

HANJIANG WU (ORCID: 0009-0002-3259-2920)¹

NING SHAN (ORCID: 0009-0005-6766-7429)¹

SHANSHAN LI (ORCID: 0009-0009-5955-7130)¹

JIAHAO LIU (ORCID: 0009-0006-0880-868X)²

DIANJI WANG (ORCID: 0009-0006-4505-9120)³

YINA ZOU (ORCID: 0000-0002-3100-3541)³

LICHAO LU (ORCID: 0000-0002-3204-9509)²

MICROBIAL MIGRATION BETWEEN TWO ENGINEERING PROJECTS. FEASIBILITY ANALYSIS, PERFORMANCE, AND MICROBIAL COMMUNITY SUCCESSION

Existing sewage treatment plants are expected to be the abundant storage and valuable source of naturally functional microorganisms for the proposed ones. This study attempted to migrate the microbial flora in a biological aerated filter (BAF) treating reverse osmosis concentrated water to a proposed BAF treating photovoltaic pickling wastewater, both systems-designed to mainly remove nitrate. The removal efficiency of nitrate nitrogen in total nitrogen increased significantly from 7.3 to 79.5% after the enriched microorganisms were inoculated into the pilot plant. The effluent was stably controlled to meet the required standards (ammonium nitrogen < 35 mg/dm³, total nitrogen < 45 mg/dm³) in practical engineering. The enrichment of the important denitrifying and phosphorus-accumulating the genus *Thauera* and the genus *Flavobacterium* involved in the nitrogen cycle was observed as one of the important reasons for performance improvement. This study is expected to provide theoretical guidance for the efficient control of industrial sewage.

1. INTRODUCTION

Population growth, industrial development, and climate change have exerted enormous pressure on water resources. Water demand for manufacturing and household use

¹Zhejiang Fuchun Ziguang Environmental Protection Co., Ltd. Hangzhou 310000, China.

²Zhejiang Key Laboratory of Pollution Control for Port-Petrochemical Industry, Zhejiang Ocean University, Zhoushan 316004, China.

³State Key Laboratory of Water Disaster Prevention, Yangtze Institute for Conservation and Development, Hohai University, Nanjing 210098, China, corresponding author Y. Zou, email address: yinazou@hhu.edu.cn

is expected to increase by 130% over the next 25 years, leading to water scarcity that will affect more than 40% of the world's population [1]. An optimal solution to address water scarcity is to reuse water. As a crucial component of modern environmental engineering systems, urban sewage treatment plants have become increasingly prominent in the urbanization process. With the global urbanization rate increasing by 1.5% annually, daily municipal wastewater generation has exceeded 930 million m³ [2]. However, in developing countries, 38% of sewage is directly discharged without treatment, and the imbalance between treatment demand and capacity has posed a severe ecological and environmental challenge while increasing the demand for sewage treatment plants.

The physical and chemical processes of sewage treatment are generally complex and costly in terms of installation, startup, and maintenance, especially in large-scale plants. Consequently, biological processes have become indispensable in sewage treatment, as they are efficient, environmentally friendly, and economical. Microorganisms use pollutants as a source of nutrients and energy for growth and metabolism, playing a critical role in biological sewage treatment. As an abundant and comprehensive source of functional microorganisms, existing sewage treatment plants are expected to provide multifunctional microorganisms with activated metabolism for proposed engineering plants.

However, directional microbial migration presents technical challenges related to the differences between various sewage sources and artificially constructed biochemical reaction environments. According to ecological kinetic models, the enrichment efficiencies of functional microorganisms in activated sludge systems are typically lower than expected [3]. This limitation probably stems from three aspects. First, functional bacteria with the ability to denitrify and remove phosphorus are highly sensitive to dissolved oxygen gradients (0.2–2.0 mg/dm³), temperature fluctuations (± 3 °C), and pH (6.8–7.5). In a previous study, fluctuation in chemical oxygen demand (COD) to a range of 0.35–0.82 mg/dm³ led to a 28–65% decrease in microbial growth [4]. Hence, the spatiotemporal heterogeneity between the two engineering projects should be considered before microbial migration. In addition, inhibitory factors, such as heavy metals (e.g., Cr⁶⁺ > 0.5 mg/dm³) and phenols (> 200 mg/dm³), introduced with the industrial sewage, reduce expression of ammonia monooxygenase subunit A (*amoA*) in ammonia-oxidizing bacteria (AOB) [5]. Nitrite accumulation increased from 15 to 82 mg/dm³ in a coking sewage treatment plant when the AOB experienced quorum quenching under a phenol shock load (300 mg/dm³). The nitrification rate remained 56% of the theoretical value even with mitigation measures, such as adding humic acid (50 mg/dm³) and extending the sludge retention time to 25 days. Interspecific competition is also a significant challenge in forming a microbial community with the expected functions. For example, denitrifying bacteria and glycogen-accumulating organisms compete for volatile fatty acids. The denitrification efficiency was reported to decline by more than 40% at a C/N ratio of < 5 [6]. Therefore, for newly constructed wastewater plants or those facing significant changes in wastewater sources, seeking appropriate microbial communities for inoculation is crucial. Therefore, a strategy for finding an adaptable inoculation for a proposed sewage treatment plant is urgently needed.

Microbial migration between sewage treatments presents a viable solution to this problem. The performance of a proposed sewage treatment may be enhanced by inoculating it with microorganisms from an existing treatment facility that employs similar functions. However, indiscriminate microbial migration would result in failures primarily due to non-negligible differences in environmental parameters between the source and target systems. This technical challenge is particularly pronounced between municipal and industrial sewage treatment plants. The underlying mechanisms of these challenges include (1) suppression of metabolic activity caused by differences in environmental stressors such as dominant pollutant concentration and fluctuations in redox potential, (2) limited microbial colonization due to competition with pre-existing microbial communities; and (3) failure in synergistic metabolism caused by an imbalance of ecological niches undergoing different metabolic processes. A case study demonstrated this by transferring a nitrifying microbial community (containing 28% *Nitrosomonas europaea*) from a municipal wastewater treatment plant to a petrochemical wastewater treatment plant. Microbial activity was inhibited by 30–70% when the residual sulfides exceeded 15 mg/dm³, despite the influent NH₄⁺-N load being adjusted to an optimal range [7]. Simultaneously, the abundance of the migrated strains declined due to competition with native sulfur-oxidizing bacteria (*Thiobacillus* spp.) after 12 sludge retention cycles. As a result, the NH₄⁺-N removal rate declined to less than half of the expected values in petrochemical sewage treatment [8]. These documented failures underscore the need for feasibility evaluation and microbiota acclimatization in the context of microbial migration.

