

Sensor-guided Real-time Monitoring of Microbial Succession in Anaerobic Baffled Reactors

Limei Jiang,^{1,2} Wei Sun,^{2,3*} Yinbo Xiang,^{2,3}
Zihan Chen,^{2,3} Lifeng Zheng,^{2,3} and Zhongzhen Li³

¹School of Environmental Science and Engineering, Guangdong University of Petrochemical Technology,
Maoming, Guangdong 525000 China

²Guangdong Provincial Key Laboratory of Green and Intelligent Agricultural Production,
Maoming, Guangdong 525000, China

³College of Biology and Food Engineering, Guangdong University of Petrochemical Technology,
Maoming, Guangdong 525000, China

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This study was carried out to explore how sensors can be applied to monitor and predict microbial succession in anaerobic baffled reactors (ABRs) that treat high-strength agricultural wastewater. A laboratory-scale ABR equipped with pH sensors, oxidation–reduction potential (ORP) sensors, ion-selective electrodes, and dissolved oxygen sensors was operated through four phases: baseline, nitrogen loading, wastewater introduction, and recovery. Sensor data showed that the nitrogen removal rates of the ABR increased from 90.2% ($\text{NH}_4^+\text{-N}$) and 99.8% ($\text{NO}_2^-\text{-N}$) at moderate loading to 96.1 and 100% under peak loading ($0.289 \text{ kg/m}^3\cdot\text{d}$). For organic material introduction, removal rates decreased significantly [73.4% of $\text{NH}_4^+\text{-N}$, 66.1% of $\text{NO}_2^-\text{-N}$, and 60.3% of chemical oxygen demand (COD)], coinciding with negative ORP changes and NO_2^- accumulation. Recovery was captured in real time, with efficiencies increased to 94.2, 100, and 94.1% for $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and COD, respectively. Ribosomal RNA sequencing results showed microbial changes. Operational taxonomic unit richness decreased to 31159 in the wastewater introduction phase but increased to 38564 in the recovery phase. The thriving of *Candidatus Kuenenia* was suppressed and replaced by heterotrophic *Limnobacter*. The correlation between sensor data and microbial diversity indices showed that sensors can be used as proxies for ecological transitions. The results of this study serve as a reference for the sensor-data-based diagnostics and prediction of microbial diversity changes, enabling bioreactors to achieve resilient, automated nitrogen removal under variable organic shocks.

1. Introduction

With the rapid growth of the livestock and aquaculture industries, the management of highly concentrated wastewater has become a critical issue to address. In particular, effluents are

*Corresponding author: e-mail: sunwei@gdupt.edu.cn
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characterized by fluctuating, high-strength chemical oxygen demand and elevated ammonium (NH_4^+) levels, posing serious challenges to aquatic ecosystems. Traditional biological nitrogen removal processes are energy-intensive, requiring continuous external organic carbon dosing for denitrification.⁽¹⁾ To overcome the economic and operational limitations in traditional processes, anaerobic ammonium oxidation (anammox) technology has been widely used owing to its autotrophic nature and the omission of aeration and external carbon sources.^(2,3)

However, the slow doubling time for functional microbial growth, such as anammox bacteria, complicates reactor operations and makes the operation vulnerable to organic or volatile toxic shocks. To optimize reactor performance and stability, anaerobic baffled reactors (ABRs) are used.⁽⁴⁾ The structure of an ABR enables hydrodynamic stages that naturally segregate microbial communities, offering superior biomass retention and resistance to shock loads.⁽⁵⁾ Despite these structural advantages, the high-precision monitoring of chemical parameters, such as pH, oxidation–reduction potential (ORP), and nitrogen species concentrations, is required to maintain an optimal operational balance in each ABR compartment.⁽⁶⁾ In traditional monitoring methods, manual sampling and destructive chemical assays are used, which introduce significant time delays and prevent the automated, real-time feedback and control of hydraulic retention times (HRTs) or influent dilution ratios.⁽⁷⁾ To solve such problems, electrochemical, optical, and state-variable sensors are adopted for automated biological process monitoring.⁽⁸⁾ By implementing sensors in the ABR system, changes in chemical parameters are continuously monitored, and indicators of microbial inhibition (volatile fatty acid accumulation or nitrite toxicity) are identified before an irreversible system failure occurs.⁽⁹⁾ Although sensors are widely implemented in large-scale municipal wastewater facilities, the application of multichannel sensor data to monitor microbiome distributions in multistage anaerobic processes in the ABR system is scarce. Specifically, it is necessary to explore how sensor data loops can be utilized to enhance the functional transition between autotrophic anammox activity and heterotrophic denitrification during variable organic substrate shocks.

