH	7.6	6-9
TDS (mg/L)	110	1,000

Water quality parameter	Measurement Result	Water Quality Standard
Turbidity (NTU)	186	-

Note: The water quality standards refer to Class III water quality standards for aquaculture activities according to Government Regulation of the Republic of Indonesia Number 22 of 2021 concerning the Implementation of Environmental Protection and Management.

Changes in TOM concentration during the remediation process

The results showed that all treatments reduced TOM concentrations, both in treatments with aquatic plants and in the control. This indicates that organic matter decomposition occurred in the treatment media during the incubation period, which was likely influenced by microbial activity from the addition of EM4, oxygen availability from aeration, and the presence of aquatic plants in each treatment. The highest TOM reduction was observed in the water hyacinth (*Eichhornia crassipes*) treatment, decreasing from 25.3 ± 0.0 mg/L to 15.8 ± 0.3 mg/L, corresponding to a reduction of 9.5 mg/L or 37.5 ± 1.2% (Table 2). The water spinach (*Ipomoea aquatica*) treatment reduced TOM from 25.3 ± 0.0 mg/L to 18.1 ± 0.4 mg/L, with a reduction of 28.3 ± 1.4%. Although this reduction was lower than that observed in the water hyacinth treatment, it still indicates relatively good effectiveness in supporting the remediation process. Meanwhile, the yellow velvetleaf (*Limnocharis flava*) treatment reduced TOM to 19.8 ± 0.4 mg/L, equivalent to a reduction of 21.7 ± 1.4%, whereas the water lettuce (*Pistia stratiotes*) treatment reduced TOM to 20.9 ± 0.6 mg/L, equivalent to 17.5 ± 2.5%. In the control treatment without aquatic plants, TOM also decreased from 25.3 ± 0.0 mg/L to 22.1 ± 0.3 mg/L, with a reduction of 12.7 ± 1.0%. Statistical analysis showed that the ability of each treatment to reduce TOM differed significantly at the 95% confidence level. The reduction observed in the control indicates that organic matter decomposition still occurred even without aquatic plants, mainly due to aeration and the addition of EM4 Septic Tank, which supported the activity of decomposing microorganisms.

Table 2. TOM concentration and reduction during the remediation process

Treatment	TOM Concentration (mg/L)		Reduction
	Day 1	Day 14	(%)
Water hyacinth (*Eichhornia crassipes*)	25.3 ± 0.0	15.8 ± 0.3	37.5 ± 1.2[a]
Yellow velvetleaf (*Limnocharis flava*)	25.3 ± 0.0	19.8 ± 0.4	21.7 ± 1.4[c]
Water spinach (*Ipomoea aquatica*)	25.3 ± 0.0	18.1 ± 0.4	28.3 ± 1.4[b]
Water lettuce (*Pistia stratiotes*)	25.3 ± 0.0	20.9 ± 0.6	17.5 ± 2.5[d]
Control	25.3 ± 0.0	22.1 ± 0.3	12.7 ± 1.0[e]

Note: Different superscript letters in the same column indicate significant differences at the 95% confidence level.

Changes in nitrate concentration during the remediation process

The results showed that the water spinach (*Ipomoea aquatica*) treatment had the highest nitrate reduction efficiency, decreasing from 0.35 ± 0.0 mg/L to 0.07 ± 0.01 mg/L, with a reduction of 81.0 ± 1.6% (Table 3). The water hyacinth (*Eichhornia crassipes*) treatment also showed high effectiveness, reducing nitrate concentration from 0.35 ± 0.0 mg/L to 0.08 ± 0.01 mg/L, with a reduction percentage of 76.6 ± 1.9%. Meanwhile, yellow velvetleaf (*Limnocharis flava*) and water lettuce (*Pistia stratiotes*) showed lower effectiveness than water spinach and water hyacinth. Yellow velvetleaf reduced nitrate concentration from 0.35 ± 0.0 mg/L to 0.19 ± 0.02 mg/L, corresponding to a reduction of 44.8 ± 4.4%, while water lettuce reduced nitrate concentration to 0.21 ± 0.02 mg/L, corresponding to a reduction of 39.7 ± 5.7%. Based on the statistical analysis, the water spinach and water hyacinth treatments were not significantly different in reducing nitrate concentration. However, both treatments were significantly more effective than yellow velvetleaf, water lettuce, and the control.