The similarity of pollutant components between the existing and new plants, including the dominant pollutants and their concentrations, is a prerequisite for achieving effective microbial migration. Indeed, the success rate can increase to 68±5% by optimizing the migration strategy [9]. A similarity of 70% between the existing and proposed plants enabled successful migration [10, 11]. For example, benzene-degrading microorganisms were successfully migrated from a chemical industrial park to a pharmaceutical sewage treatment plant. In this case, the dominant pollutant components in both systems were aromatic hydrocarbons, including phenol and toluene, and the HPLC chromatogram similarity of metabolic intermediates (e.g., catechol and *cis*-dihydrodiol) reached 82%. Consequently, metabolic activity, measured by dioxygenase gene expression, was maintained, leading to considerable benzene removal performance. In contrast, the relative abundance of functional microorganisms declined by 97% within 15 days when the microorganisms were migrated from a sewage treatment plant treating textile dyeing sewage (dominated by azo dyes) to a plant treating coking sewage (dominated by polycyclic aromatic hydrocarbons) [12]. Therefore, establishing a microbial migration strategy between two engineering systems and investigating the changes it generates in the microbial community structure are essential.

This study analyzed the feasibility of migrating microbial flora from an existing sewage treatment plan treating steel wastewater to a proposed sewage treatment plan treating

photovoltaic wastewater. Performance characteristics were validated in a pilot-scale reactor after optimizing the acclimatization of microbiota. The microorganisms were finally inoculated into a sewage treatment plant to explore the performance and microbial community. The findings of this study are expected to provide theoretical support for reducing costs and improving efficiency in sewage treatment projects.

2. MATERIALS AND METHODS

Microorganisms and existing plants. The initial flora was taken from a biologically aerated filter (BAF) used to treat reverse osmosis (RO)-concentrated water in a steel manufacturing plant. The enterprise has a central wastewater treatment station that utilizes ultrafiltration, RO, and an aerated BAF to treat wastewater from its iron and steel production processes. This station has a daily treatment capacity of 45,000 m³, resulting in RO-concentrated water characterized by high total dissolved solid (5,000–6,500 mg/dm³) and high salt (1,900–2,200 mg/dm³) contents. The influent nitrate nitrogen concentration of the aerated BAF is approximately 30 mg/dm³, and the effluent concentration is less than 3 mg/dm³.

The proposed project. The target system for colony migration is located in a photovoltaic plant, which features an aerated BAF treating photovoltaic pickling wastewater. The nitrate concentration in the wastewater is approximately 45 mg/dm³, and the daily treatment volume is 6,000 m³. Since the drainage from this plant needs to be connected to the municipal wastewater treatment plant and the water volume is large (approximately 30% of the daily capacity of the municipal wastewater treatment plant), it is necessary to reduce the nitrate nitrogen concentration in the pickling wastewater to less than 10 mg/dm³. The process flow is illustrated in Fig. 1 and involves photovoltaic wastewater, fine grids, and an aeration BAF. The wastewater passes through the fine grating to a primary sedimentation tank, followed by a regulating tank and an aerated BAF before reaching the municipal wastewater treatment plant.

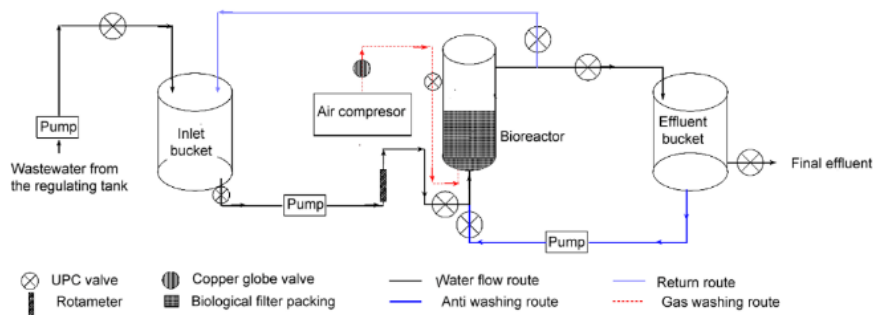


Fig. 1. The scheme of the pilot-scale reactor

Microbial acclimatization. The change in influent conditions resulted in a significant increase in nitrate concentration, making it necessary to acclimatize the initial flora to further enhance its denitrification ability. In this study, two different nutritional conditions were used to acclimatize the initial flora: beef extract + peptone and acetic acid + potassium nitrate. The grouping for lab-scale test was as follow:

- S1 – inoculated with microorganisms direct collected from the BAF of the steel manufacturing plant;
- S2 – inoculated with microorganisms enriched with beef extract + peptone with addition of wastewater from reverse osmosis (RO)-concentrated water in the steel manufacturing plant,
- S3 – inoculated with microorganisms enriched with beef extract + peptone with sterile water,
- S4 inoculated with microorganisms enriched with acetic acid + potassium nitrate with addition of wastewater from reverse osmosis (RO)-concentrated water in the steel manufacturing plant,
- S5 – inoculated the microorganisms enriched with acetic acid + potassium nitrate with sterile water.

Operating conditions of the pilot-scale reactor. The BAF of the industrial wastewater pretreatment plant employs a three-stage denitrification process for biological treatment. The design flow (Q) is 6,000 m³/day. The single-stage BAF is divided into two grids, with a plane size of 5×7 m. The surface hydraulic load is 3.5 m³/(m²·h), filter material filling height is 3.8 m, empty bed hydraulic retention time is 60 min, and denitrification load is 0.45 kg nitrate/(m³·day). The intensity of backwash gas is 15 dm³/(m²·s), and the intensity of backwash water is 5 dm³/(m²·s). The primary equipment includes a pebble-bearing layer, $d = 16\text{--}32$ mm, 30 m³, a lift pump pebble-bearing layer, $d = 8\text{--}16$ mm, 30 m³, ceramsite filter material, $d = 6\text{--}9$ mm, 532 m³, ceramsite filter material, $d = 4\text{--}6$ mm, 266 m³; a standard filter plate, 210 m³, antiblocking long-handle filter head, 25 × 430 mm, filter rod inner diameter 20 mm, acrylonitrile butadiene styrene (ABS) material, 7560; an aeration system, $Q = 12$ m³/min, two sets; and three backwash pumps, single flow $Q = 320$ m³/h, head $H = 22$ m, motor power $N = 30$ kW.

