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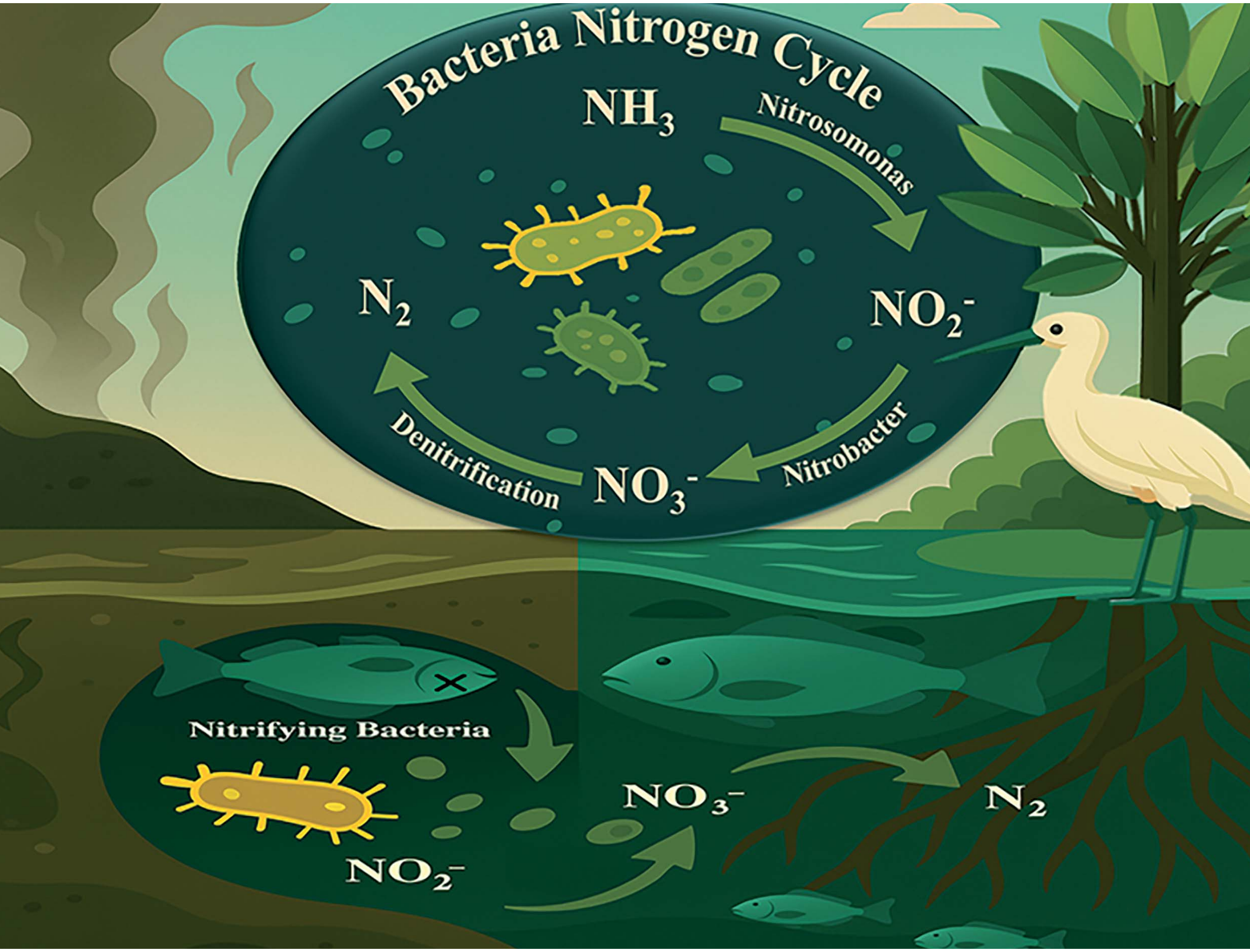

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Kelvin A. Sanoja-López, Rafael Luque *et al.*

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Bioremediation of nitrogen-rich wastewaters: microbial efficiency and environmental assessment

Viviana Tamay, Jorge Flores, Oscar Navia-Pesantes, Carlos Camba, Marianela Barona-Obando, Cesar F. Suarez, Kelvin A. Sanoja-López * and Rafael Luque *

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This research addresses a critical challenge in environmental sustainability: the remediation of contaminated wastewater in the Estero Salado, a vital ecosystem in Guayaquil, Ecuador. An innovative treatment strategy was employed using nitrifying bacteria, activated biomass and efficient microbial consortia. The study uniquely relied on real, non-simulated effluent and sediment samples, ensuring direct applicability of results. Significant reductions in nitrogenous compounds were achieved, particularly ammonia, approaching internationally allowed environmental thresholds. Nonetheless, persistent hydrocarbons and high chemical oxygen demand remained critical limitations, requiring further complementary treatments. Overall, the findings demonstrate the potential of biotechnology-based strategies for sustainable water remediation. This work provides a replicable approach for restoring highly polluted aquatic systems and underscores their positive implications for vulnerable coastal communities.

Environmental significance

Contamination in the Estero Salado, a critical ecosystem in Guayaquil, Ecuador, threatens biodiversity, water quality, and the well-being of surrounding communities. Addressing this issue is essential for environmental sustainability and public health. This study tested a biotechnology-based treatment using nitrifying bacteria, activated biomass, and microbial consortia directly on real effluents and sediments. Results showed marked reductions in nitrogenous pollutants, particularly ammonia, nearing international environmental thresholds. However, hydrocarbons and high COD persisted, highlighting the need for integrated treatment approaches. These findings demonstrate both the potential and limitations of microbial bioremediation strategies, offering a replicable model to restore highly polluted systems and protecting vulnerable urban-coastal environments.

1. Introduction

Water is the most crucial molecule on Earth, sustaining life for humans, animals, and plants.[1] It is a vital resource for societal development, not only supporting human survival but also providing nourishment through animals and plants.[2] Water is broadly categorized into two types: groundwater and surface water.[3] However, the degradation of surface water quality poses a severe threat to human health and ecosystem stability.[4] This essential resource is increasingly compromised by the accumulation of toxic waste, which disrupts natural environments. Human activities, as the primary drivers of this pollution, have rendered the preservation of water resources one of society's greatest challenges.[5] The deteriorating quality of this vital resource endangers not only human health but also the biodiversity and stability of ecosystems.[6,7]

Research reveals that over 4 billion people reside in regions facing severe water scarcity.[8] Additionally, in 2020, approximately 2.2 billion individuals lacked access to safe water supply services. The rapid urbanization, industrialization, and increased reliance on fossil fuels, among other factors, have led to a 65% rise in environmental pollution over the past two decades.[9] This surge in pollution has emerged as a significant risk factor for premature mortality, contributing to approximately one in six deaths.[10,11] In response to this crisis, the scientific community has focused on developing multifaceted solutions that address social, economic, legislative, and practical lifestyle dimensions.[12] Various methods are present for decontaminating polluted and wastewater, including techniques such as reverse osmosis, solvent extraction, electrochemical precipitation, chemical precipitation, ion exchange and evaporation.[13] Other approaches include coagulation, membrane filtration,[14] coagulation–flocculation, photocatalysis, advanced oxidation processes, and adsorption.[15]

