Analysis of Changes In Biofloc Wastewater Quality Following Eco-Enzyme Application

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Abstract.
The biofloc system improves water- and feed-use efficiency but generates wastewater containing suspended solids and nitrogenous compounds that require treatment before reuse. This study evaluated changes in biofloc wastewater quality after the addition of eco-enzyme at concentrations of 0% (control), 5%, 10%, 15%, 20%, and 25% (v/v). The wastewater and eco-enzyme were homogenized, incubated for 24 h, and analyzed for pH, dissolved oxygen (DO), ammonia (NH_3), total suspended solids (TSS), total nitrogen, total phosphorus, total potassium, and the C/N ratio. Data from two measurement replicates were analyzed using one-way analysis of variance followed by Fisher's least significant difference (LSD) test at the 5% significance level. Eco-enzyme had a highly significant effect on pH ($p < 0.001$), a significant effect on DO ($p = 0.012$), and a highly significant effect on NH_3 ($p < 0.001$). The pH increased from 2.760 in the control to 3.145 at the 25% dose, whereas DO decreased from 2.650 to 2.500 mg/L and NH_3 increased from 0.500 to 2.000 mg/L. TSS, total nitrogen, and total phosphorus did not differ significantly among treatments ($p > 0.05$). Total potassium increased from 0.0015% to 0.0705% ($p < 0.001$), while the C/N ratio increased from 0.460 to 6.310 ($p = 0.004$). These findings indicate that, after 24 h of contact, eco-enzyme functions more as a carbon source and a mineralization bioactivator than as a stand-alone treatment capable of directly producing water suitable for aquaculture. The treated water still requires neutralization, aeration, ammonia control, and a longer maturation period before reuse.
Keywords: *Aquaculture Wastewater, Biofloc, Eco-Enzyme, Water Quality, Mineralization, C/N Ratio.*

I. INTRODUCTION

Intensive aquaculture has expanded as a strategy for meeting protein demand; however, higher stocking densities and increased feed use also raise the loads of organic matter and nitrogen in culture water (Boyd, 2003). Biofloc technology was developed to convert inorganic nitrogen into microbial biomass through the management of carbon sources and the C/N ratio, thereby reducing water-exchange requirements and allowing some nutrients to be reused by cultured organisms (Avnimelech, 2009). Nevertheless, the accumulation of flocs, uneaten feed, feces, and metabolic products can still generate effluent with high concentrations of TSS, ammonia, nitrite, nitrate, and phosphorus when the system's assimilative capacity is exceeded (Burford et al., 2004).

Discharging untreated aquaculture wastewater may increase turbidity, oxygen demand, organic-matter sedimentation, and nutrient enrichment in receiving waters (Cripps & Bergheim, 2000). Excessive suspended solids can impair light penetration, organism respiration, and aeration efficiency, while un-ionized ammonia may damage gills and inhibit fish growth (Akinremi., 2020). Therefore, the reuse of biofloc wastewater requires treatment that controls toxic compounds without eliminating all residual nutrients that could still be recovered within a circular production system (Müller et al., 2018).

Eco-enzyme is a fermented liquid produced from fruit and vegetable waste, a sugar source, and water. It contains organic acids, microbial metabolites, and enzymatic activity that may accelerate the hydrolysis and mineralization of organic matter (Widyastuti., 2023). Its use is attractive because it links household organic-waste reduction with relatively low-cost wastewater treatment and is consistent with circular-economy principles (Dirgahayu et al., 2024). However, eco-enzyme composition is strongly influenced by the raw materials, mixture ratio, fermentation period, temperature, and product maturity; consequently, its effects on water quality are not always uniform (Julinar, 2025).

Adding fermented material to wastewater may initiate two simultaneous processes: the breakdown of complex organic compounds and an increase in microbial respiration that consumes dissolved oxygen (Junaidi., 2021). Protein hydrolysis may accelerate ammonification, whereas the conversion of ammonia to nitrite and nitrate becomes effective only when nitrifying bacteria have adequate pH, alkalinity, and oxygen (Hargreaves, 1998). Consequently, increased biodegradation activity is not always immediately followed by lower ammonia concentrations or higher DO during a short contact period (Yu et al., 2023).