The pilot-scale simulation replicates the process flow of a BAF. The influent (effluent from the regulating tank of the industrial wastewater pretreatment plant) is pumped to the inlet bucket by the submersible sewage pump. The water is pumped to the upper part of the reaction column by the influent magnetic pump, and the produced water is filtered through the filler and discharged into the outlet bucket. The outlet bucket also provides backwash water. During backwashing, the water is cleaned from bottom to top by the backwash pump through valve switching. The sewage is discharged through the sewage pipeline, and the fan provides backwash air. During biofilm formation, switching the valve returns the bacterial liquid in the reaction column to the inlet bucket for circulation. The pilot process is illustrated in Fig. 2.

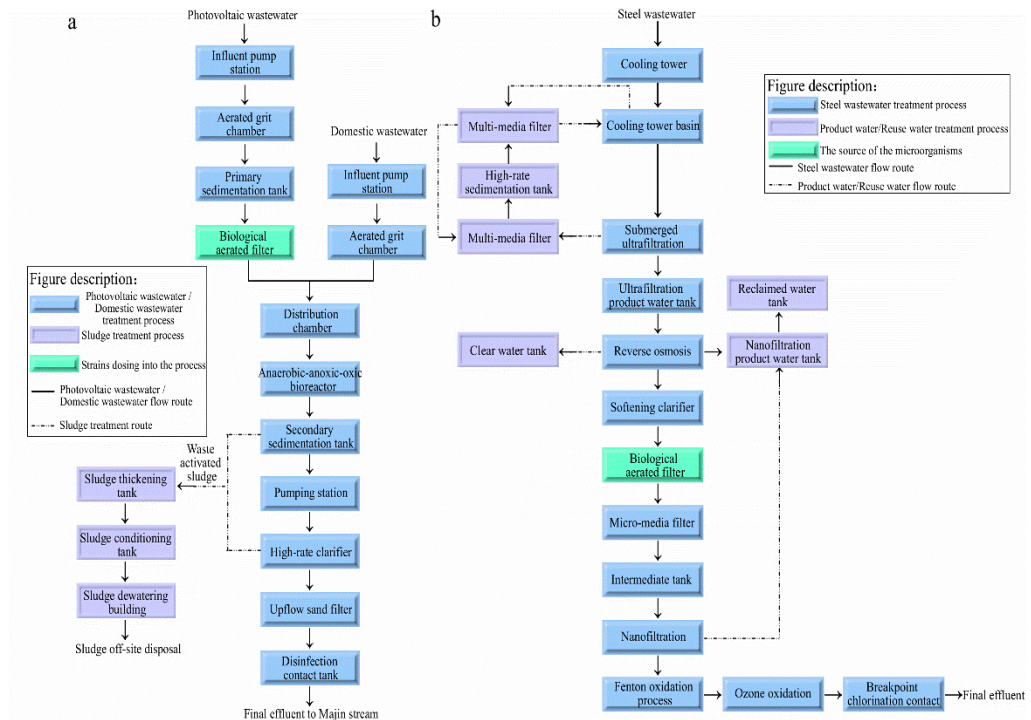


Fig. 2. Process flow diagram of the wastewater treatment plants treating photovoltaic wastewater (a) and steel wastewater (b)

The bacteria hanging drop membrane. Bacterial suspension and filler are placed in the reaction column. After the pilot plant was started, the bacterial suspension followed the reflux route, and the bacterial strain was added to the reaction column using the hanging drop method over a 2-day cycle. A mixture of 80 cm³ of 99% acetic acid (carbon source) and 4.2 g of dipotassium hydrogen phosphate (phosphorus source) was added to the inlet bucket daily.

After starting the pilot plant, the bacterial suspension follows the reflux route, and the bacterial strain in the reaction column is added using the hanging drop method over a 2-day cycle. A mixture of 80 cm³ of 99% acetic acid (carbon source) and 4.2 g of dipotassium hydrogen phosphate (phosphorus source) was added to the inlet bucket daily.

To operate the device, water is injected from the regulating tank into the inlet bucket, simulating the project situation and following the water flow route, with continuous operation for 15 days. The pH, COD, total phosphorus (TP), total nitrogen (TN), and NH₄⁺-N were measured in water samples obtained from the inlet and outlet buckets daily.

Operating conditions in the engineering application. Bacterial enrichment: Domestic bacteria and fillers were collected from the pilot plant and evenly distributed into

10 one-ton barrels. The barrels were filled with regulating pool water, to which 300 cm³ of 99% acetic acid (carbon source) and 16 g of dipotassium hydrogen phosphate (phosphorus source) were added. The barrels were closed and incubated for 7 days. Fresh filler was added daily to support the growth of surface biofilms.

Engineering the hanging membrane. The single-stage BAF was divided into two grids, designated as group A and group B. The A1 and B1 pools were filled with water from the regulating pool, and the inlet and outlet water valves were closed. The A1 and B1 pools were injected with 5 t of filler and bacterial suspension, respectively. Then, 3 dm³ of 99% acetic acid (carbon source) and 160 g of dipotassium hydrogen phosphate (phosphorus source) were added, and the mixture was allowed to stand for 12 h. During the 5 days' biofilm formation period, 84 dm³ of 99% acetic acid and 1 kg of dipotassium hydrogen phosphate were added to each group of ponds daily to provide the carbon and phosphorus sources required for denitrifying bacteria. Engineering operation and debugging: After successfully constructing the hanging film, the inlet flow of the BAF was determined to be 6,000 m³/day according to the design. The pH, COD, TP, TN, and NH₄⁺-N levels of the influent and effluent were monitored daily, and acetic acid and phosphate were added as needed to maintain optimal water quality.

Analytical methods. The glass electrode method was used for pH determination [13], the dichromate method for COD determination [14], the ammonium molybdate spectrophotometric method for TP determination [15], the alkaline potassium persulfate elimination UV spectrophotometric method for TN determination [16], and the distillation-neutralization titration method for NH₄⁺-N determination [17].

The microbial community structure was analyzed for initial and postmigration flora. Bacterial samples were collected from the initial system (BAF-treated, RO-concentrated water, referred to as NGBIO) and the operationally stable target system (BAF-treated photovoltaic pickling wastewater, referred to as KHBIO). They were centrifuged at 10,000g for 10 min to pellet microbial biomass. Genomic DNA was extracted using the DNeasy PowerSoil Pro Kit (QIAGEN, Germany) according to the manufacturer's protocol, with an additional bead-beating step (5 min at 30 Hz) to enhance cell lysis. DNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), with A260/A280 ratios between 1.8 and 2.0 considered acceptable.