However, one of the primary challenges faced by communities is the unpleasant odors emitted by wastewaters. These odors not only cause discomfort to residents but can also have adverse health effects.[16] Prolonged exposure to these odors has been linked to various health issues, including emotional

Universidad ECOTEC, Km. 13.5 Samborondón, Samborondón, EC092302, Ecuador. E-mail: ksanoja@ecotec.edu.ec; rluque@ecotec.edu.ec; Tel: +593 963385271

stress, headaches, depression, eye and respiratory irritation, and nausea.[17]

The nitrogen cycle plays a key role in maintaining ecological balance. It begins with nitrogen fixation, where microorganisms convert atmospheric nitrogen into usable forms. Nitrogen-containing organic matter is then broken down into ammonia or ammonium.[18] During nitrification, these compounds are oxidized to nitrites and subsequently to nitrates, which plants can assimilate.[19] The cycle concludes with denitrification, where bacteria transform nitrites and nitrates back into gaseous nitrogen, releasing it into the atmosphere (Fig. 1).

Each of these approaches targets specific pollutants, including pharmaceuticals,[20] organic matter,[21] metals,[22] and others[23] Despite their effectiveness, these treatments do not substantially mitigate odors emanating from polluted water bodies. Consequently, recent years have witnessed a growing global interest in microorganisms such as fungi, yeasts, algae, and particularly bacteria due to their environmental effectiveness, cost-efficiency, and sustainable nature.[24] In this context, microbiological bioremediation technologies have emerged as promising alternatives for tackling such pollutants. Bacteria, as natural decomposers, have evolved over time to play a crucial role in breaking down contaminants. Their potential is now harnessed as a biotechnological tool to combat environmental pollution through bioremediation processes.[24] These processes employ bacteria as a treatment medium, as demonstrated by studies like those of,[25] which highlight the efficacy of microorganisms in addressing environmental pollution through bioremediation (NO_2^-).

Nitrifying bacteria (NB) are fundamental autotrophic microorganisms in the nitrogen cycle, playing a pivotal role in the nitrification process, which converts ammonia (NH_3/NH_4^+) into nitrates (NO_3^-), a plant-assimilable form.[26] This transformation occurs in two stages: first, ammonia is oxidized to nitrite (NO_2^-) by ammonium-oxidizing bacteria such as *Nitrosomonas* and *Nitrosospira*; subsequently, nitrite is converted to nitrate by nitrite-oxidizing bacteria such as *Nitrobacter* and *Nitrospira*. These bacteria derive energy from these chemical reactions, using carbon dioxide as their carbon source, and require aerobic conditions for their metabolism.[27] NB are vital in both natural ecosystems and human-engineered systems like wastewater treatment plants. They help prevent the accumulation of toxic compounds such as ammonia and nitrites, improve soil fertility, enhance water quality, and support agricultural sustainability while mitigating environmental impacts.[28]

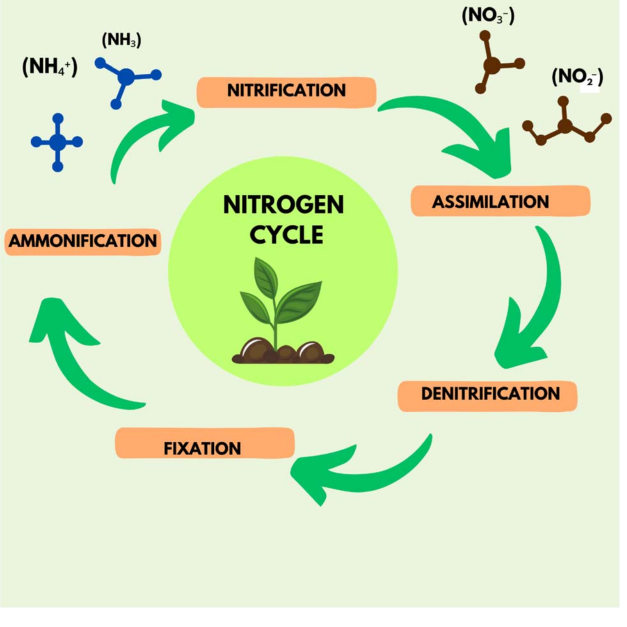

Fig. 1 General outline of the nitrogen cycle.

Despite this potential, most studies on bacterial mechanisms have been conducted under controlled or simulated conditions, often overlooking the complexity of real wastewater bodies, which frequently contain contaminants such as hydrocarbons and high chemical oxygen demand (COD). In light of these challenges, scalable and effective remediation strategies are urgently needed for direct application in urban estuarine ecosystems. The Estero Salado, located in the heart of Guayaquil, Ecuador, exemplifies the environmental and social burden of untreated wastewater discharges. Nearby communities often report intolerable odors, visible pollution, and related health concerns, underscoring the need for site-specific and cost-effective solutions. Developing and validating biotechnological treatments under real environmental conditions could provide a valuable alternative to conventional methods and enable broader application in other tropical coastal regions facing similar challenges. Therefore, this study seeks to bridge that gap by examining the activity of NB and other microorganisms in highly contaminated surface waters, specifically within a coastal estuarine environment in Guayaquil. This site is particularly affected by strong odors and visible degradation, which harm both the environment and surrounding communities. To address this, water and sediment samples were directly collected from the estuary to investigate microbial dynamics, assess nitrogen compound removal, and explore effective biotechnological strategies under field conditions. The findings aim to support scalable and sustainable bioremediation approaches applicable to similar urban water bodies.

2. Materials and methods

2.1 Agents employed

Commercially available microbial formulations were applied for bioremediation treatments; each selected for its potential to degrade aquatic contaminants. EM-1 (Embioecsa), a commercial patented consortium of lactic acid bacteria, photosynthetic bacteria, and yeasts, was used for its synergistic ability to decompose organic matter and control odors. Star Pond N (Zamub), a commercial patented nitrification-specific formulation containing Nitrosomonas and Nitrobacter, facilitated the aerobic oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-). CEPA AERO24-Z (BioService), an aerobic activated sludge enriched with nitrifying strains again dominated by Nitrosomonas and Nitrobacter was also tested, according to distributor specifications. All microorganisms were directly applied to estuarine water and sediment samples without prior cultivation, simulating field conditions in a highly contaminated environment. Distilled water was used to prepare and ensure uniform dosing of treatments (BioBase-SCSJ-I).