Previous research has proven the utility of online sensors in conventional activated sludge systems,^(10,11) but multichannel sensor networks have not been integrated to monitor compartment-specific microbiome successions in ABRs. In this study, by implementing real-time electrochemical sensors for micro-scale functional microbial transitions under sudden organic loading, macroscale physical diagnostics are conducted. In addition, a multistage, sensor-monitored ABR process was developed in this study to treat wastewater. The process comprises unacclimated baseline, nitrogen loading acceleration, organic/wastewater introduction perturbation, and sensor-guided autotrophic activity recovery phases. The developed process was implemented to explore how sensor data (pH/ORP/nitrogen species concentrations) can be used to enhance denitrification and anammox. By coupling sensor data with the high-throughput molecular sequencing of microbial activities, functional bacterial groups were identified. The results of this study provide an engineering principle for the design of smart, sensor-based biological reactors capable of automated nitrogen removal from agricultural wastewater.

2. Materials and Methods

2.1 System architecture

The experimental system was constructed, including a laboratory-scale ABR fabricated from acrylic glass, divided into six compartments. The reactor's dimensions were 90 cm in length, 30 cm in width, and 45 cm in height, with each compartment having an effective volume of 6.75 L, giving a total working volume of 40.5 L. Each compartment contained an upflow and downflow chamber separated by baffles to ensure staged hydrodynamics and biomass retention (Fig. 1).^(12,13) The reactor was covered with opaque material to maintain dark conditions, and influent was supplied via a peristaltic pump to control HRT.

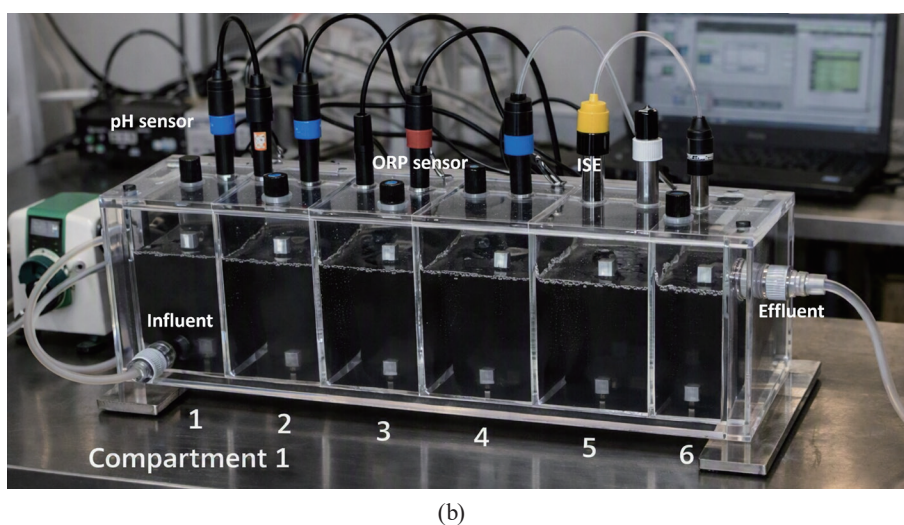
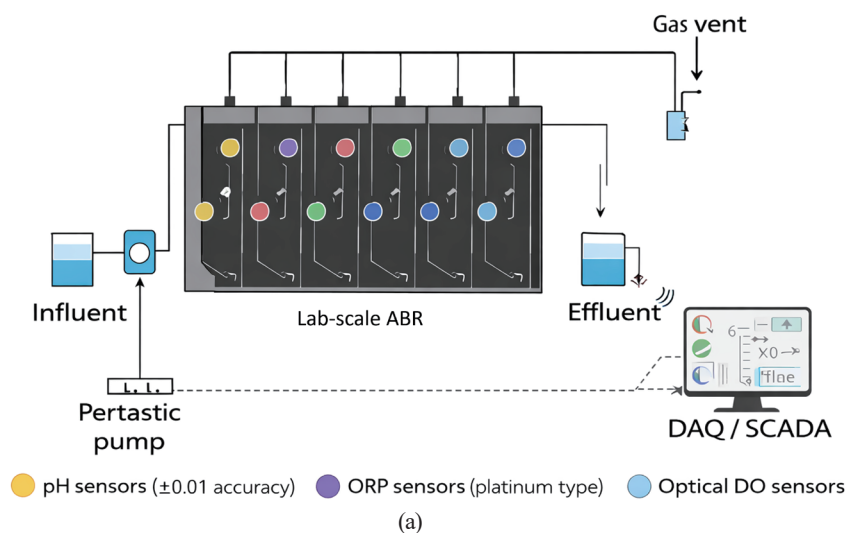


