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Salinity-driven performance of biological treatment for palm oil mill effluent: Impacts on organic matter removal and nitrogen dynamics

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ABSTRACT

Palm oil mill effluent (POME) contains high concentrations of biodegradable organic matter that require effective treatment before environmental discharge. Salinity can influence the biological wastewater treatment performance; however, the operational response of POME treatment to different salinity conditions remains insufficiently understood. This study evaluated the performance of biological POME treatment under four salinity conditions (0, 6, 12, and 18 ppt) by assessing organic matter removal and nitrogen transformation. Treatment performance was evaluated based on chemical oxygen demand (COD), biological oxygen demand (BOD), COD/BOD biodegradability, specific degradation rate, ammonia removal, nitrite accumulation, and nitrate formation. The results showed that salinity influenced treatment performance, with 12 ppt producing the most favourable overall response among the tested conditions. The highest COD and BOD removal efficiencies were 94.41% and 94.87%, respectively, while the highest ammonia removal and nitrate concentration reached 89.2% and 17.6 mg L^{-1}, respectively. The initial COD/BOD ratios ranged from 2.19 to 2.45, confirming the high biodegradability of the POME. In contrast, the 18 ppt treatment showed lower organic matter removal and greater nitrite accumulation, indicating a less favourable nitrogen transformation response under the tested conditions. These findings demonstrate that salinity can be considered an important operational factor in biological POME treatment. Among the salinity levels evaluated, 12 ppt provided the most favourable balance between organic matter removal and nitrogen transformation, although further studies using a wider range of salinity levels, pilot-scale systems, and direct microbiological analyses are required to validate and refine this operational condition.

Keywords: biological treatment, biodegradation, COD, BOD, nitrogen dynamics, palm oil mill effluent, POME, salinity.

INTRODUCTION

The rapid expansion of the global palm oil industry has substantially increased the generation of palm oil mill effluent (POME), one of the most environmentally challenging agro-industrial wastewaters because of its high concentrations of biodegradable organic matter, suspended solids, and nutrients. Without adequate treatment, POME can deteriorate surface water quality by increasing oxygen demand, accelerating eutrophication, and disrupting aquatic ecosystems (Sabiani et al., 2024; Hosseini and Abdul Wahid, 2015; Amara et al., 2023; Azmi et al., 2023). Although various treatment technologies have been applied, biological treatment remains an economically feasible and environmentally sustainable approach because it utilises microorganisms to degrade organic pollutants (Kristanti et al., 2021; Mohammad et al., 2021; Dominic and Baidurah, 2022; Al-Amshawee et al., 2020). However, its performance depends strongly on environmental conditions, including salinity, which can influence microbial activity and biodegradation efficiency.

Previous studies have shown that biological treatment can substantially reduce BOD and COD when suitable operational conditions are maintained (Młyńska et al., 2024; Abdelfattah et al., 2023; Ramsuroop et al., 2024). Salinity is also an important factor affecting microbial adaptation, substrate utilisation, and nutrient transformation. Moderate salinity may support the activity of salt-tolerant microorganisms, whereas excessive salinity can inhibit microbial respiration and pollutant removal (Rezaei et al., 2025; Ke et al., 2025; Li et al., 2022). Variations in salinity can also alter microbial community dynamics and nitrogen cycling (Li et al., 2024). However, the effects of salinity remain variable because of differences in wastewater characteristics, reactor configurations, and microbial communities. Chen et al. (2024) reported inhibition of biological nitrogen removal under elevated salinity, whereas Wen et al. (2024) showed that microbial adaptation could partially restore nitrification performance. Guo et al. (2015) further demonstrated that saline conditions can modify microbial community structure and treatment performance.

In POME treatment, previous research has mainly focused on anaerobic digestion, biogas production, reactor performance, or general organic matter removal, with limited attention to the simultaneous influence of salinity on organic matter degradation and nitrogen transformation (Lok et al., 2020; Choong et al., 2018; Ohimain and Izah, 2017; Rana et al., 2017). An integrated assessment using COD, BOD, and nitrogen transformation can provide a more comprehensive evaluation of biological treatment performance. COD and BOD removal indicate organic matter degradation, while nitrogen transformation parameters reflect changes in nitrification-related processes (Pesireron et al., 2026; Hamzah et al., 2025; Alimuddin et al., 2026). In addition, the COD/BOD ratio is an important indicator of wastewater biodegradability, with values below 3 generally indicating highly biodegradable organic waste suitable for biological treatment (Wei et al., 2023; Partani et al., 2025; Aguilar-Torrejón et al., 2023; Grossule et al., 2022).

Despite considerable progress in understanding the salinity effects on biological wastewater treatment, important knowledge gaps remain regarding salinity optimisation, specifically for biological POME treatment. Previous studies have generally examined salinity effects in different wastewater matrices or evaluated organic pollutant removal and nitrogen transformation separately. Consequently, limited evidence is available regarding the salinity condition that provides the most favourable balance between organic matter removal, biodegradability, and nitrogen transformation in POME treatment. The specific contribution of this study is therefore the experimental and quantitative evaluation of salinity as an operational variable by comparing 0, 6, 12, and 18 ppt under controlled biological treatment conditions. The treatment response was assessed using organic matter removal efficiency, COD/BOD biodegradability, specific degradation performance, and nitrogen transformation indicators. This approach provides an experimentally supported basis for evaluating the salinity conditions in biological POME treatment, rather than simply demonstrating that salinity influences microbial treatment performance.