Table 3. Nitrate concentration and reduction during the remediation process

Treatment	Nitrate Concentration (mg/L)		Reduction (%)
	Day 1	Day 14	
Water hyacinth (*Eichhornia crassipes*)	0.35 ± 0.0	0.08 ± 0.01	76.6 ± 1.9[a]
Yellow velvetleaf (*Limnocharis flava*)	0.35 ± 0.0	0.19 ± 0.02	44.8 ± 4.4[b]
Water spinach (*Ipomoea aquatica*)	0.35 ± 0.0	0.07 ± 0.01	81.0 ± 1.6[a]
Water lettuce (*Pistia stratiotes*)	0.35 ± 0.0	0.21 ± 0.02	39.7 ± 5.7[b]
Control	0.35 ± 0.0	0.31 ± 0.02	12.4 ± 4.4[c]

Note: Different superscript letters in the same column indicate significant differences at the 95% confidence level.

Changes in phosphate concentration during the remediation process

The analysis showed that the water spinach (*Ipomoea aquatica*) treatment had the highest phosphate reduction capacity, decreasing phosphate concentration from 1.29 ± 0.0 mg/L to 0.27 ± 0.06 mg/L, with a reduction of 79.4 ± 5.0% (Table 4). The water hyacinth (*Eichhornia crassipes*) treatment also showed high effectiveness, reducing phosphate concentration from 1.29 ± 0.0 mg/L to 0.32 ± 0.03 mg/L, with a reduction of 75.6 ± 0.3%. The yellow velvetleaf (*Limnocharis flava*) treatment reduced phosphate concentration by 71.0 ± 0.5%, from 1.29 ± 0.0 mg/L to 0.37 ± 0.01 mg/L. Meanwhile, the water lettuce (*Pistia stratiotes*) treatment reduced phosphate concentration from 1.29 ± 0.0 mg/L to 0.41 ± 0.04 mg/L, with a reduction of 68.1 ± 2.8%. In the control treatment, phosphate concentration decreased only slightly, from 1.29 ± 0.0 mg/L to 1.28 ± 0.00 mg/L, with a reduction of 1.0 ± 0.2%. The statistical analysis showed that the water spinach treatment was the most effective in reducing phosphate concentration compared with the other treatments. The water hyacinth treatment was not significantly different from the yellow velvetleaf treatment, but it differed significantly from the water lettuce and control treatments. The very small reduction observed in the control treatment indicates that aeration and EM4 addition without aquatic plants were not sufficient to effectively reduce phosphate concentration.

Table 4. Phosphate concentration and reduction during the remediation process

Treatment	Phosphate Concentration (mg/L)		Reduction (%)
	Day 1	Day 14	
Water hyacinth (*Eichhornia crassipes*)	1.29 ± 0.0	0.32 ± 0.03	75.6 ± 0.3[ab]
Yellow velvetleaf (*Limnocharis flava*)	1.29 ± 0.0	0.37 ± 0.01	71.0 ± 0.5[bc]
Water spinach (*Ipomoea aquatica*)	1.29 ± 0.0	0.27 ± 0.06	79.4 ± 5.0[a]
Water lettuce (*Pistia stratiotes*)	1.29 ± 0.0	0.41 ± 0.04	68.1 ± 2.8[c]
Control	1.29 ± 0.0	1.28 ± 0.00	1.0 ± 0.2[d]

Note: Different superscript letters in the same column indicate significant differences at the 95% confidence level.