The C/N ratio is an important indicator because carbon availability determines the capacity of heterotrophic bacteria to assimilate inorganic nitrogen and form floc biomass (Avnimelech, 2009). In biofloc systems, appropriate carbon addition can suppress dissolved nitrogen; however, a ratio that is too low limits assimilation, whereas excessive carbon may increase oxygen demand and microbial-solids production (Avnimelech, 2012). Eco-enzyme should therefore be evaluated using multiple parameters rather than a single indicator such as pH or TSS (Akinremi., 2023).

The biofloc wastewater used in this study originated from a culture unit in Nungka Village, Awayan District, Balangan Regency, where a simple treatment alternative suitable for community-scale application is needed. The organic-matter- and nutrient-rich wastewater has potential for reuse, but treatment must ensure that pH, DO, ammonia, and suspended solids do not pose risks to cultured organisms or the receiving environment (Boyd, 2003). Evaluating changes across a broad dose range is necessary to distinguish mineralization and nutrient-enrichment effects from genuine water-quality improvements that support reuse (Pardiansyah et al., 2019). This study aimed to analyze changes in the water quality and nutrient content of biofloc wastewater after the addition of eco-enzyme at concentrations of 0%, 5%, 10%, 15%, 20%, and 25% for 24 h. The analysis focused on pH, DO, NH_3, TSS, total nitrogen, total phosphorus, total potassium, and the C/N ratio to determine whether eco-enzyme functions primarily as a water-treatment agent, a mineralization bioactivator, or an additional carbon source.

II. METHODS

Study site and analytical measurements

Biofloc wastewater was collected from Nungka Village, Awayan District, Balangan Regency, South Kalimantan. TSS and nutrient analyses were performed at the BSPJI Laboratory in Banjarbaru, while pH, DO, and NH_3 were measured using field instruments and a spectrophotometer in accordance with the study procedures.

Eco-enzyme preparation

Eco-enzyme was obtained from the Banjarbaru Eco Enzyme Community and was prepared from fruit and vegetable waste, brown sugar, and water at a ratio of 3:1:10. The mixture was fermented for approximately three months at room temperature. The mature product was dark brown, had an acidic odor, and had a pH of approximately 2.7-4.0.

Experimental treatments

The wastewater received six treatments based on the percentage of total volume: K = 0%, E5 = 5%, E10 = 10%, E15 = 15%, E20 = 20%, and E25 = 25% eco-enzyme. Each mixture was stirred until homogeneous, left for 24 h, and analyzed in two measurement replicates.

Table 1. Parameters, analytical methods, main equipment, and measurement units.

Parameter	Analytical method/reference	Main equipment	Unit

Parameter	Analytical method/reference	Main equipment	Unit
pH	Electrometry; APHA 4500-H^+	pH meter	-
DO	Electrometry; APHA 4500-O	DO meter	mg/L
NH_3	Indophenol spectrophotometry; APHA 4500-NH_3 F	Spectrophotometer	mg/L
TSS	Gravimetry; APHA 2540 D	Filter paper and analytical balance	mg/L
Total N	Kjeldahl; APHA 4500-N	Kjeldahl unit	%
Total P	Ascorbic acid; APHA 4500-P E	Spectrophotometer	%
Total K	AAS; APHA 3111 B	Atomic absorption spectrophotometer	%
C/N ratio	Ratio of organic carbon to total N	Laboratory analytical results	-

Data analysis

Data are presented as the mean ± standard deviation. Differences among treatments were analyzed using one-way ANOVA at a 5% significance level, and parameters showing significant differences were further evaluated using Fisher's least significant difference (LSD) test. The raw data and Minitab outputs reported in the thesis were rechecked to ensure consistency among the results tables, p-values, and interpretations.

Fig. 1. Study materials and stages: (A) the biofloc unit from which wastewater was collected, (B) eco-enzyme, and (C) treatment sample bottles. Source: research documentation, 2024-2026.

III. RESULTS AND DISCUSSION

Changes in water-quality parameters

After the 24-h contact period, the parameters showed different responses. pH increased progressively with eco-enzyme concentration, DO tended to decrease, and NH_3 increased to 2.000 mg/L beginning at E10. TSS varied widely without a consistent dose-response pattern.

Table 2. Mean ± standard deviation of water-quality parameters after eco-enzyme treatment (n = 2).