2.2 Sampling and use of microorganisms

The experimental samples were collected from a designated area within Branch 9 of the Estero Salado, located in Guayaquil, Ecuador. A specific section was selected for sampling, where water and sediment samples were collected from multiple points. For the water sample, these were combined in controlled proportions of 90% water and 10% sediment by weight. For the section designated as sludge, samples were collected as pure sludge without any additional aggregates. This composite mixture served as the baseline matrix for microbial treatments. The microorganisms utilized in this study were categorized into three groups: Efficient Microorganisms (EM), Nitrifying Bacteria (NB), and Activated Biomass (AB). The experimental design incorporated varying concentrations and combinations of these microbial treatments, as detailed in Table 1, to thoroughly evaluate their effects on the water-sediment matrix. To ensure data reliability and statistical robustness, all experiments were conducted in triplicate, providing mean values for each data point collected during the experimental phase. The corresponding statistical analyses (standard deviations and error bars) are additionally provided in the SI. This systematic methodology aimed to optimize the quality of the data and facilitate an in-depth exploration of microbial interactions in the remediation of water and sediment within the estuary ecosystem.

3. Sample analysis

The analysis of the selected samples was based on the best-performing experimental results, as detailed in Table 1 with a total of fourteen[14] samples selected to analyze different parameters of interest. These analyses were conducted by ELICROM, a specialized laboratory accredited under ISO 17025 by the Ecuadorian Accreditation Body (SAE), ensuring the highest precision and reliability in the results. The evaluated analytical parameters included key environmental and chemical indicators: total petroleum hydrocarbons (mg L^{-1}), dissolved oxygen (%), nitrites (mg L^{-1}), nitrates (mg L^{-1}), chemical oxygen demand (mg L^{-1}), ammonia (mg L^{-1}), total phosphorus (mg L^{-1}), total Kjeldahl nitrogen (mg L^{-1}), hydrogen sulfide (mg L^{-1}), total alkalinity (mg L^{-1}), total organic carbon (mg L^{-1}), and salinity (%). This comprehensive analysis provided critical insights into the efficacy of the microbial treatments and their impact on water and sediment quality, forming the foundation for interpreting the experimental results.

4. Results

The results of the analyzed parameters for the selected combinations and experimental trials are presented in Table 2. These findings demonstrate that the different bacterial combinations and dosages applied had distinct effects on water quality parameters. Notably, treatments incorporating NB resulted in a significant reduction in nitrogenous organic compounds, along with marked improvements in other key water quality indicators. These outcomes underscore the effectiveness of this methodology as a targeted treatment approach designed to address specific contaminants in water systems.[29] The observed efficacy highlights the adaptability and precision of microbial treatments, particularly those involving NB, in mitigating pollution and enhancing water quality, offering promising potential for broader application in environmental remediation efforts.[30]

All variables were analyzed with a one-way ANOVA by dose, using Rep as a blocking factor ($n = 3$ per dose). For each treatment, we report mean ± SD and display error bars as ±SE, computed from the model's pooled residual standard deviation (see SI). Dose had a highly significant effect on every variable ($p < 0.001$), while the replicate factor was negligible or small.

The pooled values, used as the basis for SE and error bars, were: Total Petroleum Hydrocarbons (TPH) = 1.04 mg L^{-1} (treatment means ∼10.5–21.1 mg L^{-1}), nitrates = 0.275 mg L^{-1} (0.06–4.95 mg L^{-1}), nitrites = 0.00384 mg L^{-1} (0.006–0.086 mg L^{-1}), COD = 188.68 mg L^{-1} (347–2785 mg L^{-1}), BOD = 128.63 mg L^{-1} (190–1598 mg L^{-1}), phosphorus = 8.28 mg L^{-1} (28.6–128 mg L^{-1}), total alkalinity = 26.22 mg L^{-1} (199–418 mg L^{-1}), salinity = 0.042% (0.231–0.821%), dissolved oxygen = 5.08% (1.56–75.7%), and TKN = 8.12 mg L^{-1} (22.9–125 mg L^{-1}). These residual dispersions are small relative to the corresponding treatment means, confirming that triplicates were consistent and that the observed differences are reproducible.

4.1 Ammonia, petroleum hydrocarbons, total phosphorus, and total organic carbon

Ammonia concentrations ranged from 1.15 to 27.5 mg L^{-1}. Several treatments, particularly those involving NB, reduced levels to near the U.S. EPA threshold for treated wastewater (<20 mg L^{-1}) (EPA, 2000), while others exceeded this limit. Elevated concentrations pose serious ecological risks, including toxicity to aquatic organisms and disruption of ecosystem balance.[31] These results emphasize the need to optimize microbial dosages to achieve consistent nitrification and encourage the exploration of combined methods. For example, Nishi *et al.*[32] reported that coupling microalgae with NB achieved nearly 100% ammonia removal through uptake by microalgae.

Total petroleum hydrocarbons (TPHs) ranged from 7.468 to 20.622 mg L^{-1}, values generally considered environmentally concerning because levels above 10 mg L^{-1} are persistent and toxic, even though no strict regulatory limit exists for surface waters (U.S. EPA, 2000). The microbial formulations applied here, while effective for nitrogenous compounds, did not significantly reduce hydrocarbon content. This limitation is consistent with previous reports showing that hydrophobic hydrocarbons, particularly under oxygen-limited conditions, exhibit poor biodegradability by conventional microbial

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Table 1 Quantities and combinations studied in the dosing of micro-organisms in water and sludge samples, and observations found[a]