Changes in bacterial abundance in water during the remediation process

The analysis showed that bacterial abundance increased in all treatments. The highest increase was observed in the water spinach (*Ipomoea aquatica*) treatment, from (3.2 ± 0.0) × 10^3 CFU/mL to (38.9 ± 2.0) × 10^3 CFU/mL (Table 5). The water hyacinth (*Eichhornia crassipes*) treatment also showed high bacterial abundance, increasing to (35.0 ± 3.3) × 10^3 CFU/mL after 14 days. In the yellow velvetleaf (*Limnocharis flava*) treatment, bacterial abundance increased to (30.9 ± 1.4) × 10^3 CFU/mL, while in the water lettuce (*Pistia stratiotes*) treatment, it increased to (22.9 ± 2.2) × 10^3 CFU/mL. Although both treatments showed an increase in bacterial abundance compared with the initial condition, their values were lower than those observed in the water spinach and water hyacinth treatments. In the control treatment, bacterial abundance also increased from (3.2 ± 0.0) × 10^3 CFU/mL to (11.7 ± 2.2) × 10^3 CFU/mL. Based on the statistical analysis, the water spinach treatment was not significantly different from the water hyacinth treatment, but it differed significantly from the other treatments. These results indicate that water spinach provided a suitable and optimal habitat for bacterial growth in the water. The increase in bacterial abundance in the control treatment suggests that EM4 addition, the presence of organic matter in the wastewater, and aeration were still able to support bacterial growth even in the absence of aquatic plants. However, bacterial abundance in the control treatment was the lowest among all treatments with aquatic plants.

Table 5. Bacterial abundance and increase in water during the remediation process

Treatment	Bacterial abundance ($\times 10^3$ CFU/mL)		Increase ($\times 10^3$ CFU/mL)
	Day 1	Day 14	
Water hyacinth (*Eichhornia crassipes*)	3.2 ± 0.0	35.0 ± 3.3	31.8 ± 3.3[ab]
Yellow velvetleaf (*Limnocharis flava*)	3.2 ± 0.0	30.9 ± 1.4	27.8 ± 1.4[b]
Water spinach (*Ipomoea aquatica*)	3.2 ± 0.0	38.9 ± 2.0	35.7 ± 2.0[a]
Water lettuce (*Pistia stratiotes*)	3.2 ± 0.0	22.9 ± 2.2	19.8 ± 2.2[c]
Control	3.2 ± 0.0	11.7 ± 2.2	8.5 ± 2.2[d]

Note: Different superscript letters in the same column indicate significant differences at the 95% confidence level.

Leaf chlorophyll content during the remediation process

The chlorophyll content in aquatic plant leaves decreased after 14 days of wastewater remediation. The highest initial chlorophyll content was observed in water hyacinth (*Eichhornia crassipes*), at 1.53 ± 0.06 mg/mL, which decreased to 1.27 ± 0.06 mg/mL on day 14, corresponding to a reduction of 17.2% (Table 6). The yellow velvetleaf (*Limnocharis flava*) treatment showed a decrease in chlorophyll content from 1.43 ± 0.04 mg/mL to 1.07 ± 0.03 mg/mL, with a reduction percentage of 25.5%. The water spinach (*Ipomoea aquatica*) treatment showed the best physiological response, as it had the lowest decrease in chlorophyll content, with a reduction of only 6.7 ± 2.9%, from 1.65 ± 0.03 mg/mL to 1.54 ± 0.04 mg/mL. In contrast, water lettuce (*Pistia stratiotes*) showed the greatest decrease in chlorophyll content, from 1.51 ± 0.04 mg/mL to 0.89 ± 0.03 mg/mL, with a reduction percentage of 41.1%. The lowest reduction percentage observed in water spinach indicates its greater ability to maintain photosynthetic pigment stability during exposure to wastewater. In contrast, the greater reduction observed in water lettuce suggests that this species experienced higher physiological stress during the remediation process.

Table 6. Chlorophyll content and reduction in aquatic plant leaves during remediation

Treatment	Chlorophyll Content (mg/mL)		Reduction (%)
	Day 1	Day 14	
Water hyacinth (*Eichhornia crassipes*)	1.53 ± 0.06	1.27 ± 0.06	17.2 ± 6.7
Yellow velvetleaf (*Limnocharis flava*)	1.43 ± 0.04	1.07 ± 0.03	25.5 ± 2.6
Water spinach (*Ipomoea aquatica*)	1.65 ± 0.03	1.54 ± 0.04	6.7 ± 2.9
Water lettuce (*Pistia stratiotes*)	1.51 ± 0.04	0.89 ± 0.03	41.1 ± 3.0