**NH_3*

Parameter	K (0%)	E5	E10	E15	E20	E25	ANOVA p
pH	2.760 ± 0.014	2.810 ± 0.042	2.920 ± 0.028	2.945 ± 0.021	3.035 ± 0.007	3.145 ± 0.021	<0.001
DO (mg/L)	2.650 ± 0.071	2.550 ± 0.071	2.500 ± 0.000	2.500 ± 0.000	2.400 ± 0.000	2.500 ± 0.000	0.012
NH_3 (mg/L)	0.500 ± 0.000	1.500 ± 0.000	2.000 ± 0.000	2.000 ± 0.000	2.000 ± 0.000	2.000 ± 0.000	<0.001*
TSS (mg/L)	1,315 ± 247	1,340 ± 509	1,340 ± 57	1,360 ± 57	1,930 ± 721	1,780 ± 85	0.475

replicate values were identical within each treatment; therefore, the inferential result should be interpreted together with the descriptive pattern.

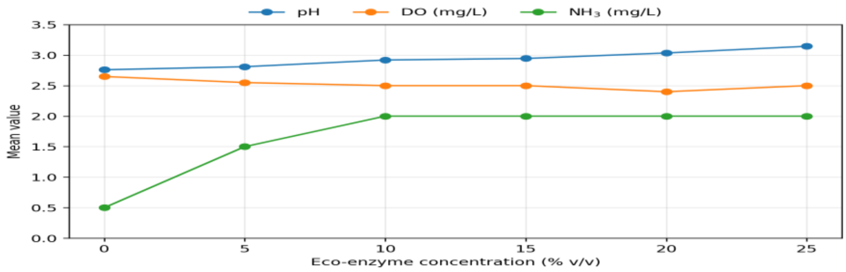

Fig 2. Responses of pH, DO, and NH_3 to eco-enzyme concentration after a 24-h contact period.

Acidity (pH)

Eco-enzyme addition increased pH highly significantly from 2.760 in the control to 3.145 at E25, demonstrating a clear dose response even though all media remained strongly acidic. This range is far below the values generally considered suitable for freshwater fish and stable nitrification, approximately pH 6.5-8.5 (Boyd, 2003). The pH increase at higher doses cannot be interpreted directly as adequate neutralization because eco-enzyme itself contains fermentation-derived organic acids and has a low pH (Widyastuti., 2023). The observed change probably reflects interactions among dilution, wastewater buffering capacity, mineral release, and ammonification during incubation (Junaidi., 2021). Thus, treatment success should be evaluated not only by statistical increases but also by how closely the final values approach the safe and stable range required for the intended biological process (Akinremi., 2023).

Excessively low pH can inhibit nitrifying bacteria and slow the oxidation of ammonia to nitrite and nitrate, although the proportion of un-ionized ammonia generally decreases under acidic conditions (Hargreaves, 1998). Inhibition of nitrification may cause nitrogen released through decomposition to remain accumulated as ammonium or total ammonia during the initial treatment stage (Yu et al., 2023). Therefore, the increase in pH to 3.145 was insufficient to demonstrate that the water was ready for reuse as an aquaculture medium (McCusker et al., 2023).

These findings indicate that eco-enzyme is more appropriately regarded as a bioactivation material requiring subsequent conditioning than as a stand-alone neutralizing agent (Dirgahayu et al., 2024). Gradual neutralization, alkalinity supplementation, or blending with water of higher buffering capacity should be tested to shift pH toward a range that supports nitrification (Akinremi., 2020). Periodic pH monitoring is also required because the 24-h response does not represent medium-term stability after microbial activity has developed (Yu et al., 2023).

Dissolved oxygen (DO)

DO decreased significantly from 2.650 mg/L in the control to the lowest value of 2.400 mg/L at E20, while E25 produced 2.500 mg/L. This decline is consistent with increased microbial respiration when more carbon sources and organic substrates are available (McCusker et al., 2023). During the initial biodegradation phase, heterotrophic bacterial activity may increase biological oxygen demand faster than diffusion and aeration can replace the oxygen consumed (Cripps & Bergheim, 2000).