Tests in water					
Number	Dosage	Bacteria and/or combination	Observed changes		
			Turbidity	Odor	Color
		Waters with sediments			
1		EM	X	X	X
2	5 µL	NB	X	X	X
3		AB	X	X	X
4		EM	X	X	X
5	10 µL	NB	X	X	No
6		AB	X	X	No
7		EM	X	X	No
8	20 µL	NB	X	X	No
9		AB	X	X	No
10		EM	No	No	No
11	50 µL	NB	No	No	No
12		AB	No	No	No
13	100 µL	EM	No	No	No
14		NB	No	No	No
15		AB	No	No	No
16		EM	No	No	No
17	500 µL	NB	No	No	No
18		AB	No	Yes	Yes
19	1000 µL	EM	No	Yes	Yes
20		NB	No	Yes	No
21		AB	Yes	Yes	Yes
22	2000 µL	EM	No	Yes	Yes
23		NB	No	Yes	No
24		AB	Yes	Yes	Yes
25		EM : NB 75 : 25	No	No	No
26		EM : AB 75 : 25	No	No	No
27	100 µL	EM : NB 50 : 50	No	No	No
28		EM : AB 50 : 50	Yes	Yes	Yes
29	500 µL	EM : NB 75 : 25	No	No	No
30		EM : AB 75 : 25	Yes	Yes	Yes
31		EM : NB 50 : 50	No	No	No
32		EM : AB 50 : 50	Yes	Yes	Yes
33		EM : NB 75 : 25	No	No	No
34	1000 µL	EM : AB 75 : 25	Yes	Yes	Yes
35		EM : NB 50 : 50	No	Yes	Yes
36		EM : AB 50 : 50	Yes	Yes	Yes
37	2000 µL	EM : NB 75 : 25	Yes	Yes	Yes
38		EM : AB 75 : 25	Yes	Yes	Yes
		Sludge			
39	1 mL	EM	—	Yes	—
40		NB	—	Yes	—
41		BioService	—	Yes	—
42		EM	—	Yes	—
43	5 mL	NB	—	Yes	—
44		AB	—	Yes	—
45		EM	—	Yes	—
46	10 mL	NB	—	Yes	—
47		AB	—	Yes	—
48		EM	—	Yes	—
49	20 mL	NB	—	Yes	—
50		AB	—	Yes	—
51		EM	—	Yes	—
52	30 mL	NB	—	Yes	—
53		AB	—	Yes	—
54	1 mL	EM : NB 75 : 25	—	Yes	—
55		EM : AB 75 : 25	—	Yes	—
56	5 mL	EM : NB 75 : 25	—	Yes	—
57		EM : AB 75 : 25	—	Yes	—
58	10 mL	EM : NB 75 : 25	—	Yes	—
59		EM : AB 75 : 25	—	Yes	—
60	1 mL	EM : NB 50 : 50	—	Yes	—
61		EM : AB 50 : 50	—	Yes	—
62	5 mL	EM : NB 50 : 50	—	Yes	—
63		EM : AB 50 : 50	—	Yes	—
64	10 mL	EM : NB 50 : 50	—	Yes	—
65		EM : AB 50 : 50	—	Yes	—

Little change
Good change
Significant change
No positive change
No change

[a] EM: effective bacteria; NB: nitrifying bacteria; AB: activated biomass.

Table 2 Results obtained from the analysis of each of the samples studied

Sample	TPH (mg L^{-1})	DO (%)	NO_2^- (mg L^{-1})	NO_3^- (mg L^{-1})	COD (mg L^{-1})	BOD (mg L^{-1})	NH_3 (mg L^{-1})	TP (mg L^{-1})	TKN (mg L^{-1})	H_2S (mg L^{-1})	TA (mg L^{-1})	TOC (mg L^{-1})	Sal (%)
Mother solution	16.27	66.9	0.01	0.5	394	197	27.3	36.8	104.5	2.24	370	84	0.7
44	15.28	2.8	0.03	—	865	183	25.8	40.0	84.3	1.06	360	82	0.7
42	11.73	4.0	0.01	0.1	382	190	24.0	30.4	62.0	1.46	366	70	0.7
43	12.80	1.2	0.01	0.5	411	205	27.5	59.7	124.8	1.09	380	126	0.7
47	12.23	4.0	0.02	—	372	186	25.0	36.8	87.3	1.15	356	77	0.7
45	12.05	2.7	0.02	—	840	421	19.3	40.8	79.5	5.25	392	121	0.7
46	16.06	1.2	0.01	0.3	871	440	23.5	74.6	64.0	1.67	400	140	0.7
62	20.62	3.0	0.01	0.5	503	258	25.0	57.2	70.0	1.75	380	136	0.7
64	7.47	23.7	0.02	—	2885	1462	1.3	104.0	88.5	8.50	200	407	0.3
63	10.54	0.8	0.01	—	356	178	23.5	96.0	67.5	0.40	386	69	0.7
65	13.10	11.6	0.09	—	2490	1246	1.5	143.0	17.8	6.13	264	421	0.3
56	12.83	1.6	0.05	0.5	567	283	25.3	46.0	107.3	1.60	392	97	0.7
58	12.78	6.7	0.02	0.1	1610	805	1.5	78.2	32.0	8.55	274	291	0.3
59	12.66	2	0.02		2155	1084	1.5	136.0	40.3	6.69	320	314	0.3

consortia.[24,25] Integrating complementary treatments such as advanced oxidation processes is critical in order to reduce TPHs concentration.

Excessive phosphorus contributes to eutrophication and algal blooms, a pattern well-documented in coastal ecosystems.[33] Since the bacterial formulations did not achieve phosphorus removal, incorporating chemical precipitation (*e.g.*, alum or ferric salts) or biosorption methods is necessary. Previous studies demonstrate that precipitation achieves rapid phosphorus reduction, though at the cost of sludge production, whereas biosorption can provide more sustainable long-term options.[15]

Total organic carbon (TOC) reached up to 400 mg L^{-1}, reflecting a substantial organic load capable of depleting dissolved oxygen and generating anoxic conditions. Similar findings have been reported in bioremediation systems where high TOC persists despite microbial activity.[21] These results, summarized in Fig. 2, highlight the importance of designing multidimensional treatment trains that integrate biological consortia with oxygenation and hybrid processes. Future pilot-scale implementations should combine microbial treatments with physical–chemical polishing to achieve regulatory compliance, particularly in tropical estuarine environments such as the Estero Salado.

4.2 Nitrite, nitrates and hydrogen sulphide

Nitrite and nitrate are key indicators of water quality. According to WHO and U.S. EPA guidelines, nitrite should remain <1 mg L^{-1} and nitrate <10 mg L^{-1} in potable water.[34] In this study, concentrations across all treatments remained well within these limits (Fig. 3), confirming the effectiveness of the microbial strategies in controlling these nitrogenous compounds and minimizing risks to water safety. By contrast, hydrogen sulfide (H_2S) peaked at 9 mg L^{-1}, likely due to the absence of anoxic zones and the accumulation of sulfidic soils that enhance organic matter sequestration.[35] Although no strict drinking-water standard exists for H_2S, elevated levels are linked to foul odors, infrastructure corrosion, and anaerobic conditions that impair aquatic ecosystems.[36] The observed variability in H_2S underscores the need to investigate drivers such as microbial metabolism and organic matter decomposition.

4.3 COD, BOD, dissolved oxygen

Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) values in the Estero Salado samples were far higher than international discharge standards (Fig. 4). The U.S. EPA sets a COD limit of 125 mg L^{-1} for treated wastewater (U.S Environmental Protection Agency, 2000), yet our samples reached 2885 mg L^{-1}, a pattern consistent with highly polluted urban effluents.[37] Similarly, BOD exceeded 1400 mg L^{-1}, compared to recommended values of 25–40 mg L^{-1}.[38] Comparable exceedances have been documented in tropical estuaries receiving mixed industrial and domestic inputs, where natural self-purification is insufficient.[39] These results confirm that microbial consortia alone are not enough under such high organic loads.