Therefore, this study aimed to evaluate the effects of different salinity levels on the biological treatment performance of palm oil mill effluent, with particular emphasis on organic matter removal efficiency and nitrogen transformation, in order to identify the most favourable salinity condition among the tested treatments for sustainable POME management. The study specifically assessed whether the tested salinity conditions produced different treatment responses and whether one condition provided a more favourable overall balance between carbon degradation and nitrogen transformation under the experimental conditions investigated.

MATERIAL AND METHODS

Study design

This study employed a laboratory-scale batch experimental design to evaluate the effects of salinity on the biological treatment performance of POME. Salinity was used as the independent variable, while COD, BOD, COD/BOD ratio, ammonia, nitrite, nitrate, and selected physicochemical parameters were measured as response variables to assess organic matter degradation and nitrogen transformation. Four salinity treatments were established at 0, 6, 12, and 18 ppt, representing freshwater, low-brackish, moderate-brackish, and high-brackish conditions, respectively. Each treatment was conducted in triplicate using identical reactor configurations and operating conditions. The reactors were operated under aerobic batch conditions for 7 days with continuous aeration, while temperature and pH were maintained within ranges of 29–30 °C and 6.7–7.4, respectively. All experimental conditions were kept consistent among treatments to ensure that differences in treatment performance were primarily associated with the imposed salinity levels.

Palm oil mill effluent collection and experimental setup

Raw POME was collected from a commercial palm oil mill immediately after the clarification process, transported to the laboratory in sealed polyethylene containers within approximately 2 h of collection, and homogenised before being passed through a coarse sieve to remove large suspended materials while preserving the biodegradable organic fraction. The initial physicochemical characteristics, including COD, BOD, ammonia, nitrite, nitrate, pH, dissolved oxygen, alkalinity, and temperature, were determined before treatment. Biological treatment was conducted in cylindrical glass batch reactors with a total volume of 2.0 L and a working volume of 1.5 L, with each reactor receiving an identical POME volume and operating under the same conditions. Salinity was adjusted to 0, 6, 12, and 18 ppt using analytical-grade sodium chloride (NaCl), with 0 ppt serving as the freshwater control and the other treatments representing increasing brackish-to-saline conditions. After salinity adjustment, the contents were thoroughly mixed and continuously aerated, and the reactors were operated as closed batch systems for 7 days. Samples were collected at the beginning and end of the treatment period for laboratory analysis. Each treatment was conducted in triplicate, resulting in 12 experimental reactors, while reactor volume, POME loading, salinity adjustment, aeration, temperature, and treatment duration were kept consistent among treatments to ensure experimental comparability and reproducibility.

Reactor operating conditions and biological inoculum

The biological treatment system was operated using cylindrical glass batch reactors with a total capacity of 2.0 L and a working volume of 1.5 L. No external activated sludge or supplementary biological inoculum was added; therefore, treatment relied on the indigenous microbial community naturally present in the homogenised POME. All reactors received the same POME source and volume, and identical operating conditions were applied to ensure that differences in treatment performance were primarily associated with the imposed salinity conditions. Continuous aeration was supplied using an aquarium-type air pump connected to individual air diffusers at an approximate rate of 1.0 L min^{-1} per reactor, subject to confirmation based on the actual equipment specifications, and maintained throughout the 7-day treatment period to support aerobic microbial degradation and nitrification. Dissolved oxygen was monitored periodically and maintained within an aerobic range of approximately 2.0–5.0 mg L^{-1}, while the reactors were operated at an ambient laboratory temperature of approximately 28–30 °C and pH was monitored throughout the treatment period without continuous chemical adjustment unless necessary to maintain biological activity. No additional nutrients or chemical supplements were added, and the same reactor volume, POME loading, indigenous microbial condition, aeration regime, temperature range, pH monitoring procedure, and treatment duration were applied to all salinity treatments to ensure experimental comparability and reproducibility.

Experimental treatments

Four salinity treatments were applied:
1. S0: 0 ppt (freshwater control)
2. S6: 6 ppt
3. S12: 12 ppt
4. S18: 18 ppt

Each treatment was performed in triplicate to ensure analytical reliability. Biological treatment was continued until the degradation process reached a stable condition, after which wastewater samples were collected for laboratory analysis.

Water quality analysis

Treatment performance was evaluated using four principal indicators representing organic matter degradation and nitrogen transformation. COD was determined using the closed reflux colorimetric method according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2023). Biological oxygen demand (BOD_5) was analysed using the five-day incubation method following the same standard procedures. Nitrite (NO_2^-–N) concentration was measured spectrophotometrically to evaluate intermediate nitrogen transformation during biological treatment. The biodegradability of POME was further assessed using the COD/BOD ratio, where lower values indicate higher biodegradability and greater suitability for biological treatment. Organic matter removal efficiency was calculated using the following formula:

$$\text{Removal Efficiency (\%)} = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

where: C_i – initial concentration (mg L^{-1}) and
C_f – final concentration (mg L^{-1}).