Changes in other water quality parameters during the remediation process

The water quality parameters measured during the remediation process generally remained within the acceptable range based on the Class III water quality standards for aquaculture activities. DO, pH, and TDS values remained within the water quality standards used as references, whereas turbidity had no standard value listed in the table. Nevertheless, turbidity remained an important parameter to monitor because it is associated with the presence of suspended particles in wastewater. DO concentrations during the remediation process ranged from 5.4 to 6.1 mg/L, exceeding the minimum water quality standard of 3.0 mg/L (Table 7). The pH values ranged from 7.6 to 7.9, remaining within the water quality standard range of 6–9. TDS values ranged from 50 to 110 mg/L, which were substantially below the water quality standard of 1,000 mg/L. Meanwhile, turbidity values ranged from 75 to 186 NTU. These values indicate that the wastewater still contained suspended particles, including residual feed, fish feces, fine organic matter, microorganisms, and other particles originating from the aquaculture medium. However, compared with the initial turbidity value of 186 NTU, the observed range suggests a decreasing trend in turbidity in several treatments after remediation.

Table 7. Water quality of Nile tilapia aquaculture wastewater during the remediation process

Water Quality Parameter	Measurement Result	Water Quality Standard
DO (mg/L)	5.4-6.1	3.0
pH	7.6-7.9	6-9
TDS (mg/L)	50-110	1,000
Turbidity (NTU)	75-186	-

Note: Class III water quality standards for aquaculture activities are based on Government Regulation of the Republic of Indonesia Number 22 of 2021 concerning the Implementation of Environmental Protection and Management.

DISCUSSION

Remediation capacity of aquatic plants for reducing organic matter and nutrient loads in Nile tilapia aquaculture wastewater

The results showed that the aquatic plants used in this study were able to reduce total organic matter (TOM), nitrate, and phosphate in Nile tilapia aquaculture wastewater. Water spinach and water hyacinth reduced TOM concentrations by more than 25% (Table 2), which was substantially higher than the reductions observed in yellow velvetleaf and water lettuce. The addition of EM4 in the experimental procedure was presumed to contribute to the decomposition of organic matter in Nile tilapia aquaculture wastewater. Arfiati et al. (2021) reported that the addition of EM4 to shrimp aquaculture wastewater reduced TOM content by more than 90%. The lower TOM reduction observed in the present study compared with that study was presumably related to differences in the characteristics of the organic matter fractions in the two types of wastewater. Nile tilapia aquaculture wastewater may contain particulate organic fractions derived from uneaten feed and feces, including crude fiber, non-starch polysaccharides, lipids, complex proteins, and feed materials that are not completely digested (Amirkolaie et al., 2005; Schneider et al., 2004; Meriac et al., 2014). These fractions generally require a hydrolysis step before they can be utilized by microorganisms; therefore, their decomposition may proceed more slowly (Alvarado et al., 2021; Rajagopal et al., 2019). Thus, although EM4 was added, the presence of more complex and particulate organic matter may have resulted in a lower TOM reduction compared with wastewater dominated by readily biodegradable organic matter.

The nutrient removal mechanism, particularly for nitrate and phosphate, in Nile tilapia aquaculture wastewater was clearly reflected in the water spinach (*Ipomoea aquatica*) treatment. In this treatment, nitrate concentration was reduced by more than 80% (Table 3). This finding indicates that water spinach is an effective phytoremediation agent for reducing nitrate concentration in wastewater. This species has creeping or partially floating stems, with adventitious and fibrous roots that can develop from stem nodes in contact with water or moist substrates (Xu et al., 2021; Maranho et al., 2024). The adventitious roots of water spinach play an important role in nitrate assimilation and photosynthesis (Rich et al., 2011). These roots develop relatively rapidly, actively absorb nutrients, and respond strongly to nutrient-rich environments. In addition, water spinach has a rapid biomass growth rate and a high nitrogen demand for the formation of new tissues (Endut et al., 2016; Ibrahim et al., 2018). These physiological and morphological characteristics may explain its higher remediation effectiveness compared with other aquatic plants, such as water hyacinth, yellow velvetleaf, and water lettuce.