Persistent COD indicates the presence of refractory organic compounds. Advanced oxidation processes (AOPs) have been shown to effectively mineralize such pollutants, outperforming biological treatments in heavily contaminated matrices.[40] Likewise, while microbial treatments reduce BOD in moderate-load systems, in high-load scenarios the excessive oxygen demand overwhelms microbial capacity, requiring hybrid approaches that combine biological and chemical steps.[38] Our observed temporary increase in COD/BOD mirrors reports from other bioremediation studies where microbial growth and lysis release additional organic matter before stabilization. This reinforces the need for post-treatment polishing, such as adsorption or AOPs, to achieve effluent standards.

Dissolved oxygen (DO) ranged from 0.8% to 23.7%, well below the ≥5 mg L^{-1} needed for efficient aerobic activity.[41] Similar oxygen deficiencies have been reported in bioreactors treating high-strength effluents, where oxygen limitation

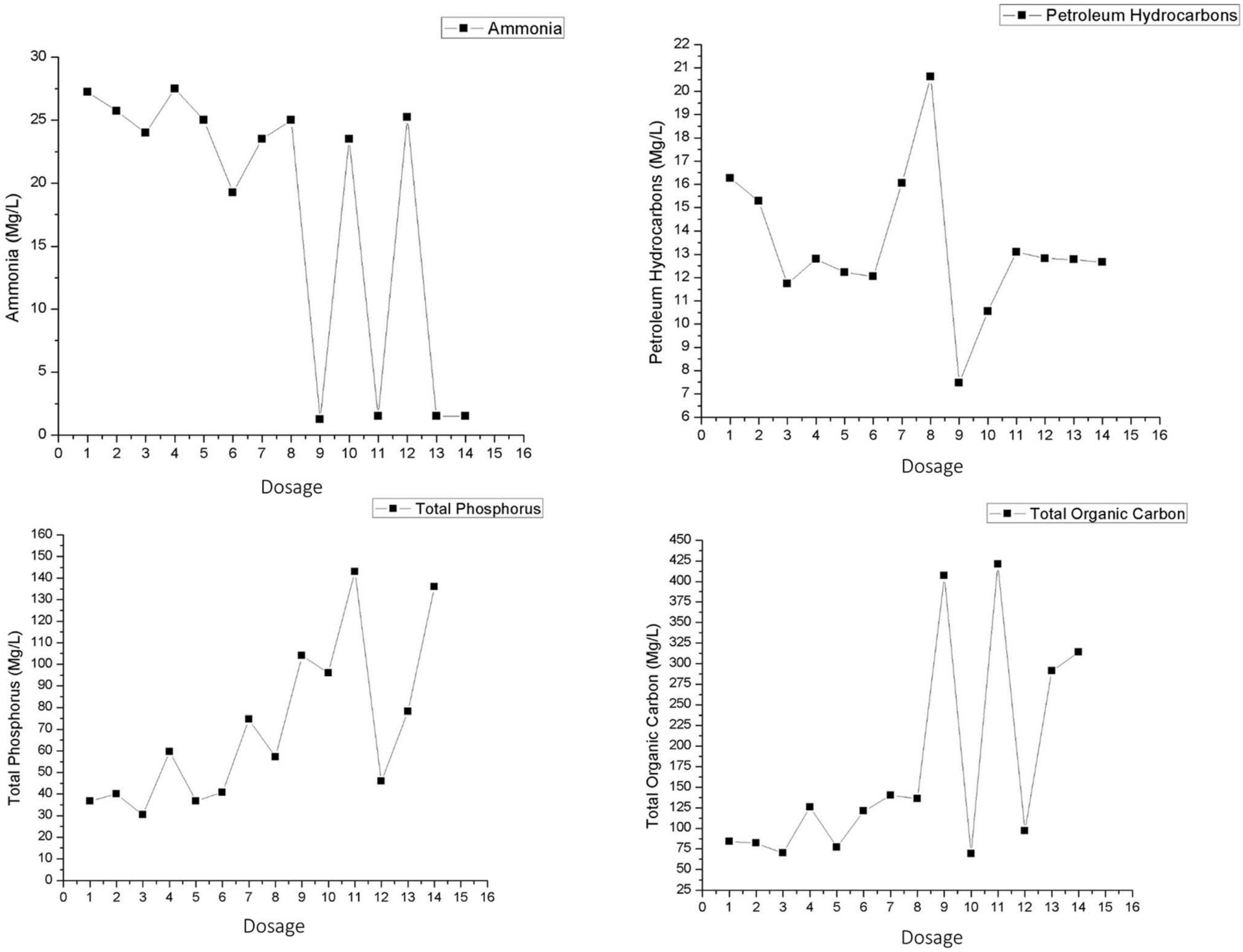

Fig. 2 Results for selected combinations: ammonia, petroleum hydrocarbons, total phosphorus, and total organic carbon.

impairs nitrification and shifts microbial communities.[42] In our case, the absence of artificial aeration reproduced field conditions but also constrained microbial performance. Studies using aerated systems in comparable tropical watersheds have demonstrated improved COD/BOD reduction and microbial efficiency.[37,43] Therefore, future implementations should integrate cost-efficient aeration systems to sustain aerobic consortia and ensure compliance with regulatory standards.

4.4 Kjeldahl nitrogen, alkalinity and salinity

The treatments reduced Total Kjeldahl Nitrogen (TKN), but concentrations remained well above the U.S. EPA threshold of 10–15 mg L^{-1} for treated effluents (EPA, 1994). The most effective configuration (25/75 Biocompost/Embioecsa) achieved 40.25 mg L^{-1}, still more than double the permissible limit. Comparable challenges have been reported in other bioremediation systems where high organic loads limit complete nitrogen removal, suggesting that optimization of microbial ratios or integration with denitrification processes is required.[44] Studies using hybrid biological–chemical systems have shown improved nitrogen removal efficiency compared to biological treatments alone, supporting the need for combined approaches in estuarine environments.

Alkalinity reached 400 mg L^{-1} in several treatments, exceeding the recommended 100–200 mg L^{-1} range.[44] Similar increases in alkalinity have been observed in anaerobic and nitrification-dominated systems, where ammonia oxidation generates hydroxides that shift buffering capacity. Elevated alkalinity can impair downstream polishing processes and destabilize aquatic ecosystems. Control measures such as lime dosing, acid neutralization, or stepwise aeration have proven effective in maintaining stable pH-alkalinity balances in comparable wastewater treatment scenarios.

Salinity values (0.25–0.74%) were consistent with brackish estuarine conditions, aligning with previous reports in tropical coastal systems.[45] While salinity in this range may not directly breach regulatory thresholds, it poses challenges for effluent reuse, particularly in agriculture, where high salinity restricts crop suitability. Elevated salinity has also been shown to inhibit nitrifying and denitrifying bacteria, reducing overall bioremediation efficiency.[45] Strategies such as electrodialysis or dilution with lower-salinity sources have been proposed as complementary measures to expand reuse potential.