The V4 hypervariable region of the bacterial 16S rRNA gene was amplified using the universal primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Each 25 µL PCR reaction contained 12.5 µL of 2× KAPA HiFi HotStart ReadyMix (Roche, Switzerland), 1 µM of each primer, and 10–20 ng of template DNA. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 3 min; followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s; with a final extension at 72 °C for 5 min.

PCR products were purified using the AMPure XP bead system (Beckman Coulter, USA) and quantified with the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, USA).

Purified amplicons were pooled in equimolar amounts and sequenced on an Illumina NextSeq 2000 platform (Illumina, USA) using 2×150 bp paired-end chemistry at Biozeron Biotechnology Co., Ltd. (Shanghai, China) [18]. Raw sequence data were processed using QIIME2 (version 2023.5). After demultiplexing, primers were trimmed, and reads were quality-filtered (quality score ≥ 20), denoised, and merged using DADA2 to generate amplicon sequence variants (ASVs). Taxonomy was assigned against the Silva 138.1 rRNA database at a 99% similarity threshold. Sequences were clustered into operational taxonomic units (OTUs) at 97% similarity for downstream analysis of microbial community composition. Alpha diversity indices, including Shannon, Simpson, and Chao1, were calculated based on the OTU table using the “vegan” package in R.

3. RESULTS AND DISCUSSION

3.1. MICROBIAL MIGRATION

The influent wastewater quality and effluent requirements of the existing project (steel wastewater treatment) and the proposed project (photovoltaic wastewater treatment) were compared to evaluate feasibility (Table 1). Nitrates were the dominant pollutants in both steel and photovoltaic wastewater.

Table 1

The quality of the steel wastewater and the photovoltaic wastewater

Wastewater category	Parameter	Steel wastewater	Photovoltaic wastewater
Influent wastewater	pH	7–9	6–9
	COD, mg/dm ³	≤ 50	≤ 400
	NH ₄ ⁺ -N, mg/dm ³	≤ 20	≤ 35
	TN, mg/dm ³	≤ 50	≤ 80
	TP, mg/dm ³	≤ 1	≤ 6
	Nitrates, mg/dm ³	≤ 30	≤ 45
Required effluent wastewater	pH	7–9	6–9
	COD, mg/dm ³	≤ 50	≤ 350
	NH ₄ ⁺ -N, mg/dm ³	≤ 2	≤ 35
	TN, mg/dm ³	≤ 5	≤ 45
	TP, mg/dm ³	≤ 1	≤ 4
	Nitrates, mg/dm ³	≤ 3	≤ 10

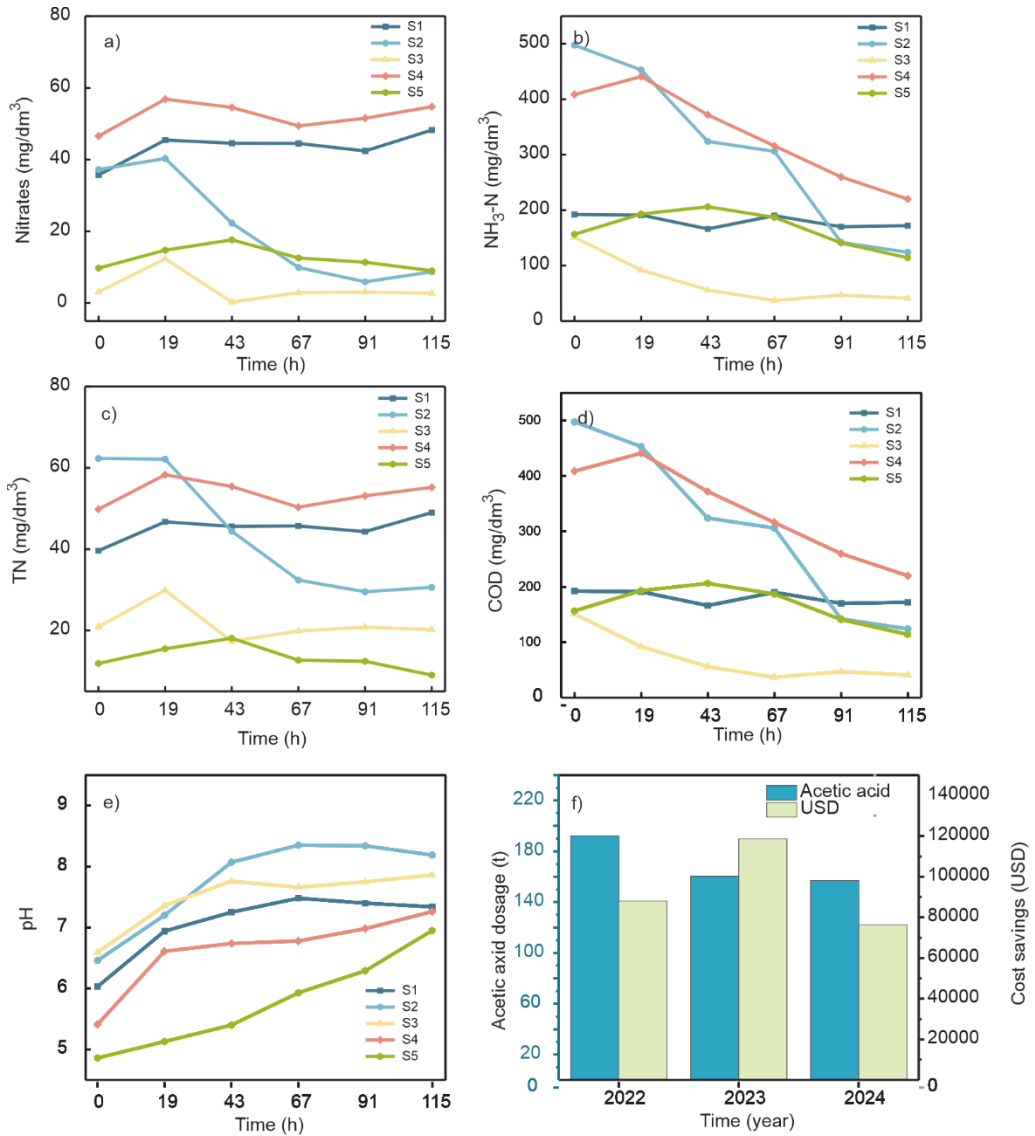


Fig. 3. Operation and maintenance costs in the existing project and the performance of the enriched microorganisms: a) nitrates, b) $\text{NH}_4^+\text{-N}$, c) TN, d) COD, e) pH, f) the cost expected to be saved

The predominant pollutants were nitrates, with peak TN values reaching 50 and 80 mg/dm³ in the two sewage sources, respectively. Additional carbon sources and trace elements were required in treating the steel wastewater because the organic pollutants (COD) and TP levels could not meet the growth needs of the bacterial population. In the

existing project, an average annual addition of 170 tons of acetic acid was required as an external carbon source from 2022 to 2024, which resulted in an average annual investment cost of approximately USD 94,000 (Fig. 3f). In contrast, the cost was expected to be saved as COD and TP were higher in photovoltaic wastewater than in steel wastewater. Although there are differences in the specific water quality of the two systems, their main pollutant categories and operating scenarios are comparable, which is sufficient to support the feasibility analysis of microbial migration. Therefore, microbial migration from the existing steel wastewater project to the proposed photovoltaic wastewater project is feasible and is expected to result in savings in operational and maintenance costs.