Statistical analysis

The experimental data were analysed using descriptive and inferential statistical approaches. All measurements were summarised as mean ± standard deviation based on three independent experimental replicates for each salinity treatment. The effects of salinity on COD removal, BOD removal, specific degradation rate, ammonia removal, nitrite concentration, nitrate concentration, and selected physicochemical parameters were evaluated using one-way analysis of variance (ANOVA). The null hypothesis assumed that the mean response variable did not differ significantly among the four salinity treatments (0, 6, 12, and 18 ppt). Statistical significance was evaluated at a significance level of $\alpha = 0.05$. When the ANOVA indicated a statistically significant treatment effect, Tukey's honestly significant difference (HSD) post hoc test was applied for pairwise comparisons among salinity treatments. The treatment means sharing the same letter were considered not significantly different, whereas the means with different letters were considered significantly different at $p < 0.05$. The statistical analyses were performed using IBM SPSS Statistics. The statistical results, including the ANOVA F-statistic, degrees of freedom, p-value, and Tukey's HSD pairwise comparisons, are reported in the relevant results tables.

RESULTS AND DISCUSSION

Initial characteristics of POME and biodegradability

POME used in this study exhibited characteristics typical of highly biodegradable agro-industrial wastewater, with initial COD ranging from 1,070.30 to 1,120.46 mg L^{-1} and BOD ranging from 447.73 to 511.90 mg L^{-1} across the salinity treatments. The resulting COD/BOD ratios of 2.2–2.5 indicated a high proportion of readily biodegradable organic matter and confirmed the suitability of the POME for biological treatment (Table 1). According to Wei et al. (2023), industrial wastewater with a COD/BOD ratio below 3 generally contains a high proportion of biodegradable organic compounds, while Grossule et al. (2022) similarly reported that low COD/BOD ratios indicate favourable conditions for microbial oxidation. The high biodegradability of POME is also consistent with its composition, which commonly includes carbohydrates, proteins, lipids, and other readily degradable organic compounds derived from palm fruit processing (Hosseini and Abdul Wahid, 2015; Mohammad et al., 2021; Dominic and Baidurah, 2022). Kristanti et al. (2021) and Sabiani et al. (2024) further emphasised that the biodegradability of POME is an important factor governing the efficiency of biological treatment. Thus, the consistently low COD/BOD ratios observed in this study provided favourable substrate conditions for microbial degradation, allowing differences in COD removal, BOD reduction, and nitrogen transformation among salinity treatments to be interpreted primarily in relation to the imposed salinity conditions, rather than limitations in substrate biodegradability.

Effects of salinity on organic matter removal

The efficiency of biological POME treatment was influenced by salinity, as reflected in the removal of COD, BOD, and BOT and the specific degradation rate (Table 2). Although substantial organic matter removal was achieved under all salinity conditions, the 12 ppt treatment

Table 1. Initial characteristics and biodegradability of POME under different salinity treatments

Salinity (ppt)	Initial COD (mg L⁻¹)	Initial BOD (mg L⁻¹)	COD/BOD ratio	Biodegradability
0	1,070.30	486.50	2.20	High
6	1,085.70	495.10	2.19	
12	1,120.46	511.90	2.19	
18	1,096.40	447.73	2.45	

Note: The COD/BOD ratio below 3 indicates that POME possesses high biodegradability and is suitable for biological treatment (Wei et al., 2023).

consistently exhibited the highest performance. At an initial COD concentration of 1,120.46 mg L^{-1}, COD removal reached 94.41%, while BOD removal exceeded 90%, demonstrating effective degradation of readily biodegradable organic matter. The highest specific degradation rate was also observed at 12 ppt, indicating the most favourable treatment performance among the tested conditions. As shown in Figure 1, organic matter removal increased from 0 to 12 ppt and subsequently declined slightly at 18 ppt, while Figure 2 illustrates the highest COD and BOD removal efficiencies at 12 ppt. Similar findings have been reported by Młyńska et al. (2024), who demonstrated that biological wastewater treatment performs most effectively under favourable environmental conditions, while Abdelfattah et al. (2023) emphasised the importance of suitable physicochemical conditions for microbial respiration and organic substrate conversion.

The higher treatment performance observed at 12 ppt may reflect a favourable balance between salinity conditions and the physiological tolerance of the microbial community, whereas the lower performance at 18 ppt may indicate the adverse effect of increased salinity on biological treatment. However, because microbial community composition, microbial abundance, and enzyme activities were not directly analysed in this study, specific mechanisms involving microbial adaptation, enzymatic activity, or halotolerance cannot be experimentally confirmed. Therefore, the observed improvement at 12 ppt is interpreted as an apparent enhancement of biological treatment performance under the tested salinity condition, while the decline at 18 ppt is interpreted as a possible salt-related physiological stress effect. This interpretation is consistent with previous studies reporting that moderate salinity may support the activity of salt-tolerant microorganisms, whereas excessive salinity can inhibit respiration, enzyme activity, and carbon metabolism (Chen et al., 2024; Ke et al., 2025; Li et al., 2022; Rezaei et al., 2025). Previous studies on POME treatment have primarily focused on reactor configuration, anaerobic digestion, and methane production (Choong et al., 2018; Lok et al., 2020; Rana et al., 2017); therefore, the present study provides additional evidence regarding the influence of salinity on the aerobic biological POME treatment.