Fig. 1. (Color online) (a) Laboratory-scale ABR with six compartments, influent/effluent flow, gas vent, and integrated sensors connected to DAQ/SCADA platform and (b) photograph of ABR.

To enable real-time monitoring and automated control, the ABR was equipped with the following sensors (Table 1): pH sensors (electrochemical probes, ± 0.01 accuracy) were installed in each compartment to monitor acid–base balance.⁽¹⁴⁾ ORP sensors (platinum electrode type) were used to obtain redox state information, which is critical for distinguishing anammox versus denitrification activity.⁽¹⁵⁾ Ion-selective electrodes (ISEs) were used to measure NH_4^+ , nitrite (NO_2^-), and nitrate (NO_3^-) concentrations. Optical dissolved (OD) oxygen sensors were used to monitor strict anaerobic conditions.⁽¹⁴⁾

All sensors were connected to a National Instruments USB-6009 data acquisition (DAQ) module, which provided 14-bit resolution and multichannel input capability. DAQ was integrated with a supervisory control and data acquisition (SCADA) platform developed in LabVIEW 2020, enabling wireless data transmission, the real-time visualization of compartment-specific chemical profiles, and the automated adjustment of influent dilution or HRT. The SCADA interface included alarm functions for inhibitory signals such as nitrite accumulation and ORP changes, ensuring proactive operational control.^(15,16)

2.2 Sensor calibration and long-term stability

To ensure temporal stability across the multimonth operational phases, pH, ORP, and DO sensors and ISEs underwent routine maintenance and recalibration protocols. Glass pH and platinum ORP electrodes were recalibrated bi-weekly using standard buffer solutions (pHs 4.01, 7.00, and 10.01) and an ORP reference solution of +220 mV with Ag/AgCl. The optical DO sensors were zero-point and air-calibrated monthly. To reduce signal drift and surface biofouling caused by continuous exposure to anaerobic sludge, ISE probes were calibrated weekly, with automated physical rinsing with deionized water followed by baseline drift correction against standard chemical assays.⁽¹⁷⁾ Calibrations were conducted to maintain signal drift within $\pm 3\%$, ensuring that sensor measurements were reliable.⁽¹⁸⁾

2.3 Materials

Activated sludge was collected from the secondary sedimentation tank of Longyan City Longjin Wastewater Treatment Plant, China. Sixty percent of the reactor volume was filled with

Table 1
Specifications of sensors used in ABR monitoring system.

Measurement device	Measurement mechanism	Model	Manufacturer	Country
pH sensors	Potentiometric glass electrode	EasyFerm Bio HB K8 225	Hamilton Bonaduz AG	Switzerland
ORP sensors	Platinum redox combination electrode	InPro 3253i/SG/225	Mettler-Toledo International Inc.	Switzerland
ISE sensors (NH_4^+ , NO_2^- , NO_3^-)	Potentiometric ion-selective membrane	CS5520 Series	Clean Instruments Co., Ltd.	China
Dissolved oxygen sensors	Optical luminescence quenching	ROX 605	YSI Incorporated/Xylem Incorporated	United States

this inoculum to ensure microbial diversity.⁽¹⁹⁾ The synthetic wastewater was prepared, containing ammonium sulfate and sodium nitrite as nitrogen sources, bicarbonate as a buffer, and trace elements to support microbial growth (Table 2).⁽²⁰⁾