DO values in all treatments were below levels commonly recommended for intensive aquaculture and efficient nitrification, and the risk of hypoxia therefore remained important (Boyd, 2003). Low oxygen can slow ammonia oxidation, alter microbial-community composition, and increase the likelihood of anaerobic zones forming within flocs or sediments (Zhu & Chen, 2023). Accordingly, eco-enzyme application to biofloc wastewater should be accompanied by controlled aeration rather than relying solely on passive fermentative processes (McCusker et al., 2023).

The non-linear difference between E20 and E25 indicates that DO was governed by interactions among several processes, including the quantity of readily degradable substrate, microbial density, mixing, and oxygen transfer (Cripps & Bergheim, 2000). A longer contact period may produce a different pattern after some substrates have been depleted and the respiration rate begins to decline

(Widyastuti., 2023). Serial measurements are therefore more informative than a single observation for determining when the water reaches a stable phase (Akinremi., 2023).

Ammonia (NH_3)

NH_3 increased from 0.500 mg/L in the control to 1.500 mg/L at E5 and 2.000 mg/L at E10-E25. This pattern indicates that, within 24 h, organic-nitrogen mineralization proceeded faster than assimilation or nitrification (Hargreaves, 1998). Proteolytic enzymes and fermentative microbial activity may break proteins down into amino acids, which subsequently undergo deamination and ammonification (Junaidi., 2021).

Ammonia accumulation was also associated with the highly acidic pH and low DO, two conditions that do not optimally support the growth and activity of nitrifying bacteria (Akinremi., 2020). Although the proportion of un-ionized NH_3 decreases at low pH, the high total ammonia-nitrogen level still indicates that treatment was incomplete and may become problematic when pH is subsequently increased (Hargreaves, 1998). Raising pH without reducing total ammonia nitrogen can shift the equilibrium toward the more toxic NH_3 fraction (Boyd, 2003).

The result differs from the expectation that a carbon source would immediately increase nitrogen assimilation by heterotrophic bacteria because the effectiveness of this process depends on the C/N ratio, oxygen availability, alkalinity, and microbial-community adaptation time (Zhu & Chen, 2023). The highest C/N ratio in this study was only 6.310, which remains below the range commonly used to promote heterotrophic dominance in biofloc systems (Avnimelech, 2012). Eco-enzyme addition therefore did not provide a sufficient combination of carbon and environmental conditions to suppress ammonia accumulation over a short period (McCusker et al., 2023).

Ammonia control requires an integrated strategy involving a longer maturation period, aeration, alkalinity buffering, and, where necessary, inoculation with an adapted nitrifying community (Akinremi., 2020). Simultaneous monitoring of total ammonia, nitrite, nitrate, and alkalinity would help determine whether nitrogen is being converted to safer forms or merely accumulating in an intermediate phase (Hargreaves, 1998). Interpretation of the NH_3 changes should also consider that the two replicates in each treatment produced identical values; future studies therefore require more biological replicates to improve estimation precision (Akinremi., 2023).

Total suspended solids (TSS)

TSS ranged from 1,315 to 1,930 mg/L and did not differ significantly among treatments, with the greatest variation observed at E20. The absence of a declining pattern suggests that hydrolysis of organic solids may have been offset by the formation of new microbial biomass and flocs (Burford et al., 2004). In biofloc systems, TSS is not composed entirely of inert waste because part of it consists of bacteria, algae, protozoa, and organic particles that can serve as nutrient sources (Avnimelech, 2012).

Excessively high TSS can increase aeration requirements, interfere with respiration, and promote sludge accumulation if it is not controlled (Boyd, 2003). Eco-enzyme appeared to change particle composition or degradability without reducing the total mass of solids within 24 h (Widyastuti., 2023). If TSS reduction is the primary treatment objective, the biological process should be combined with sedimentation, filtration, or floc separation (Müller et al., 2018).

Changes in nutrients and the C/N ratio

Total nitrogen and total phosphorus did not differ significantly among treatments, whereas potassium and the C/N ratio increased highly significantly. This pattern indicates that nutrient release during fermentation and mineralization did not occur uniformly for all elements (Pardiansyah et al., 2019). Nitrogen may shift among organic and inorganic forms, while phosphorus may remain bound to solids or biomass; consequently, changes in total concentrations may not be detected over a short period (Zhao., 2018).