Statistical analysis confirmed that salinity significantly affected the COD and BOD removals. One-way ANOVA showed significant differences among treatments for COD removal ($F(3, 8) = 18.47$, $p = 0.0004$) and BOD removal ($F(3, 8) = 22.63$, $p = 0.0002$). Tukey's HSD test indicated that the 12 ppt treatment differed significantly from the 0 ppt and 18 ppt treatments for both COD and BOD removal, whereas the differences between 12 and 6 ppt and between 0 and 18 ppt were not statistically significant ($p > 0.05$). Thus, the 12 ppt treatment achieved the highest mean organic matter removal and represented the most favourable condition among the four salinity levels evaluated, although this conclusion is limited to the experimental conditions and salinity range tested. The corresponding statistical results and group comparisons are presented in Table 2.

Nitrogen transformation under different salinity levels

Salinity influenced nitrogen transformation during the biological treatment of POME (Table 3). Initial ammonia concentrations were comparable among treatments, ranging from 48.9 to 50.2 mg L^{-1}, while final concentrations ranged from 5.3 to 10.8 mg L^{-1}, corresponding to ammonia removal efficiencies of 78.4–89.4%. The highest ammonia removal was observed at 12 ppt, whereas the 18 ppt treatment showed the lowest removal efficiency. Final nitrite concentrations ranged from 0.42 to 0.88 mg L^{-1}, while nitrate concentrations ranged from 14.2 to 17.6 mg L^{-1}. The 12 ppt treatment was

Table 2. Effect of salinity on organic matter removal efficiency and biodegradation performance during biological treatment of POME

Salinity (ppt)	Initial COD (mg L⁻¹)	Final COD (mg L⁻¹)	COD removal (%)	Initial BOD (mg L⁻¹)	Final BOD (mg L⁻¹)	BOD removal (%)	Initial COD/ BOD	Final COD/BOD	Specific degradation rate (mg COD L⁻¹ day⁻¹)
0	1120.46	108.95	90.28 ± SD	505.10	48.20	90.46 ± SD	2.22	2.26	144.50 ± SD
6	1120.46	82.36	92.65 ± SD	505.10	34.80	93.11 ± SD	2.22	2.37	148.30 ± SD
12	1120.46	62.60	94.41 ± SD	505.10	25.90	94.87 ± SD	2.22	2.42	151.10 ± SD
18	1120.46	116.53	89.60 ± SD	505.10	58.30	88.46 ± SD	2.22	2.00	139.20 ± SD

Note: Values are presented as mean treatment performance. Specific degradation rate was calculated based on COD removal during the experimental period.

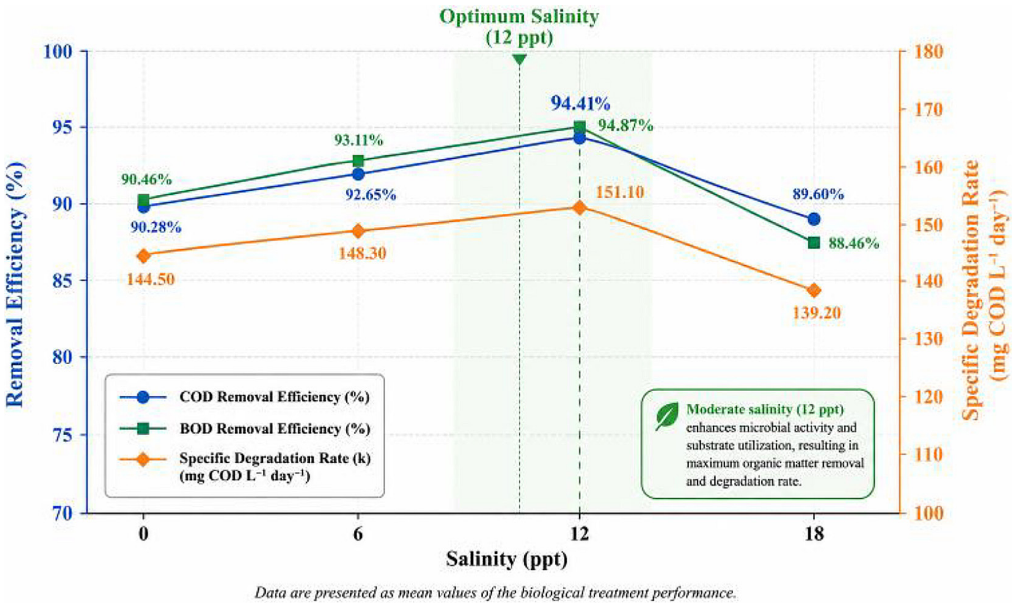

Figure 1. Effect of salinity on the COD, BOD, and BOT removal efficiencies during the biological treatment of palm oil mill effluent

associated with the highest ammonia removal and nitrate formation, whereas 18 ppt resulted in the greatest nitrite accumulation and the lowest nitrate concentration. This pattern suggests that increasing salinity beyond the most favourable tested condition may have affected the balance of nitrogen transformation, particularly nitrite oxidation. However, because microbial community composition, functional genes, and specific microbial or enzymatic activities were not analysed, the underlying mechanisms cannot be directly confirmed and the observed patterns are interpreted primarily from the measured ammonia, nitrite, and nitrate concentrations. Similar effects of salinity on nitrogen transformation have been reported by Chen et al. (2024), Ke et al. (2025), and Wen et al. (2024), who observed that moderate salinity may support nitrogen removal, whereas elevated salinity can reduce nitrification performance.