The ABR was initially operated at an HRT of 48 h. Nitrogen load was increased by the stepwise addition of NH_4^+ and NO_2^- salts, and sensor data were used to maintain stable redox balance and microbial activity.⁽¹⁴⁾ The nitrogen load was increased from 0.014 to 0.289 $\text{kg/m}^3\cdot\text{d}$ for 150 days. Inhibitory nitrite accumulation was monitored using the sensors for the controlled adjustment of influent composition.⁽¹⁵⁾ Wastewater was introduced after ABR stabilization. Table 3 shows the composition of the wastewater used in the experiment: pH of 7.7–8.4, chemical oxygen demand (COD) of 135–492 mg/L, NH_4^+ -N of 170–260 mg/L, and NO_2^- -N of 70–355 mg/L. The monitoring of the composition using sensors was critical to detect organic shock loads and guide transitions between autotrophic anammox and heterotrophic denitrification.⁽¹⁶⁾

Table 2
Simulated wastewater prepared in experiment.

Chemical	Concentration (g/L)
NaHCO_3	1.25
$\text{MgSO}_4\cdot 7\text{H}_2\text{O}$	0.3
$\text{CaCl}_2\cdot \text{H}_2\text{O}$	0.136
KH_2PO_4	0.0255
$(\text{NH}_4)_2\text{SO}_4$	0.7
NaNO_2	0.75
Trace elements	1 mL of stock solution containing ethylenediaminetetraacetic acid (5.0 g/L) and $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ (5.0 g/L), 1 mL of stock solution containing $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ (0.43 g/L), $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ (0.24 g/L), $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$ (0.99 g/L), H_3BO_3 (0.314 g/L), $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ (0.25 g/L), and $\text{NiCl}_2\cdot 6\text{H}_2\text{O}$ (0.19 g/L)

Table 3
OTU number and alpha diversity index of each compartment in different operation phases of ABR.

Phase	Compartment	Number of OTUs	Chao1 richness estimator	Shannon diversity index
Phase I	Overall	38315	625	7.34
	1	28045	686	6.15
	2	28803	725	6.56
	3	54917	771	6.78
	4	46810	819	6.52
	5	39765	766	7.59
Phase II	6	24769	787	7.82
	1	29612	721	6.62
	2	32675	776	4.92
	3	32102	829	5.99
	4	31629	881	6.83
	5	25263	876	7.33
Phase III	6	35675	914	7.87
	1	35315	708	7.15
	2	33079	803	7.58
	3	31340	864	7.66
	4	65086	864	7.52
	5	32312	818	7.45
Phase IV	6	34254	820	7.47

The experiment was conducted in four phases. In the baseline phase, the chemical and microbial profiles of the unacclimated sludge were monitored using sensors to establish a baseline. In the nitrogen loading phase, nitrogen concentration was increased, and HRT was reduced, with sensors guiding the adjustments to ensure stable reactor performance. In the wastewater introduction phase, real wastewater was introduced into the ABR system, and sensors were used to identify inhibition signals, such as NO_2^- accumulation and redox changes. Finally, in the recovery phase, synthetic wastewater was introduced to restore autotrophic activity.

Sensor data were validated through comparison with chemical assay results to ensure accuracy. These assays included measurements with a pH meter, a COD analyzer, and spectrophotometric methods for chemical and microbial profiles.⁽²⁰⁾ Through sensor monitoring and chemical validation, calibration accuracy and the reliability of the sensor readings were ensured.^(14,15) Sludge samples were collected from each compartment of the reactor and preserved at -80°C for subsequent analysis. DNA was extracted from the samples and sequenced using the Illumina MiSeq platform. The sensor data were correlated with microbial diversity indices, including both α -diversity and β -diversity, to identify functional transitions between anammox and denitrification communities.^(21,22)

2.4 Microbial analysis

To evaluate the spatial and temporal changes in microbial consortia across the compartments of the ABR, highthroughput 16S rRNA gene amplicon sequencing data were analyzed.⁽²³⁾ Microbial community α -diversity was quantified by using the number of operational taxonomic units (OTUs), the Chao1 richness estimator, and the Shannon diversity index. The number of OTUs is defined as the absolute count of unique sequence clusters grouped at a 97% nucleotide identity threshold, providing a direct inventory of taxonomic categories within each compartment. To account for rare or low-abundance species that may evade direct sequencing detection, the non-parametric Chao1 richness estimator was calculated as

$$S_{\text{Chao1}} = S_{\text{obs}} + \frac{n_1(n_1 - 1)}{2(n_2 + 1)}. \quad (1)$$