Prior to application in the pilot-scale reactor, the microorganisms were acclimatized and enriched using different carbon sources, and their denitrification performance was validated. The growth of the microorganisms in the suspension can be judged by visually observing its turbidity [19]. The microbial suspension enriched with “beef extract + peptone” was visually observed better than that enriched with “acetic acid + potassium nitrate” after 48 hours (Fig. 3a–e). The TN removal efficiency was 28.73% (from 62.3 to 44.4 mg/dm³) in S2 after 43 h of denitrification. The denitrification process led to a noticeable increase in pH. The decrease in COD confirmed that the consumption of the carbon source accompanied the denitrification process. By 67 h, the TN decreased to 32.4 mg/dm³ (with 22.5 mg/dm³ ammonium nitrogen), suggesting significant consumption of nitrate nitrogen. In contrast, the TN remained 50.3 mg/dm³ in S4 after 67 h. We therefore verified that beef extract and peptone are the optimal carbon sources for the enrichment and denitrification performance of denitrifying microorganisms.

3.2. PERFORMANCE AFTER MICROBIAL MIGRATION

The enriched microbial suspension, supplemented with beef extract and peptone, was used as the inoculum in the pilot-scale reactor. Six days after the inoculation, the nitrates removal efficiency achieved 73.15% (the outlet concentration was 12.45 mg/dm³ when the inlet concentration was 46.37 mg/dm³) (Fig. 4b). Concurrently, the denitrification process was accompanied by carbon source consumption, with a COD removal efficiency of approximately 55% (Fig. 4c). All indicators met the designated effluent requirements and local standards after the 15th day of operation in the pilot-scale reactor. These results provided further evidence on the feasibility of microbial migration.

The enriched microorganisms were inoculated into the BAF of the photovoltaic sewage treatment plant (Fig. 5). The TN removal efficiency was stably maintained at values exceeding 50%, along with the nitrate’s removal efficiency exceeding 80%, after a 3-day startup period. The average effluent concentrations of NH₄⁺-N, TN, COD, and TP from the BAF (Fig. 6) were 23.5, 33.5, 52.5, and 0.7 mg/dm³, respectively, consistent with designated effluent requirements (NH₄⁺-N < 35 mg/dm³, TN < 45 mg/dm³, COD < 340 mg/dm³, and TP < 4 mg/dm³). Fluctuations and a decline in removal efficiencies were

observed after 6 months of stable operation (in July) (Fig. 5) due to the decreased influent loads resulting from the partial shutdowns and production reductions of the industry.

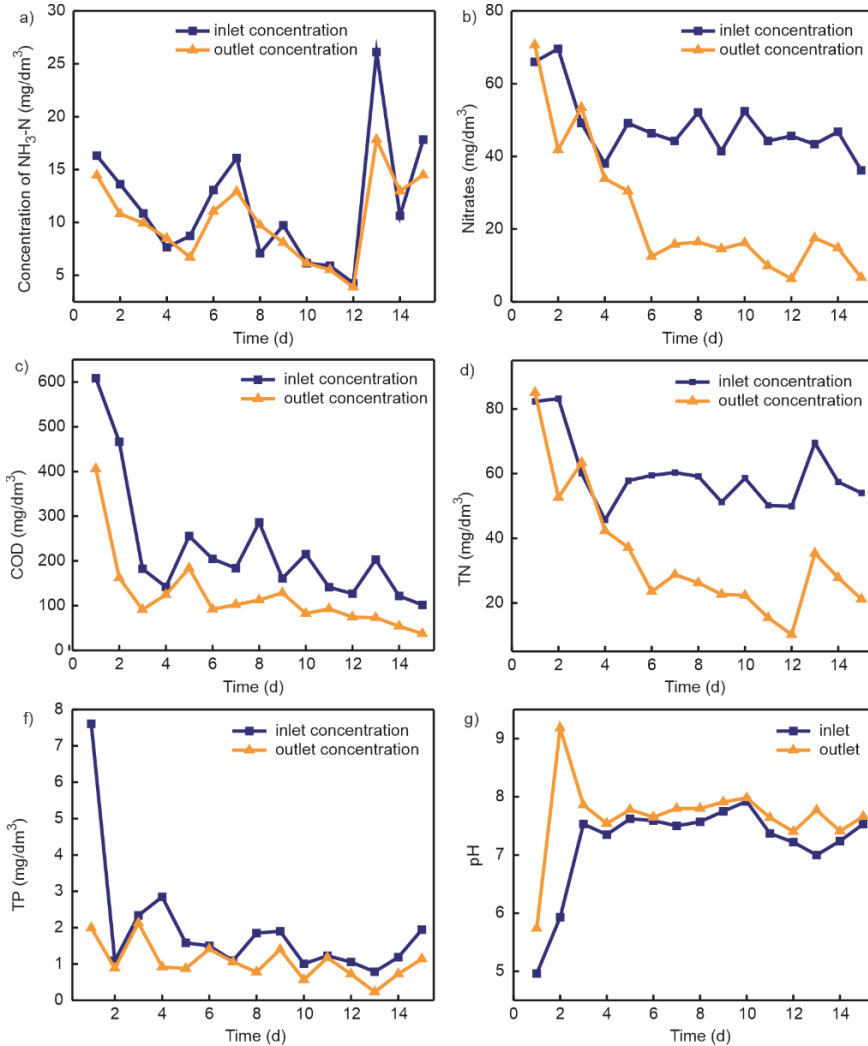


Fig. 4. Performance of the pilot-scale reactor with the migration of the enriched microorganisms: a) $\text{NH}_4^+\text{-N}$, b) nitrates, c) COD, d) TN, e) TP, f) pH

No external carbon source was required, resulting in savings in operation and maintenance costs compared with the steel sewage treatment plant. These results indicated that successful microbial migration resulted in efficient wastewater purification. In addition, the microorganisms were sensitive to fluctuations in the influent loads, demonstrating the importance of feasibility analysis and verification before microbial migration.