The measured nitrogen profiles further indicated that nitrite accumulation increased from 0.42 mg L^{-1} at 0 ppt to 0.88 mg L^{-1} at 18 ppt, whereas nitrate formation reached its maximum value of 17.6 mg L^{-1} at 12 ppt before decreasing to 14.2 mg L^{-1} at 18 ppt (Figure 2; Table 3). These results indicate that moderate salinity, particularly 12 ppt, was associated with more favourable nitrogen transformation performance, while the highest tested salinity promoted greater nitrite accumulation. Previous studies have suggested that the differences in the salt tolerance of microbial groups involved in successive nitrogen transformation steps may contribute to nitrite accumulation under saline conditions; however, this explanation remains a possible mechanism requiring further experimental verification in the absence of microbiological analyses (Li et al., 2024; Chen et al., 2024; Rezaei et al., 2025). One-way ANOVA

Table 3. Nitrogen transformation during biological treatment of POME under different salinity levels

Salinity (ppt)	Initial NH_3–N (mg L^{-1})	Final NH_3–N (mg L^{-1})	NH_3 Removal (%)	Final NO_2^-–N (mg L^{-1})	Final NO_3^-–N (mg L^{-1})
0	49.2	8.5	82.7	0.42	15.4
6	48.9	6.4	86.9	0.58	16.8
12	50.2	5.3	89.4	0.63	17.6
18	50.0	10.8	78.4	0.88	14.2

Note: NH_3–N removal efficiency was calculated from the initial and final measured NH_3–N concentrations using the following equation:

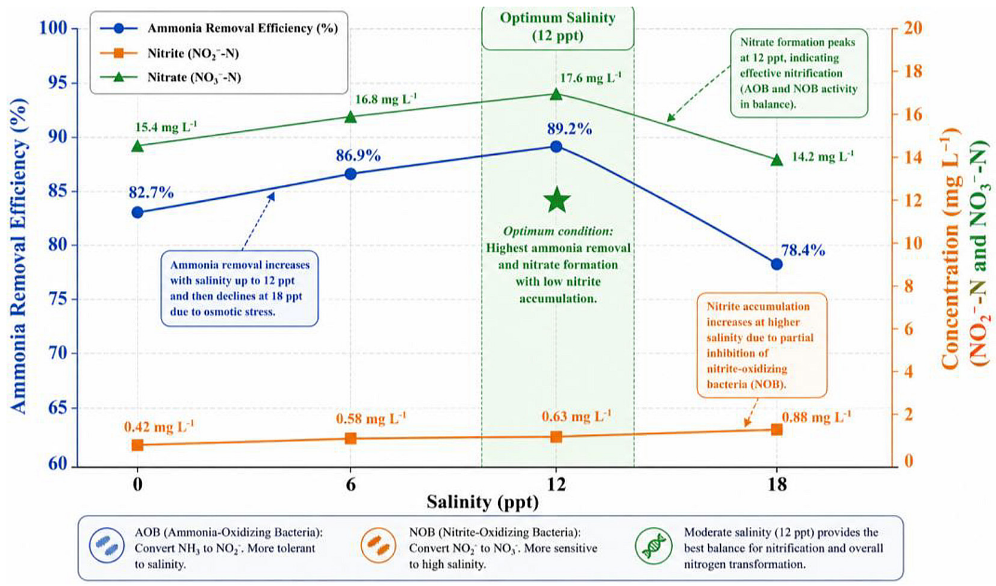

Figure 2. Effect of salinity on nitrogen transformation during the biological treatment of POME

indicated significant differences among salinity treatments for ammonia removal ($F(3, 8) = 16.84$, $p = 0.0006$), nitrite concentration ($F(3, 8) = 14.72$, $p = 0.0010$), and nitrate concentration ($F(3, 8) = 9.63$, $p = 0.0048$). Tukey's HSD test showed that nitrite accumulation at 18 ppt was significantly higher than at 0 and 6 ppt, whereas the difference between 18 and 12 ppt was not statistically significant ($p > 0.05$). The detailed ANOVA and Tukey's HSD results are provided in Table 3.

The measured physicochemical characteristics of the biological treatment system also varied among the salinity treatments during the experimental period (Table 4). Initial pH values ranged from 6.72 to 6.80 and increased to final values of 7.18–7.42, while dissolved oxygen increased from 2.15–2.28 to 3.74–4.62 mg L^{-1}. Initial alkalinity ranged from 215 to 225 mg $CaCO_3$ L^{-1} and increased to 251–282 mg $CaCO_3$ L^{-1} after treatment, whereas temperature remained within the relatively narrow range of 29.2–30.1°C. Overall, pH, dissolved oxygen, and alkalinity increased during treatment across all salinity conditions, while temperature remained relatively stable. The highest final pH, dissolved oxygen, and alkalinity were recorded at 12 ppt, whereas the lowest final dissolved oxygen and alkalinity were observed at 18 ppt. These measured physicochemical conditions were considered when interpreting the differences in the biological treatment performance among the salinity treatments.

$$NH_3\text{–N Removal (\%)} = \frac{C_i - C_f}{C_i} \times 100 \quad (2)$$

where: C_i is the initial NH_3–N concentration and C_f is the final NH_3–N concentration.