Here, S_{obs} is the number of directly observed OTUs, n_1 represents the number of singletons (OTUs appearing only once in the sequence pool), and n_2 represents the number of doubletons (OTUs appearing exactly twice). OTU and Chao1 richness estimators are used to estimate the total number of distinct taxonomic types (species richness), but they do not represent population distribution. Therefore, the Shannon diversity index (H') was calculated to integrate richness and evenness into a single heterogeneous structural metric.

$$H' = - \sum_{i=1}^{S_{\text{obs}}} p_i \ln p_i \quad (2)$$

Here, p_i is the proportion of total sequences belonging to the i -th OTU. Chao1 richness estimators and the Shannon diversity index are used to determine differentiation between a decrease in absolute species variety (richness) and an ecological shift toward dominance by specific heterotrophic functional groups (evenness). Such a differentiation is critical for interpreting how external substrate perturbations, such as high-strength organic introduction, affect the microbial community structure in the ABR.

To quantify the spatiotemporal variations and evolutionary distances of microbial community structures across the different operational phases and the compartments of ABR, nonmetric multidimensional scaling (NMDS) and principal coordinate analysis (PCoA) were conducted to calculate taxonomic distance matrices generated from normalized sequence abundance profiles. (20) NMDS and PCoA were applied to predict the multidimensional rank-order dissimilarity of samples into a simplified two-dimensional ecological space. To ensure the reliability of the spatial distances, the stress coefficient was monitored, with a strict threshold of <0.1 adopted to guarantee accurate ordination. Under this criterion, the geometric distances in the NMDS graph present taxonomic divergence between compartments, enabling sensor-detected gradients (ISE and ORP data) to be cross-validated against ecological clustering.

PCoA was used to extract the coordinate axes that accounted for maximal variation within the microbial population matrix. Eigenvalues along the first two principal axes were used to calculate the percentage of cumulative variance explained by dominant environmental constraints, such as organic loading rates or compartmental partitioning. The results are used for the interpretation of sensor-monitored data such as nitrogen concentration, redox potential, pH changes, and structured microbial community divergence. NMDS and PCoA were used to calculate abundance-weighted differences and evaluate whether the microbial community underwent localized spatial changes along compartments or transitioned into distinct ecological steady states.

Sensor data were used to calculate the microbial parameters and validate transitions at the microbial community level. The results were interpreted as biological phenomena and sensor-based monitoring in wastewater treatment systems.

3. Results

The nitrogen loading phase showed how the data from multichannel ISEs, pH electrodes, and COD analyzers were used to monitor reactor performance in real time. The removal rates of nitrogen species and the reduction rate of COD were calculated in operational phases.

$$R = \frac{C_{inf} - C_{eff}}{C_{inf}} \times 100 \quad (3)$$

Here, C_{inf} and C_{eff} represent the influent and effluent concentrations (mg/L), respectively. As the influent nitrogen loading rate increased from 0.014 to 0.150 kg/m³·d, the ISE sensors recorded nitrogen removal performance. Figure 2 shows that effluent ammonium (NH₄⁺-N) concentrations declined steadily, and the removal rate increased sharply to 90.2%, indicating

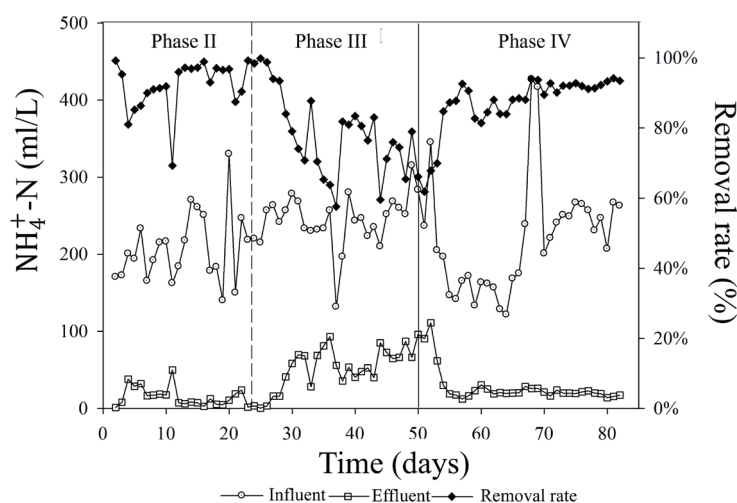


Fig. 2. $\text{NH}_4^+\text{-N}$ removal curve of ABR treating wastewater by using ISEs. Sensor signals reveal removal rate changes across nitrogen loading (phase II), wastewater introduction (phase III), and recovery (phase IV) phases.