Given that over 500,000 sewage treatment plants have already been constructed globally, which process more than 400 billion cubic meters of wastewater annually, the extensive application of BAFs has led to the establishment of an abundant and diverse library of functional microbial communities for various types of wastewater.

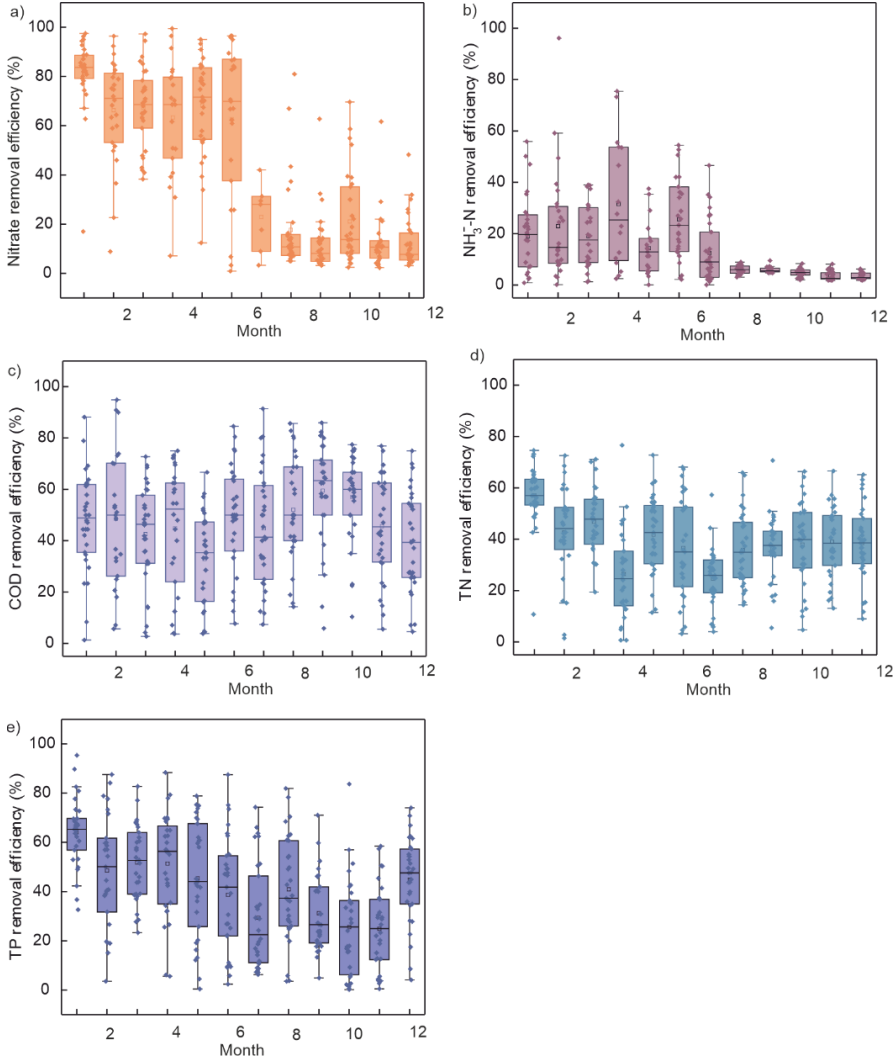


Fig. 5. Removal efficiencies of: a) nitrates, b) $\text{NH}_4^+\text{-N}$, c) COD, d) TN, e) TP

This study demonstrated the feasibility of using existing engineering projects as microbial community repositories and achieving cost-effective and efficient proposed projects through microbial migration. Bridging the information gap between different waste-

water treatment projects can enable effective microbial migration, which holds significant importance for reducing costs and enhancing efficiency in the construction of new wastewater treatment plants.