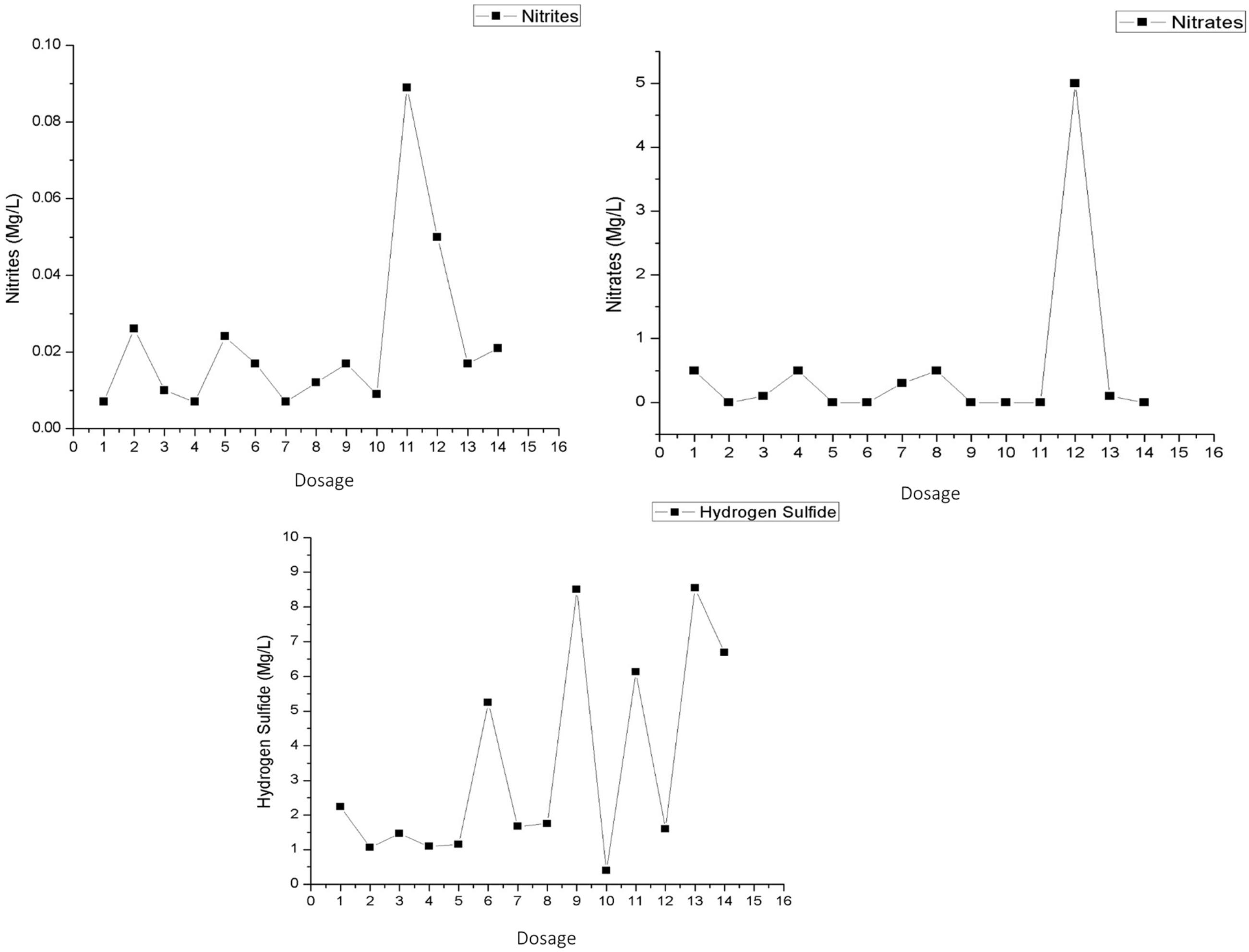

Fig. 3 Results for selected combinations: nitrite, nitrates and hydrogen sulphide.

Overall, our results parallel other estuarine bioremediation studies where partial success in nitrogen removal was offset by persistent alkalinity and salinity constraints. These findings reinforce the need for integrated management that couples microbial consortia with alkalinity adjustment and salinity control to achieve both regulatory compliance and ecological safety (Fig. 5).

The treatments tested in Estero Salado highlight both the promise and the limitations of microbial remediation under complex estuarine conditions. NB effectively reduced ammonia while keeping nitrite and nitrate within WHO and EPA safety thresholds, consistent with previous studies reporting robust nitrification in mixed microbial systems.[26,28] However, phosphorus concentrations remained elevated, underscoring that microbial formulations alone cannot address nutrient overload. Similar conclusions have been drawn in other coastal studies, where precipitation or biosorption was required to prevent eutrophication.[15,33]

TPH and high TOC persisted above acceptable levels, reflecting the poor biodegradability of hydrophobic compounds under oxygen-limited conditions. Comparative work has shown that AOPs and adsorption outperform microbial strategies for these pollutants,[24,40] supporting the need for integrated physical, chemical steps. Elevated hydrogen sulfide further indicated localized anaerobic hotspots, a phenomenon also reported in mangrove sediments.[35] Addressing this will require aeration or chemical oxidation[36] in combination with microbial treatments.

Excessive COD and BOD confirmed severe organic loading, in line with reports from other polluted tropical estuaries.[37,38] In such scenarios, hybrid configurations, such as membrane bioreactors or staged bioaugmentation, have demonstrated superior pollutant removal compared to biological treatments alone.[40] Although TKN levels decreased, they remained above EPA thresholds, mirroring difficulties reported in high-strength wastewater systems where additional biological nitrogen removal or ion exchange was necessary.[38] Elevated alkalinity and brackish salinity similarly constrained performance; both factors are known to inhibit microbial activity,[45] highlighting the importance of monitoring and corrective control.

Overall, our findings converge with other studies in showing that while microbial consortia are effective for nitrogen management, comprehensive estuarine remediation requires a multifaceted approach. Combining microbial bioremediation