efficient ammonium oxidation under increasing nitrogen stress. Figure 3 shows that nitrite ($\text{NO}_2^-\text{-N}$) removal rate reached 99.8%, indicating that the reactor maintained strong nitrite conversion capacity. Figure 4 shows that COD averaged 84.5%, suggesting that organic matter degradation remained stable even as nitrogen loading intensified.

When HRT was shortened and nitrogen load reached $0.289 \text{ kg/m}^3\cdot\text{d}$, the sensor data showed convergent kinetic curves, which are characteristic of balanced anammox activity. Under these conditions, the $\text{NH}_4^+\text{-N}$ removal rate increased to 96.1%, the $\text{NO}_2^-\text{-N}$ removal rate approached 100%, and COD reduction rates stabilized at 85.5%. The ISE data were used for the calculation of the stoichiometric ratio of consumed $\text{NH}_4^+\text{-N}$, consumed $\text{NO}_2^-\text{-N}$, and produced $\text{NO}_3^-\text{-N}$ (1:1.32:0.26), which matched the theoretical anammox reaction. This alignment indicates that sensor-based monitoring validates biochemical pathways without relying solely on offline assays. Continuous pH sensor data showed that effluent pH remained slightly higher than influent values in the threshold range of 6.5–8.0, validating that real-time acid–base monitoring effectively maintained reactor stability.

During the wastewater introduction phase, the system showed two distinct response profiles. In the first 30 days, ISE data indicated that $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ removal rates remained above 93%, but COD reduction rates decreased to 76.7%. At the same time, ORP sensors recorded negative voltage changes, indicating a shift toward more reduced conditions. This correlation between electrochemical signals and COD decrease showed that sensors detected substrate competition and metabolic transitions in real time.

In the subsequent 25 days, as influent $\text{NH}_4^+\text{-N}$ increased to 345 mg/L and COD to 470 mg/L, nitrogen removal rates fell to 73.4% ($\text{NH}_4^+\text{-N}$) and 66.1% ($\text{NO}_2^-\text{-N}$), and COD reduction rates decreased to 60.3%. These decreases in ISE and ORP data showed the trajectory of reactor destabilization under organic shock, providing an earlywarning capability for process inhibition.

In the recovery phase, synthetic wastewater was reintroduced to restore autotrophic activity. Over 30 days, sensor data showed a progressive recovery. $\text{NH}_4^+\text{-N}$ removal rates increased to

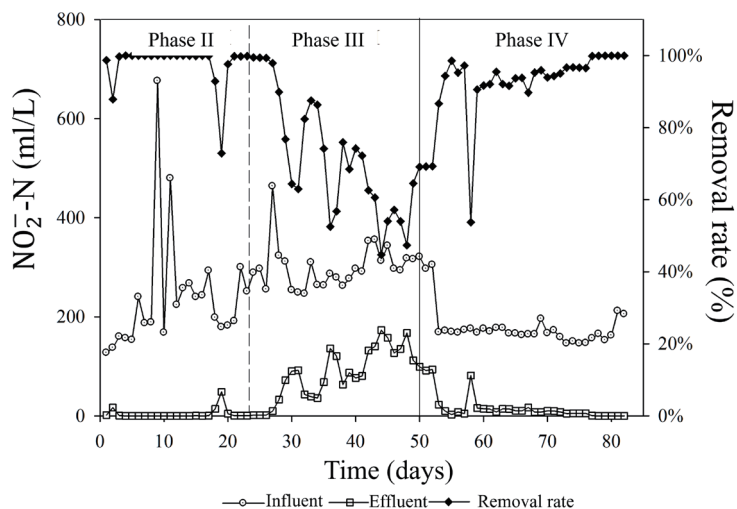


Fig. 3. NO_2^- -N removal curve of ABR treating wastewater by using ISEs. Sensor signals reveal removal rate changes across nitrogen loading (phase II), wastewater introduction (phase III), and recovery phases (phase IV).