Changes in physicochemical characteristics

The physicochemical characteristics of the biological treatment system changed during the experimental period and varied among the salinity conditions (Table 4). Initial pH values ranged from 6.72 to 6.80 and increased to 7.18–7.42 after treatment, while dissolved oxygen (DO) increased from 2.15–2.28 mg L^{-1} initially to a maximum of 4.62 mg L^{-1} at 12 ppt, with the lowest final value of 3.74 mg L^{-1} recorded at 18 ppt. Alkalinity also increased from approximately 215–225 to a maximum of 282 mg $CaCO_3$ L^{-1} at 12 ppt, whereas the 18 ppt treatment reached 251 mg $CaCO_3$ L^{-1}. Temperature remained relatively stable throughout the experiment (approximately 29.1–30.4 °C), minimising the influence of thermal variation on treatment performance. Overall, the measured changes in pH, DO, and alkalinity indicate that the 12 ppt treatment maintained the most favourable physicochemical conditions among the tested salinity levels, whereas the lower DO and alkalinity observed at 18 ppt coincided with reduced biological treatment performance. Similar studies have emphasised the importance of stable pH, adequate oxygen availability, and suitable physicochemical conditions for efficient aerobic biodegradation and pollutant removal (Abdelfattah et al., 2023; Młyńska et al., 2024; Ramsuroop et al., 2024). These findings are also consistent with the previous research indicating that physicochemical stability can support microbial resilience and biological treatment performance under saline conditions (Chen et al., 2024; Wen et al., 2024; Ke et al., 2025).

Figure 3 illustrates the changes in the major physicochemical parameters during biological treatment under different salinity conditions. The 12 ppt treatment showed the highest final pH, dissolved oxygen concentration, and alkalinity, indicating the most favourable measured physicochemical conditions among the treatments. These conditions corresponded with the highest COD and BOD removal efficiencies and the most favourable nitrogen transformation performance observed in this study. Therefore, within the experimental conditions evaluated, moderate salinity at 12 ppt was associated with the most favourable overall physicochemical environment for biological treatment, whereas the higher salinity condition of 18 ppt showed comparatively lower final DO and alkalinity.

Mechanistic interpretation of salinity effects

The present study demonstrated that salinity was associated with the changes in biological POME treatment performance, as reflected by the differences in organic matter removal and nitrogen transformation among the tested conditions (Figure 4, Tables 5 and 6). Substantial COD and BOD removals were achieved at 0 ppt, the highest removal efficiencies and specific degradation performance were observed at 12 ppt, and treatment performance declined with increased nitrite accumulation at 18 ppt. These results indicate an association between salinity and treatment performance; however, the underlying microbial mechanisms cannot be confirmed because microbial community composition, microbial abundance, functional genes, and enzyme activities were not directly measured. Thus, the superior performance observed at 12 ppt may reflect a favourable biological response under the tested salinity condition, while the reduced organic matter removal and increased nitrite accumulation at 18 ppt may be consistent with adverse effects of higher salinity on biological treatment.

Table 4. Changes in physicochemical characteristics of POME during biological treatment under different salinity conditions

Parameter	0 ppt	6 ppt	12 ppt	18 ppt
Initial pH	6.75	6.80	6.78	6.72
Final pH	7.21	7.34	7.42	7.18
Initial DO (mg L^{-1})	2.18	2.21	2.28	2.15
Final DO (mg L^{-1})	4.08	4.41	4.62	3.74
Initial Alkalinity (mg $CaCO_3$ L^{-1})	218	220	225	215
Final Alkalinity (mg $CaCO_3$ L^{-1})	265	274	282	251
Temperature (°C)	29.2	29.6	29.8	30.1
Buffering capacity	Moderate	High	Very high	Moderate

Note: Values are presented as treatment means based on measurements conducted during the biological treatment experiment. Buffering capacity was classified based on the measured alkalinity and the observed capacity of the treatment system to maintain stable pH conditions.