Nitrate uptake by water spinach occurs through direct contact with the water column. Dissolved nitrate in the wastewater moves toward the root surface and then enters root cells through nitrate transporter proteins in the cell membrane (Forde, 2000). After uptake, nitrate can be temporarily stored in root tissues or translocated to stems and leaves through the xylem (O'Brien et al., 2016). In plant tissues, nitrate is not directly used as a biomass component. It is first reduced to nitrite by nitrate reductase and then further reduced to ammonium by nitrite reductase. Ammonium is subsequently assimilated through nitrogen metabolism pathways, particularly the GS-GOGAT pathway, to form amino acids (Sanz-Luque et al., 2015; Tejada-Jimenez et al., 2019; Yoneyama and Suzuki, 2020). These amino acids are then used to synthesize proteins, enzymes, chlorophyll, nucleic acids, and new plant tissues (Forde and Lea, 2007).

Phosphate concentration in Nile tilapia aquaculture wastewater was reduced by more than 75% in this study (Table 4). Phosphate in water can also be absorbed by water spinach in the form of dissolved phosphate ions, mainly $H_2PO_4^-$ and HPO_4^{2-} (Mimura and Reid, 2024). Because the wastewater used in this study tended to be neutral (Table 1), the dominant phosphate ion absorbed was likely HPO_4^{2-} (Fang et al., 2024). Phosphate supports the formation of new tissues through its role in energy supply (Vance et al., 2003). After entering plant tissues, phosphate is translocated to stems and leaves through vascular tissues. Phosphorus is then used to form essential compounds for plant growth, such as ATP as a cellular energy source, DNA and RNA, cell membrane phospholipids, enzymes, and new plant tissues (Schachtman et al., 1998). Because water spinach has rapid vegetative growth, its phosphorus demand is also high, particularly for cell division and the formation of new roots, stems, and leaves.

The bacterial abundance analysis showed that all treatments experienced an increase in bacterial abundance during the 14-day remediation process (Table 5). The higher increase in bacterial abundance in treatments with aquatic plants compared with the control indicates that the presence of aquatic plants created suitable microhabitat conditions for bacterial growth. The root systems of aquatic plants can serve as attachment sites for bacteria and support biofilm formation; therefore, microbial activity may occur not only in the water column but also around the root zone (Pang et al., 2016). In this zone, bacteria can utilize organic matter from wastewater, root exudates, and decomposition products as sources of energy and carbon. Complex organic matter can be decomposed by bacteria into simpler compounds through mineralization. This process may produce more readily available nutrient forms, such as inorganic nitrogen and phosphate.

The observation of leaf chlorophyll content showed that all aquatic plant species experienced a decrease in chlorophyll content after 14 days of remediation (Table 6). This decrease indicates physiological stress in aquatic plants due to exposure to Nile tilapia aquaculture wastewater. Such stress may result from changes in water quality, high organic matter loads, accumulation of nitrogen and phosphorus compounds, microbial activity, and the possible decrease in dissolved oxygen availability during organic matter decomposition. These conditions may disrupt the stability of photosynthetic pigments, reduce photosynthetic efficiency, and cause chlorophyll degradation in leaves. However, the degree of chlorophyll reduction differed among aquatic plant species. Water spinach (*Ipomoea aquatica*) showed the lowest chlorophyll reduction (6.7%), indicating its greater ability to maintain chlorophyll content compared with the other aquatic plants during exposure to Nile tilapia aquaculture wastewater.

Changes in other water quality parameters during Nile tilapia aquaculture wastewater remediation

The dissolved oxygen (DO) values measured during the remediation process remained above the Class III water quality standard for aquaculture activities, with a minimum standard of 3.0 mg/L (Table 7). This condition indicates that dissolved oxygen availability during remediation was still sufficient to support aquatic organisms and the activity of aerobic microorganisms in decomposing organic matter. Dissolved oxygen plays an important role in aerobic organic matter mineralization because it serves as the final electron acceptor in microbial respiration (Cheng et al., 2024). In this process, aerobic bacteria use dissolved oxygen to decompose organic matter derived from residual feed and feces, which may potentially reduce DO levels in the water (Lopes et al., 2022). As previously described, bacterial abundance in the water increased in all aquatic plant treatments, which could potentially decrease DO values. However, the measurement results did not show this phenomenon. The relatively stable DO values also indicate that organic matter decomposition did not lead to severe oxygen depletion in the wastewater medium.