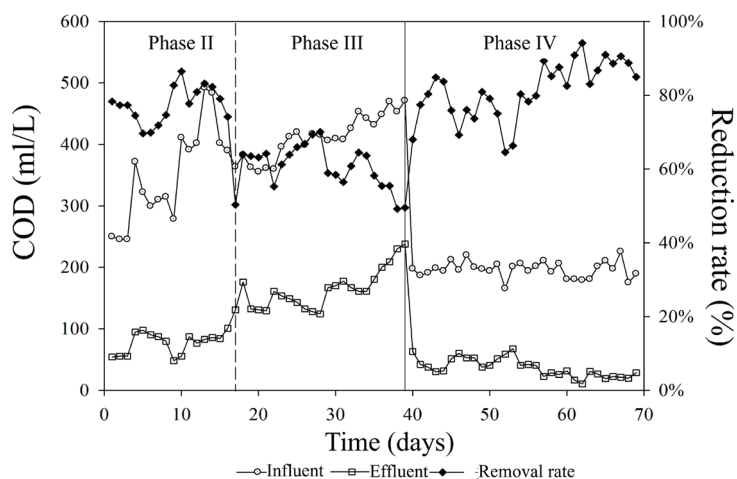


Fig. 4. COD removal curve of ABR treating wastewater (phase II: nitrogen loading phase, phase III: wastewater introduction phase, and phase IV: recovery phase).

88.9%, NO_2^- -N to 93.9%, and COD to 81.2%. At peak performance, efficiencies reached 94.2, 100, and 94.1%, respectively. These show that continuous sensor monitoring enables the monitoring of reactions in ABRs, indicating the role of sensor-guided control in maintaining stable nitrogen removal under fluctuating organic loads.

By monitoring NH_4^+ -N, NO_2^- -N, COD, pH, and ORP, early signs of destabilization, such as nitrite accumulation or negative redox shifts, can be detected before irreversible system failure. This enables proactive adjustments to hydraulic retention time or influent dilution, ensuring sustained reactor performance even under organic shock loads. Using sensor data, real-time monitoring is conducted to reduce reliance on labor-intensive offline assays. The experimental

results serve as a basis for the development of automated, resilient, and cost-effective nitrogen removal mechanisms and the design of smart bioreactors for agricultural wastewater treatment.

Table 3 and Figs. 5 and 6 show that the number of OTUs declined in the nitrogen loading and wastewater introduction phases. The alignment between sensor data (ISE, ORP, and pH) and biodiversity indices shows that sensor data are used to identify microbial richness trends, reducing reliance on frequent molecular assays.

The sequencing rarefaction curves showed that the observed number of OTUs approached an asymptote beyond 20000 reads per sample, confirming sufficient sequencing depth across all analyzed samples. This plateau validates that the biodiversity indices are robust and can be reliably correlated with real-time sensor data (ISE, ORP, and pH) without biases from undersampling (Fig. 5). The analysis of the number of OTUs of each ABR compartment in different operational phases showed strong spatial heterogeneity (Fig. 6). For instance, in Phase I, compartment 3 reached a localized maximum of approximately 55000 OTUs, whereas in Phase III, compartment 4 peaked at nearly 65000 OTUs. These localized maxima corresponded directly to sensor-detected gradients in nitrogen species concentrations ($\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$) and redox potential. The number of OTUs varied across reactor zones, demonstrating that multisensor arrays can serve as real-time proxies for identifying reactor compartments undergoing microbial community shifts or operational stress.

Overall, the number of OTUs decreased during the nitrogen loading phase (Phase II, averaging 37184 OTUs) and the wastewater introduction phase (Phase III, averaging 31159 OTUs). Correspondingly, the ISE data captured suppressed $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ removal rates, and ORP data showed destabilized redox reactions. In contrast, in the recovery phase (Phase IV), the average number of OTUs increased to 38564, with the Shannon diversity index stabilizing above 7.4. This ecological recovery was tracked in real time by the sensor network, which documented restored nitrogen removal rates and stabilized pH profiles.