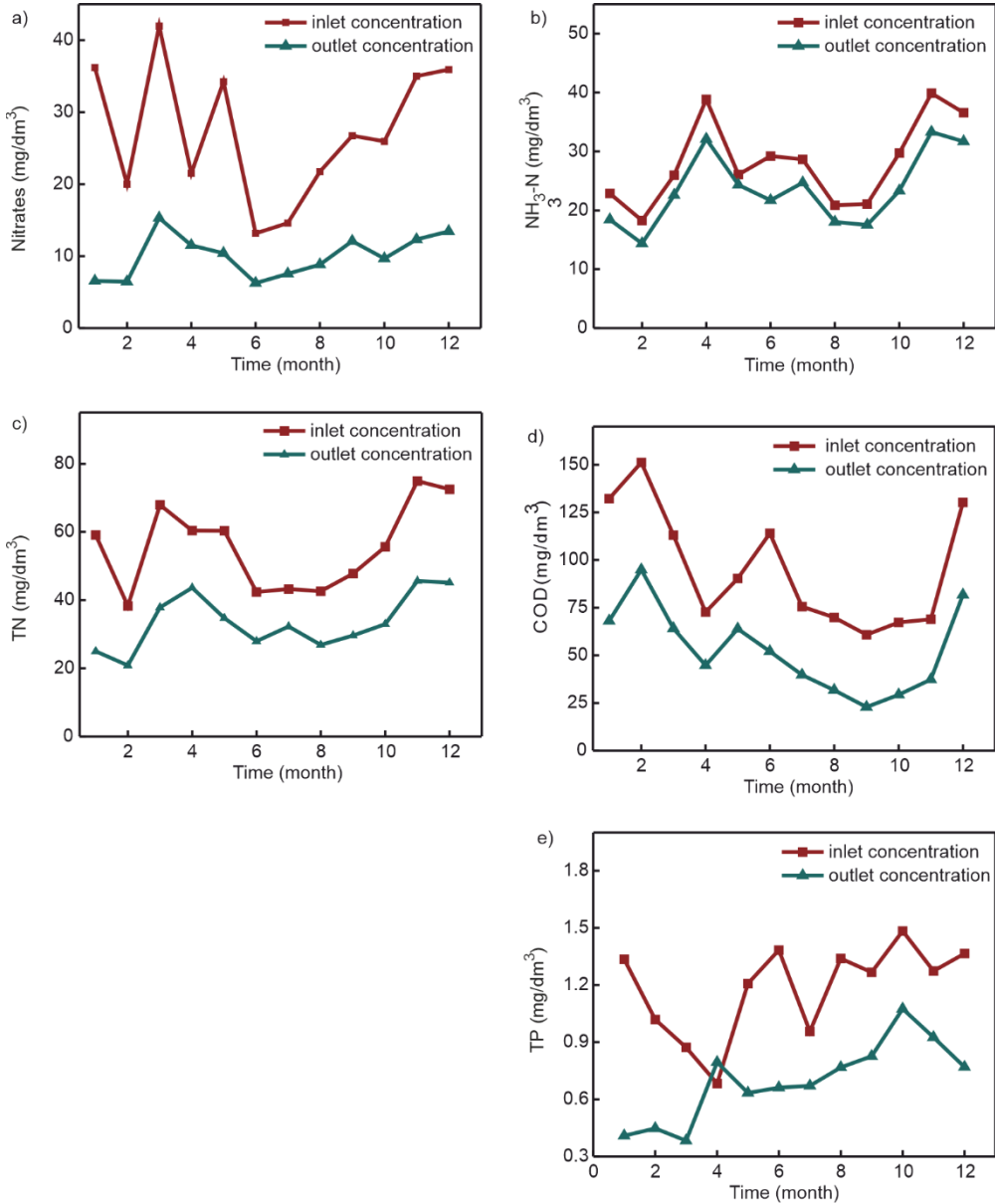


Fig. 6. Monthly average effluent concentrations including nitrates (a), $\text{NH}_4^+\text{-N}$ (b), TN (c), COD (d), and TP (e)

Fig. 7. Characteristics of the microbial communities of NGBIO and KHBIO; the phylogenetic tree was constructed at the OTU level using the 50 most abundant OTUs; from inner to outer: genus-level annotation, OTU abundance, and OTU presence/absence in KHBIO and NGBIO. The percent stacked column chart in the top right corner shows the microbial composition at the phylum level

At the OTU level, the most abundant OTUs in NGBIO belonged to the family Xanthomonadaceae and the genus *Thauera*. In contrast, in KHBIO, the most abundant OTUs belonged to the genera *Thauera* and *Flavobacterium*. *Thauera* are important denitrifying and phosphate-accumulating bacteria that can be used for removing nitrogen and phosphorus from wastewater [20]. Their higher abundance in KHBIO than in NGBIO suggested that the dominance of denitrifying bacteria in the migrated microbial community was promoted, perhaps due to the high nitrate concentration in the photovoltaic wastewater [21]. *Thauera* can adapt to various oxygen environments and regulate nitrate metabolism by adjusting the respiratory electron transport chain. In addition, the second-most abundant OTU in KHBIO belonged to the genus *Flavobacterium*, which is also important in the nitrogen cycle [22]. Half of the species in this genus possess the ability to convert nitrate to nitrite. Therefore, the presence of these two highly abundant denitrifying OTUs ensured enhanced denitrification capacity in the migrated system. NGBIO also contained an OTU from the family Xanthomonadaceae, which are typical environmental microorganisms with high hydrocarbon degradation capacity. These results indicated that the migration and acclimatization processes ensured the adaptability of the microbial community to the environment in the photovoltaic wastewater treatment system.

Furthermore, the successful migration and high activity of key genera such as *Thauera* and *Flavobacterium* from a high-salinity RO concentrate (TDS ca. 6500 mg/dm³) to a lower-salinity photovoltaic wastewater system warrant discussion regarding osmotic adaptation. A sudden decrease in external salinity can typically induce osmotic shock in microorganisms, leading to water influx, cell swelling, and potential lysis, thereby impairing microbial activity and community stability. However, in this study, no significant performance decline or community collapse was observed during or after migration. This resilience may be attributed to several underlying mechanisms. First, the source microbial community was enriched from an industrial BAF that routinely experienced fluctuations in TDS and ionic composition, potentially selecting for strains with robust osmoregulatory capabilities. Notably, members of the genus *Thauera* have been documented in various habitats with fluctuating salinities and often possess adaptive strategies such as the synthesis and accumulation of compatible solutes (e.g., glycine betaine, ectoine) to maintain cytoplasmic turgor pressure under osmotic stress. Second, the acclimatization process prior to migration – using complex organic carbon sources (beef extract and peptone) – likely provided not only nutrients for growth but also precursors for Osmo protectant synthesis, preconditioning the community to environmental change. Finally, the target photovoltaic wastewater, while lower in overall salinity, still contained a considerable ion load and organic matter, which may have mitigated the osmotic gradient compared to a freshwater environment. The absence of severe osmotic shock in this cross-system migration highlights the importance of pre-adaptation and functional selection in microbial resource banking. It also suggests that for engineering applications, salinity disparity alone may not be a prohibitive barrier if the inoculum source harbors physiologically versatile taxa and is subjected to appropriate acclimatization protocols.

4. CONCLUSION

The application and mechanisms of microbial migration were investigated in two different engineering projects. The considerable similarity in water characteristics between the existing and proposed projects demonstrated the feasibility of microbial migration. Beef extract and peptone were identified as the optimal external carbon sources in microbial acclimatization, and they enhanced the denitrification capacity of the bacterial community. The effluent parameters met the design requirements after the enriched microorganisms were applied in the pilot-scale reactor. On this basis, the performance of the BAF was explored using the enriched microorganisms as the inoculum in a proposed project treating photovoltaic wastewater. The concentrations of $\text{NH}_4^+\text{-N}$, TN, COD, and TP in the effluent met the design requirements. The bacterial genera involved in denitrification and phosphate accumulation, such as *Thauera*, as well as those involved in the nitrogen cycle, including *Flavobacterium*, were enriched during acclimatization and migration. This study demonstrated the feasibility of using existing engineering projects as microbial community repositories and achieving cost-effective and efficient new projects through microbial migration. It also provides theoretical guidance for the efficient purification of wastewater.