The pH values during the remediation process ranged from 7.6 to 7.9 (Table 7), remaining within the Class III water quality standard range of 6–9. This near-neutral to slightly alkaline pH condition was still suitable for supporting biological processes, both for aquatic plants and decomposing microorganisms. The dynamics of water pH during remediation were presumably related to the production of CO_2 and organic acids by bacteria in the water, which can affect the balance of hydrogen ions in the water (Xie et al., 2023). When CO_2 and organic acids increase, pH tends to decrease. Conversely, when nutrient assimilation by plants occurs actively, pH may become more stable or slightly increase. In addition to microbial activity, nitrogen uptake by aquatic plants can also influence pH (Feng et al., 2020). When plants absorb nitrate, they generally balance ionic charges by releasing hydroxyl or bicarbonate ions, which can increase water pH. In contrast, when plants absorb more ammonium, roots may release hydrogen ions, causing pH to decrease. Therefore, the direction of pH change is strongly influenced by the dominant form of nitrogen absorbed by plants.

The total dissolved solids (TDS) values were far below the Class III water quality standard of 1,000 mg/L (Table 7). These values indicate that the dissolved solids content in Nile tilapia aquaculture wastewater during the remediation process remained low and did not exceed the permissible threshold. The decrease in TDS observed in this study was presumably due to the capacity of aquatic plants to absorb some dissolved ions and compounds from the wastewater. During phytoremediation, some ions absorbed by aquatic plant roots are utilized for metabolic processes and the formation of new plant tissues. The main mechanism of TDS reduction occurs through nutrient uptake by roots, such as nitrate and phosphate (Su et al., 2019). However, TDS reduction by aquatic plants is usually not substantial, because not all ions can be absorbed or removed by plants. Some ions, such as sodium, chloride, sulfate, or certain dissolved minerals, may remain in the water despite the remediation process (Raza et al., 2023).

Turbidity values during the remediation process ranged from 75 to 186 NTU (Table 7). Although there is no specific water quality standard for turbidity in this context, these values indicate that the wastewater still contained suspended particles, fine organic matter, residual feed, feces, and microbial biomass (Dauda et al., 2019). Turbidity may decrease during remediation because aquatic plants help reduce suspended particles in aquaculture wastewater, such as residual feed, fine fecal particles, particulate organic matter, microbial flocs, and fine sediment particles. These particles are the main contributors to high turbidity. When aquatic plants are placed in wastewater media, their root systems in the water column can function as physical traps that retain suspended particles, allowing them to settle more easily or adhere to the root surfaces (Gomes et al., 2024).

CONCLUSIONS

Water spinach (*Ipomoea aquatica*) and water hyacinth (*Eichhornia crassipes*) showed higher remediation capacity for Nile tilapia aquaculture wastewater than yellow velvetleaf (*Limnocharis flava*) and water lettuce (*Pistia stratiotes*) in reducing total organic matter (TOM), nitrate, and phosphate. Water hyacinth reduced TOM by up to 37.5 ± 1.2%, while water spinach reduced nitrate and phosphate by 81.0% and 79.4%, respectively. Water spinach also provided suitable conditions for bacterial growth in water, as indicated by an increase in bacterial abundance from (3.20 ± 0.0) × 10^3 CFU/mL to (38.9 ± 2.0) × 10^3 CFU/mL. In addition, the water spinach treatment showed the best physiological response, with the lowest chlorophyll reduction of 6.7 ± 2.9%. Other water quality parameters during remediation remained within the water quality standards for aquaculture according to Government Regulation Number 22 of 2021, with DO, pH, and TDS values of 5.4–6.1 mg/L, 7.6–7.9, and 50–110 mg/L, respectively. Turbidity ranged from 75 to 186 NTU.

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