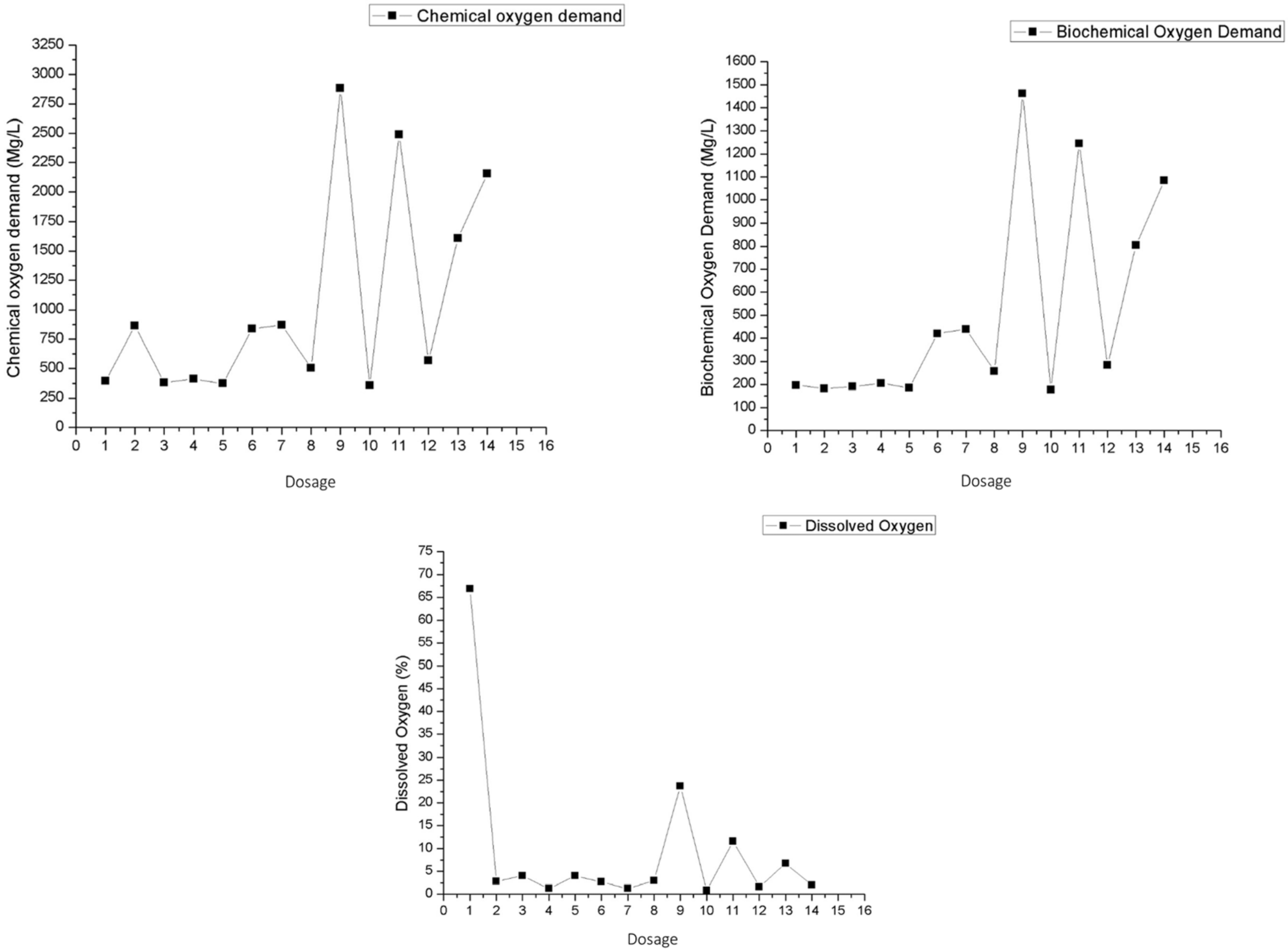

Fig. 4 Results for selected combinations: COD, BOD, dissolved oxygen.

with aeration, advanced oxidation, precipitation, or membrane-based processes appears essential to achieve regulatory compliance and ensure ecological safety in highly polluted tropical estuaries.

4.5 Comparative evaluation of microbial bioremediation and conventional treatment technologies

While our microbial treatment approach demonstrated notable efficiency in nitrogen removal under cost-effective and environmentally sustainable conditions, it showed limitations in the degradation of persistent pollutants such as petroleum hydrocarbons and phosphorus.[46] In contrast, membrane-based technologies such as membrane bioreactors (MBRs) can produce high quality effluents and are suitable for water reuse, but are constrained by high operational costs, membrane fouling, and energy demands.[47] Chemical precipitation offers effective and rapid phosphorus removal, yet it generates large volumes of chemical sludge and fails to address organic contaminants.[48] Advanced oxidation processes (AOPs) stand out for their ability to mineralize recalcitrant organic pollutants, including hydrocarbons, *via* hydroxyl radicals and other reactive species, though they are often limited by high energy input requirements and the potential generation of toxic by-products.[49,50]

These comparative insights are summarized in Table 3, which outlines the advantages and drawbacks of each technology. Based on these findings, we propose that a hybrid treatment approach, combining the scalability and adaptability of microbiological remediation with the precision and robustness of conventional methods, may offer an optimized solution for managing wastewater in tropical estuarine environments such as the Estero Salado. This integrative strategy could enhance pollutant removal while maintaining economic and ecological feasibility.

To assess the practical feasibility of scaling up microbial bioremediation, a preliminary evaluation of cost and energy requirements was conducted. Based on supplier data, the average cost of microbial consortia such as nitrifying bacteria (Star Pond N), efficient microorganisms (EM-1), and activated biomass (CEPA AERO24-Z) ranges between USD 18–35 per liter, depending on formulation and volume. For small-scale pilot systems (1–2 m^3 per day), microbial dosage requirements remain modest (∼1–2 L per week), making the approach economically attractive. However, scaling up to treat larger polluted estuarine volumes (>100 m^3 per day) could lead to