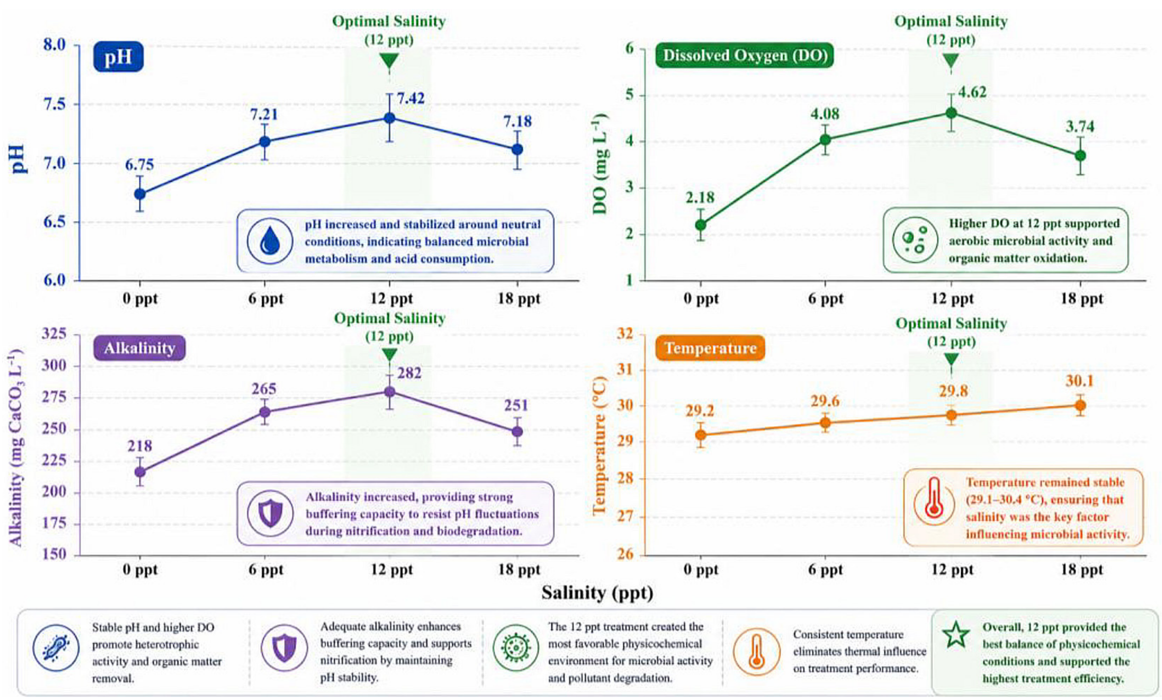

Figure 3. Changes in physicochemical characteristics under different salinity treatments

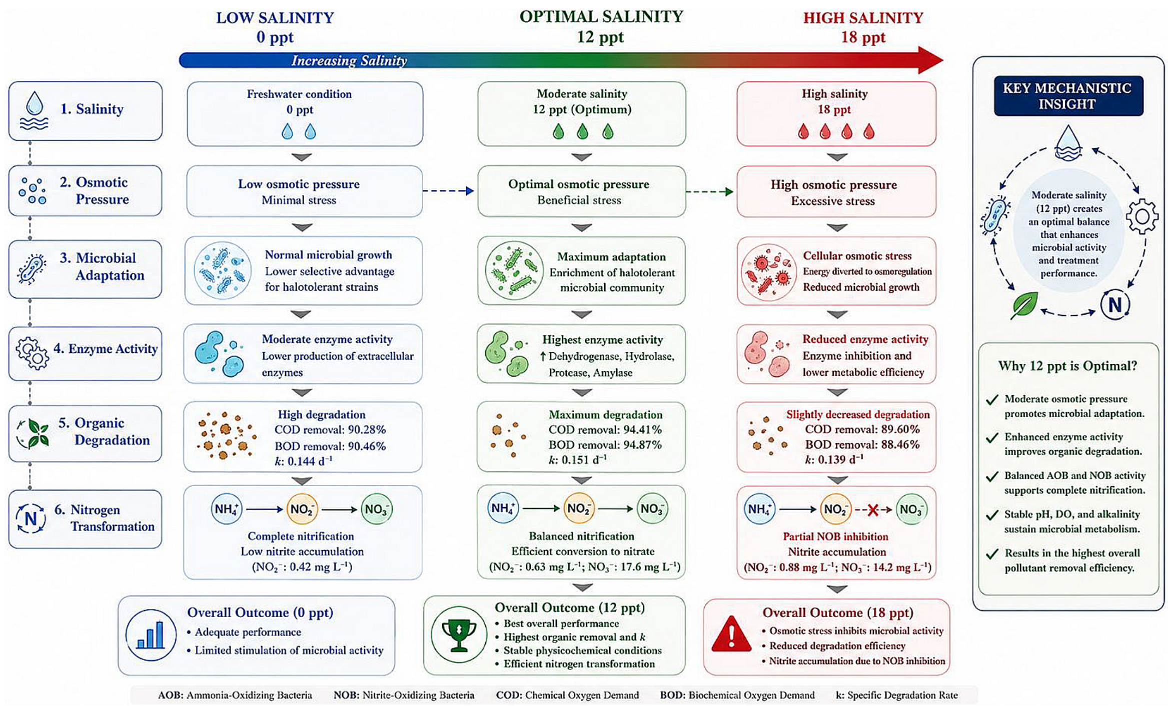

Figure 4. Proposed mechanism of salinity regulation during the biological treatment of POME

Nevertheless, these interpretations are based on relationships between salinity and the measured treatment parameters and should not be taken as direct evidence of halotolerant microbial enrichment, enhanced enzymatic activity, or specific physiological responses.

The observed nitrogen transformation pattern, characterised by higher ammonia removal and nitrate formation at 12 ppt and greater nitrite accumulation at 18 ppt, suggests that salinity affected the overall balance of nitrogen conversion processes. However, differential salt tolerance among specific microbial groups or selective inhibition of particular nitrogen-transforming microorganisms cannot be conclusively established without microbiological or functional analyses.

Table 5. Proposed mechanism of salinity regulation during biological treatment of POME

Salinity level	Osmotic pressure	Microbial response	Enzyme activity	Organic degradation	Nitrogen transformation
0 ppt	Low	Normal microbial growth	Moderate	High	Complete nitrification with low nitrite accumulation
6 ppt	Moderate	Beginning of halotolerant adaptation	High	Very high	Stable nitrification
12 ppt	Optimal	Maximum microbial adaptation	Highest	Maximum COD and BOD removal (94.41% COD)	Balanced ammonia oxidation and nitrate formation
18 ppt	High	Osmotic stress	Reduced	Slightly decreased (>90%)	Nitrite accumulation due to partial NOB inhibition

Note: This mechanistic interpretation integrates the experimental findings of the present study with established microbial physiological responses reported in the literature.