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REFERENCES

- [1] JONES E.R., BIERKENS M.F., VAN VLIET M.T.H., *Current and future global water scarcity intensifies when accounting for surface water quality*, Nat. Clim. Change, 2024, 14 (6), 629–635. DOI: 10.1038/s41558-024-02007-0.
- [2] JONES E.R., VAN VLIET M.T.H., QADIR M., BIERKENS M.F.P., *Country-level and gridded estimates of wastewater production, collection, treatment and reuse*, Earth Sys. Sci. Data, 2021, 13 (2), 237–254. DOI: 10.5194/essd-13-237-2021.
- [3] YANG F., WANG S., LI H., WANG G., WANG Y., YANG J., CHEN Y., YAN P., GUO J., FANG F., *Differences in responses of activated sludge to nutrients-poor wastewater: Function, stability, and microbial community*, Chem. Eng., 2023, 457, 141247. DOI: 10.1016/j.cej.2022.141247.
- [4] SALEH-LAKHA S., SHANNON K.E., HENDERSON S.L., GOYER C., TREVORS J.T., ZEBARTH B.J., BURTON D.L., *Effect of pH and temperature on denitrification gene expression and activity in Pseudomonas mandelii*, Appl. Environ. Microbiol., 2009, 75 (12), 3903–3911. DOI: 10.1128/AEM.00080-09.
- [5] JIANG L., LI J., KANG D., WANG H., ZHANG L., *Unraveling biological ammonium oxidation in toxic petrochemical wastewater treatment: A metagenomic exploration with practical implications*, ACS ES&T Eng., 2025, 5 (8), 2100–2107. DOI: 10.1021/acsestengg.5c00188.
- [6] OMIL F., LENS P., VISSER A., HULSHOFF POL L.W., LETTINGA G., *Long-term competition between sulfate-reducing and methanogenic bacteria in UASB reactors treating volatile fatty acids*, Biotechnol. Bioeng., 1998, 57 (6), 676–85. DOI: 10.1002/(SICI)1097-0290(19980320)57:6<676::AID-BIT5>3.0.CO;2-I.

- [7] BLACKBURN R., VADIVELU V.M., YUAN Z., KELLER J., *Kinetic characterisation of an enriched Nitrospira culture with comparison to Nitrobacter*, Water Res., 2007, 41 (14), 3033–3042. DOI: 10.1016/j.watres.2007.01.043.
- [8] TAYLOR A.E., TAYLOR K., TENNIGKEIT B., PALATINSZKY M., STIEGLMEIER M., MYROLD D.D., SCHLEPER C., WAGNER M., BOTTOMLEY P.J., *Inhibitory effects of C₂ to C₁₀ 1-alkynes on ammonia oxidation in two Nitrososphaera species*, Appl. Environ. Microbiol., 2015, 81 (6), 1942–1948. DOI: 10.1128/AEM.03688-14.
- [9] FANG Z., HUANG Y., TANG S., FAN Q., ZHANG Y., XIAO L., YONG Y., *Direct interspecies electron transfer for environmental treatment and chemical electrosynthesis: A review*, Environ. Chem. Lett., 2024, 22 (6), 3107–3133. DOI: 10.1007/s10311-024-01774-8.
- [10] POTTS L.D., DOUGLAS A., PEREZ CALDERON L.J., ANDERSON J.A., WITTE U., PROSSER J.I., GUBRY-RANGIN C., *Chronic environmental perturbation influences microbial community assembly patterns*, Environ. Sci. Technol., 2022, 56 (4), 2300–2311. DOI: 10.1021/acs.est.1c05106.
- [11] REVA O.N., WEINEL C., WEINEL M., BÖHM K., STJEPANDIC D., HOHEISEL J.D., TÜMMLER B., *Functional genomics of stress response in Pseudomonas putida KT2440*, J. Bacteriol., 2006, 188 (11), 4079–4092. DOI: 10.1128/jb.00101-06.
- [12] PATIL S.M., SURYAVANSHI M.V., CHANDANSHIVE V.V., KURADE M.B., GOVINDWAR S.P., JEON B., *Regeneration of textile wastewater deteriorated microbial diversity of soil microcosm through bioaugmentation*, Chem. Eng. J., 2020, 380, 122533. DOI: 10.1016/j.cej.2019.122533.
- [13] DAVISON W., WOOF C., *Performance tests for the measurement of pH with glass electrodes in low ionic strength solutions including natural waters*, Anal. Chem., 1985, 57 (13), 2567–2570. DOI: 10.1021/ac00290a031.
- [14] ZHANG S., CHEN W., LIU Y., LUO P., GU H., *A modified method for the accurate determination of chemical oxygen demand (COD) in high chloride oilfield wastewater*, Open J. Yangtze Oil Gas, 2018, 3 (4), 263–77. DOI: 10.4236/ojogas.2018.34023.
- [15] ADELOWO F.E., OLADEJI S.O., *Spectrophotometric analysis of phosphate concentration in agricultural soil samples and water samples using molybdenum blue method*, Brazilian J. Biol. Sci., 2016, 3 (6), e241. DOI: 10.21472/bjbs.030616.
- [16] ZHANG N., LIU Z., GUO Y., SUN H., *Influential factors and related countermeasures of the determination of TN in mine leaching wastewater*, Ind. Water Treat., 2016, 36 (5), 102–105. DOI: 10.11894/1005-829x.2016.36 (5).102.
- [17] SAHA U.K., SONON L., BISWAS B.K., *A comparison of diffusion-conductimetric and distillation-titration methods in analyzing ammonium- and nitrate-nitrogen in the KCl-extracts of Georgia soils*, Commun. Soil Sci. Plant Anal., 2018, 49 (1), 63–75. DOI: 10.1080/00103624.2017.1421647.
- [18] SUNAGAWA S., COELHO L.P., CHAFFRON S., KULTIMA J.R., LABADIE K., SALAZAR G., DJAHANSCHIRI B., ZELLER G., MENDE D.R., *Structure and function of the global ocean microbiome*, Science, 2015, 348 (6237), 1261359. DOI: 10.1126/science.1261359.
- [19] BLACK C., AL MAHMUD H., HOWLE V., WILSON S., SMITH A.C., WAKEMAN C.A., *Development of a polymicrobial checkerboard assay as a tool for determining combinatorial antibiotic effectiveness in polymicrobial communities*, Antibiotics, 2023, 12 (7), 1207. DOI: 10.3390/antibiotics12071207.
- [20] REN T., JIN X., DENG S., GUO K., GAO Y., SHI X., XU L., BAI X., SHANG Y., JIN P., *Oxygen sensing regulation mechanism of Thauera bacteria in simultaneous nitrogen and phosphorus removal process*, J. Cleaner Prod., 2024, 434, 140332. DOI: 10.1016/j.jclepro.2023.140332.
- [21] LIU F., WANG S., ZHANG X., QIAN F., WANG Y., YIN Y., *Nitrate removal from actual wastewater by coupling sulfur-based autotrophic and heterotrophic denitrification under different influent concentrations*, Water, 2021, 13 (20), 2913. DOI: 10.3390/w13202913.
- [22] CHANG M., WANG Y., PAN Y., ZHANG K., ZHU T., *Nitrogen removal from wastewater via simultaneous nitrification and denitrification using a biological folded non-aerated filter*, Biores. Technol., 2019, 289, 121696. DOI: 10.1016/j.biortech.2019.121696.