Table 3. Mean ± standard deviation of nutrients and the C/N ratio after eco-enzyme treatment (n = 2).

Parameter	K (0%)	E5	E10	E15	E20	E25	ANOVA p
Total N (%)	0.095 ± 0.021	0.095 ± 0.007	0.115 ± 0.007	0.090 ± 0.014	0.115 ± 0.035	0.090 ± 0.014	0.608
Total P (%)	0.0045 ± 0.0007	0.0030 ± 0.0014	0.0095 ± 0.0092	0.0030 ± 0.0000	0.0030 ± 0.0014	0.0035 ± 0.0007	0.548
Total K (%)	0.0015 ± 0.0007	0.0100 ± 0.0014	0.0175 ± 0.0007	0.0510 ± 0.0071	0.0590 ± 0.0014	0.0705 ± 0.0035	<0.001
C/N ratio	0.460 ± 0.113	1.730 ± 0.240	2.875 ± 0.177	4.010 ± 0.863	4.290 ± 1.315	6.310 ± 1.146	0.004

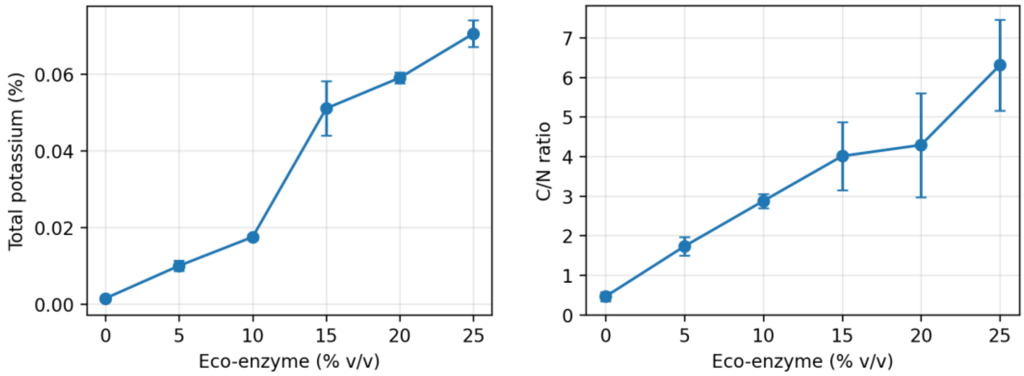

Figure 3. Increases in (A) total potassium and (B) the C/N ratio with increasing eco-enzyme concentration.

Nitrogen and phosphorus

Total nitrogen ranged from 0.090% to 0.115% and showed no consistent dose-response pattern. This variability may occur because nitrogen is distributed among dissolved fractions, biomass, and gaseous forms through different biogeochemical pathways (Zhao., 2018). The 24-h incubation period was probably too short to produce a consistent change in total nitrogen, even though the ammonia response was already evident (McCusker et al., 2023).

Total phosphorus also did not differ significantly, and the high standard deviation at E10 indicated substantial sample heterogeneity. Phosphorus can readily adsorb to solids, bind to minerals, or be incorporated into microbial biomass; therefore, total concentrations may remain relatively stable while internal redistribution occurs (Müller et al., 2018). Separate measurement of dissolved phosphate and particulate phosphorus would better explain the transformation pathways involved (Zhao., 2018).

Potassium and the C/N ratio

Potassium increased almost linearly from 0.0015% in the control to 0.0705% at E25. This increase may have resulted from the release of soluble potassium contained in fruit and vegetable raw materials and from the mineralization of microbial cells and organic matter during fermentation (Widyastuti., 2023). Unlike nitrogen, potassium is not lost through volatilization or denitrification; consequently, increasing doses of fermented material are more readily reflected in its total concentration (Zhao., 2018).

The increase in potassium indicates that the treated water has potential as a nutrient source for plants; however, this value cannot be used as the sole basis for direct reuse because pH, DO, ammonia, and TSS remained problematic (Pardiansyah et al., 2019). Use for fertigation should consider dilution, plant tolerance, salinity, and the nutrient requirements of the selected crop (Somerville et al., 2014). In aquaponic applications, water reuse must also account for the risk of toxic compounds to fish and the compatibility of nutrient concentrations with plant production (Goddek et al., 2015). Integrating treatment with nutrient reuse must balance organism safety with resource-recovery efficiency (Müller et al., 2018).