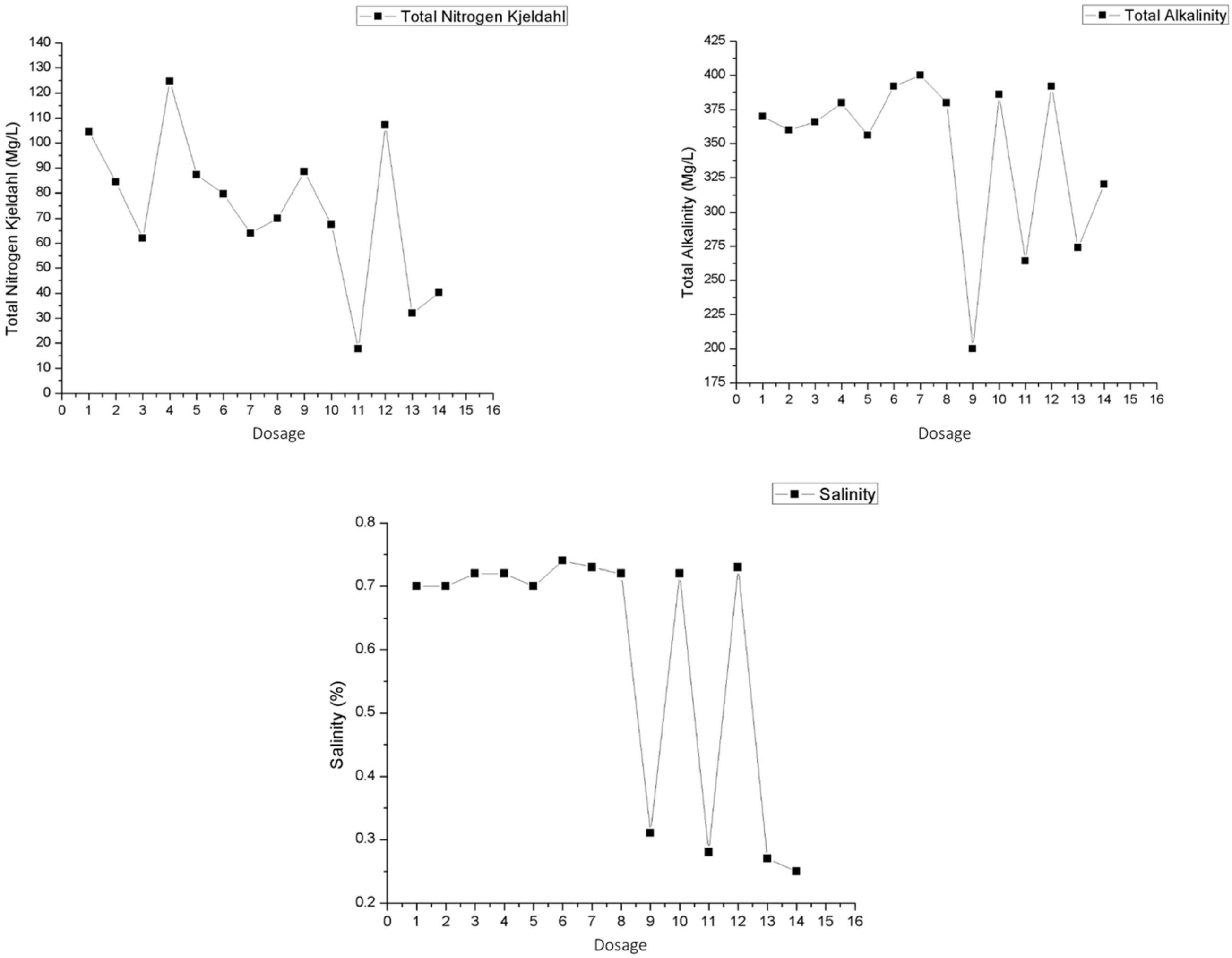

Fig. 5 Results for selected combinations: Kjeldahl nitrogen, alkalinity and salinity.

Table 3 Comparative summary: microbial bioremediation *vs.* conventional treatment methods

Technology	Main advantages	Main limitations	References
Microbial bioremediation	Sustainable, cost-efficient, adaptable, and scalable for rural or tropical settings	Low efficiency against persistent compounds like hydrocarbons and phosphorus; sensitive to oxygen levels	51 and 52
Membrane filtration/MBR	Produces high-quality effluents; compact design suitable for reuse; removes solids and pathogens	High membrane costs; fouling issues; energy-intensive operation	53 and 54
Chemical precipitation	Rapid phosphorus removal; well-established and widely applied technique	Generates chemical sludge requiring proper disposal; ineffective against organic contaminants	55
Advanced oxidation processes (AOPs)	Effective removal of recalcitrant organic pollutants, including hydrocarbons; complete mineralization	High operational cost; energy-demanding; potential formation of toxic byproducts	56

increased operational costs unless strategies such as microbial regeneration or reuse are optimized.

A critical factor affecting microbial efficiency is the availability of dissolved oxygen (DO). In this study, DO levels were consistently below the optimal threshold for aerobic microbial activity (>5 mg L^{-1}), which could limit nitrification and overall treatment performance. To overcome this limitation, forced aeration systems such as surface aerators or diffused air technologies would be necessary. These systems typically consume between 0.3–1.2 kWh m^{-3} depending on efficiency and application scale, representing a significant energy consideration in full-scale deployments. Taken together, these findings suggest that while microbial treatments are promising for decentralized or low-load scenarios due to their low cost and environmental compatibility, their application at larger scales must be supported by energy-efficient aeration and, where needed, targeted physicochemical methods. This hybridization is essential to meet regulatory thresholds for persistent pollutants such as hydrocarbons and phosphorus. Ultimately, no single technology provides a universal solution for complex wastewater matrices like those found in tropical estuarine environments. Microbial bioremediation offers a robust foundation aligned with sustainability goals, but its true potential lies in synergistic integration with conventional treatment technologies.

5. Conclusions and future perspectives

The employed treatments demonstrated a positive impact on reducing ammonia levels, particularly those involving NB, which successfully approached acceptable standards for treated water (<20 mg L^{-1}). However, some doses still exceeded this limit, highlighting the need to optimize microorganism proportions to achieve a more efficient and consistent nitrification process. Ammonia accumulation poses significant ecological risks, including toxicity to aquatic organisms and disruptions to ecosystem balance. While nitrite and nitrate levels remained within international standards, elevated total phosphorus (TP) and TOC levels indicate the limited effectiveness of the treatments in addressing these parameters. This underscores the need to integrate complementary methods, such as chemical precipitation or adsorption, to more effectively mitigate nutrients and organic compounds.

The persistent issue of TPH, with levels consistently exceeding regulatory thresholds (<10 mg L^{-1}), emphasizes the importance of combining advanced techniques, such as advanced oxidation processes, with microbiological treatments to enhance pollutant removal. Additionally, low dissolved oxygen levels highlight the urgent need for aeration systems to prevent hypoxic conditions and improve the efficiency of aerobic biological processes. While the results are promising in terms of ammonia reduction, an integrated approach that optimizes microbiological conditions and incorporates advanced physicochemical strategies is essential to ensure compliance with environmental standards and improve water quality in heavily polluted environments like Estero Salado.

Although the results showed a significant reduction in ammonium and other contaminating compounds after the application of specific microorganisms, the present study did not include microscopic validations (*e.g.*, SEM/TEM), quantifications by culture (CFU), or molecular methods (*e.g.*, PCR, qPCR, NGS) to confirm microbial proliferation *in situ*. This methodological decision was based on the exploratory and applied nature of the study, which focused on validating the efficiency of commercial treatments directly under real field conditions. However, future work should incorporate advanced microbiological techniques to confirm microbial colonization, evaluate changes in community structure, and directly correlate the presence/absence of functional groups with contaminant removal kinetics.

These findings underscore the promise of integrated biotechnological strategies for remediating nitrogenous and organic pollutants in severely impacted urban estuaries. However, future research should focus on long term pilot scale implementations, microbial community dynamics under varying environmental stresses, and the integration of molecular techniques to better monitor and optimize bioremediation efficiency. Advancing these efforts will pave the way for scalable, low cost treatment systems tailored to tropical coastal regions facing similar environmental burdens.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Data for this article are available at [Repositorio Digital Universidad Ecotec] at [**https://repositorio.ecotec.edu.ec/items/ecaab242-e63a-4afa-a42b-1c619775f4cb**].

Supplementary information is available. See DOI: **https://doi.org/10.1039/d5va00051c**.

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