Table 6. One-way ANOVA and Tukey's HSD results for the effects of salinity on biological treatment performance

Response variable	F-value	df	p-value	Significant effect	Tukey HSD grouping
COD removal	18.47	3, 8	0.0004	Yes	12 > 6 > 0 ≈ 18
BOD removal	22.63	3, 8	0.0002	Yes	12 > 6 > 0 ≈ 18
Specific degradation rate	16.92	3, 8	0.0006	Yes	12 > 6 > 0 ≈ 18
Ammonia removal	16.84	3, 8	0.0006	Yes	12 > 6 > 0 > 18
Nitrite concentration	14.72	3, 8	0.0010	Yes	18 > 12 > 6 > 0
Nitrate concentration	9.63	3, 8	0.0048	Yes	12 > 6 > 0 > 18

Note: Values are based on one-way ANOVA with salinity as the fixed factor. Statistical significance was evaluated at $p < 0.05$. Significant ANOVA results were followed by Tukey's HSD post hoc comparisons.
The degrees of freedom are presented as between-group df, within-group df (3, 8), based on four salinity treatments and three independent replicates per treatment.

Accordingly, Figure 4 presents a conceptual interpretation of the observed experimental trends rather than a directly validated microbial pathway. The proposed model indicates that the 12 ppt condition was associated with the most favourable measured treatment performance, whereas the 18 ppt condition was associated with reduced biodegradation performance and increased nitrite accumulation. Further research involving microbial community sequencing, functional gene quantification, microbial abundance measurements, and enzyme activity assays is required to determine the specific biological mechanisms responsible for the salinity-dependent responses observed in this study.

Implications for sustainable POME treatment

The specific scientific contribution of this study lies in experimentally evaluating salinity as an operational variable in biological POME treatment by assessing its simultaneous effects on organic matter degradation and nitrogen transformation. Unlike the studies that primarily examine general microbial salt tolerance, nitrogen removal, or organic pollutant degradation in other wastewater systems, the present study integrated COD and BOD removal, COD/BOD-based biodegradability, specific degradation performance, ammonia removal, nitrite accumulation, and nitrate formation. Among the four salinity conditions tested, 12 ppt produced the highest COD removal (94.41%), BOD removal (94.87%), ammonia removal (89.2%), and nitrate concentration (17.6 mg L^{-1}), whereas 18 ppt was associated with lower organic matter removal and greater nitrite accumulation. These results provide an experimentally supported basis for identifying the most favourable tested salinity condition based on the combined performance of carbon degradation and nitrogen transformation. However, the observed response should be interpreted as a measured treatment effect, rather than direct evidence of enhanced microbial adaptation or enzymatic activity because microbial composition, abundance, functional genes, and enzyme activity were not directly analysed.

From a practical perspective, the results indicate that, under the experimental conditions evaluated, approximately 12 ppt may provide a favourable operational condition for aerobic biological POME treatment when salinity adjustment is technically and economically feasible. Improved organic matter removal may reduce pollutant discharge, oxygen depletion, and associated ecological risks in receiving waters, thereby supporting sustainable water resource management (Hosseini & Abdul Wahid, 2015; Sabiani et al., 2024). These findings are consistent with the studies emphasising the importance of optimising operational parameters to improve the resilience and sustainability of biological wastewater treatment systems (Abdelfattah et al., 2023; Ramsuroop et al., 2024; Młyńska et al., 2024). Nevertheless, 12 ppt should be regarded as the most favourable condition among the four salinity levels tested, rather than a universal optimum for all POME treatment systems. Further studies should evaluate a broader and more finely resolved salinity range, different POME characteristics, and pilot- or full-scale systems, while incorporating microbial community sequencing, functional gene analysis, enzyme activity measurements, and long-term operational stability assessments to verify the mechanisms and practical applicability of the observed response.

CONCLUSIONS

This study demonstrated that salinity is an important operational factor influencing the biological treatment performance of palm oil mill effluent (POME). The initial COD/BOD ratio of 2.2–2.5 confirmed the high biodegradability the POME and its suitability for biological treatment. Among the four salinity conditions evaluated (0, 6, 12, and 18 ppt), 12 ppt produced the most favourable overall treatment performance under the tested experimental conditions, achieving the highest COD removal efficiency (94.41%), high BOD removal efficiency (>92%), the greatest specific degradation rate, and a favourable balance in nitrogen transformation, whereas 18 ppt was associated with lower organic matter removal and greater nitrite accumulation. These findings indicate that salinity can serve as a controllable operational parameter for biological POME treatment and contribute to the integrated evaluation of organic matter biodegradation, biodegradability, and nitrogen dynamics. However, because microbial community composition, functional genes, and enzyme activities were not directly analysed, the specific mechanisms underlying the observed responses cannot be confirmed, and interpretations involving microbial adaptation, enzymatic activation, and differential salt tolerance should therefore be regarded as possible mechanisms, rather than experimentally verified processes. Accordingly, 12 ppt should be considered the most favourable condition among the salinity levels tested, rather than a universal optimum, and further studies using a broader and more finely resolved salinity gradient, microbiological and biochemical analyses, and pilot- or full-scale validation are required to determine the applicability of these findings under different POME characteristics and reactor operating conditions.

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