The C/N ratio increased from 0.460 to 6.310 because eco-enzyme added organic carbon to the medium. This increase is consistent with the biofloc principle that carbon supply is a major regulator of heterotrophic bacterial growth and nitrogen assimilation (Avnimelech, 2009). However, the maximum ratio remained below the range frequently used to promote effective heterotrophic floc formation; therefore, the system's capacity to suppress ammonia remained limited (McCusker et al., 2023).

The increase in the C/N ratio also helps explain the decline in DO because greater carbon availability stimulated microbial respiration and biomass production (Yu et al., 2023). Without increased aeration, carbon addition can shift the problem from dissolved nitrogen to oxygen demand and solids accumulation (Zhu & Chen, 2023). Dose optimization should therefore be based on a balance among nitrogen reduction, oxygen consumption, floc production, and the intended final use (Akinremi., 2023).

Mechanistic synthesis and management implications

Table 4. Synthesis of water-quality changes, probable mechanisms, and management implications.

Parameter	Process interpretation	Practical implication
pH increased but remained at 2.76-3.15	Changes in buffering and mineralization did not achieve neutralization	Adjust alkalinity and allow further maturation
DO decreased to 2.40-2.65 mg/L	Microbial respiration increased after carbon addition	Provide aeration and conduct serial monitoring
NH_3 increased to 1.50-2.00 mg/L	Ammonification proceeded faster than nitrification/assimilation	Extend the contact period and manage nitrification
TSS did not change significantly	Particle degradation was offset by formation of floc biomass	Combine treatment with sedimentation or filtration when TSS reduction is the objective
K and C/N increased significantly	Eco-enzyme released minerals and supplied carbon	Potential for nutrient recovery after water quality has been made safe

Eco-enzyme accelerated organic-matter transformation and enriched carbon and potassium, but the 24-h contact period did not produce conditions suitable for direct reuse. This pattern is consistent with the concept that bioactivation is a gradual process requiring balance among carbon, oxygen, pH, alkalinity, and the microbial community (Avnimelech, 2009). Eco-enzyme should therefore be incorporated into a treatment train rather than used as a stand-alone technology replacing aeration, solids separation, and chemical stabilization (Cripps & Bergheim, 2000).

Study limitations

The study used two measurement replicates per treatment, limiting its ability to capture biological variation and wastewater heterogeneity. A larger number of replicates is required to increase statistical power and reduce the influence of extreme values, particularly for TSS and phosphorus (Akinremi., 2023).

Observations were made at only one contact time, 24 h, and therefore could not describe the adaptation phase, peak respiration, nitrification stabilization, or depletion of readily degradable substrates. Biofloc studies show that water-quality dynamics are strongly time dependent; serial measurements are therefore required to distinguish transient responses from equilibrium conditions (Yu et al., 2023).

The nitrogen parameters included only NH_3 and total nitrogen, whereas nitrite, nitrate, ammonium, alkalinity, chemical oxygen demand, biochemical oxygen demand, and microbial-community characteristics were not analyzed. Adding these parameters would clarify nitrogen-transformation pathways and the relationships among mineralization, respiration, and floc formation (Akinremi., 2020).

Eco-enzyme characteristics may vary with raw materials and fermentation processes; therefore, the findings of this study are most applicable to products with similar characteristics. Standardization of pH, carbon content, enzyme activity, and microbial load is necessary before doses can be recommended broadly (Julinar, 2025).

IV. CONCLUSION

Eco-enzyme selectively altered the initial condition of biofloc wastewater. Application at 5-25% for 24 h significantly increased pH, total potassium, and the C/N ratio, but decreased DO and increased NH_3, while TSS, total nitrogen, and total phosphorus did not change significantly. The 25% dose produced the highest pH, potassium, and C/N ratio; however, the water remained strongly acidic, oxygen deficient, and high in ammonia. None of the treatments could be considered ready for direct use as an aquaculture medium after a 24-h contact period. Eco-enzyme is more appropriately positioned as a carbon source and mineralization bioactivator within an integrated treatment train. Reuse requires neutralization, aeration, ammonia control, solids separation where necessary, and a longer maturation period.

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