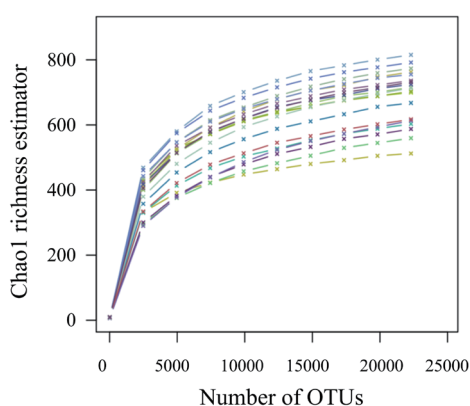


Fig. 5. (Color online) Rarefaction curves of observed OTUs across all phases (each dashed line represents data from six compartments in Phases II, III, and IV).

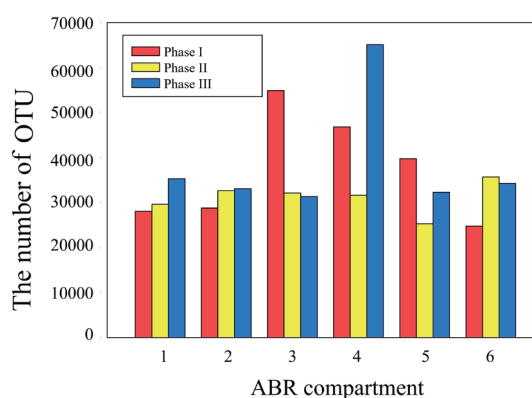


Fig. 6. (Color online) Average OTU in each ABR compartment in different operational phases (phase II: nitrogen loading phase, phase III: wastewater introduction phase, and phase IV: recovery phase).

NMDS and PCoA analysis results showed clustering patterns that matched sensor-detected operational changes (Figs. 7 and 8). NMDS1, NMDS2, PCoA1, and PCoA2 are made-up axes used to understand complex ecological data. By compressing many dimensions of information into these two axes, the samples in a two-dimensional space are plotted to easily observe patterns, similarities, or differences between microbial communities.

The nitrogen loading phase showed scattered results, reflecting sensor-detected gradients in nitrogen species. The samples collected in the wastewater introduction phase were clustered closely, consistent with sensor-detected homogenization under organic overload. The recovery phase presented stabilization. This shows that sensor data can be used to predict ecological clustering outcomes, bridging macroscale monitoring with microscale succession.

Across all phases, the ABR maintained a diverse phylum composition dominated by *Proteobacteria*, *Chloroflexi*, *Bacteroidetes*, *Acidobacteria*, *Actinobacteria*, and *Planctomycetes* (Fig. 9). At the class level, *Betaproteobacteria* increased considerably in the nitrogen loading phase up to 23.2%, reflecting heterotrophic denitrification under organic matter introduction, whereas *Alphaproteobacteria* peaked in the wastewater introduction phase (13.4%). *Planctomycetacia* showed a similar pattern to *Alphaproteobacteria*. The thriving of *Betaproteobacteria*, *Alphaproteobacteria*, and *Planctomycetacia* showed that the ABR created distinct spatiotemporal niches for them. The ABR sustained an autotrophic core (*Planctomycetacia*) during clean inorganic loading, which handed over metabolic dominance to resilient heterotrophic cohorts (*Betaproteobacteria* and *Alphaproteobacteria*) when perturbed by agricultural organic material introduction.

Three genera showed functional succession as follows. *Limnobacter* count increased in the nitrogen loading phase (7.44% on average), matching sensor-detected nitrite accumulation and ORP changes. *Burkholderia* and *Paraburkholderia* were dominant in the baseline phase, disappearing under wastewater stress. *Candidatus Kuenenia* enriched in the nitrogen loading

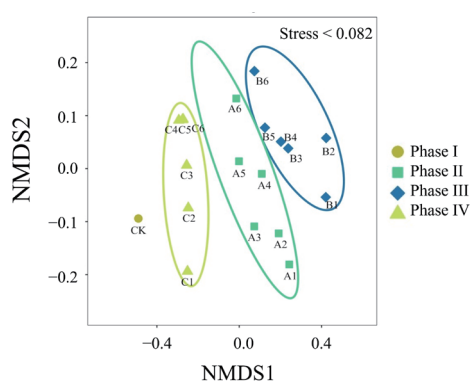


Fig. 7. (Color online) NMDS diagram of microbial community structures in ABR during different operational phases (phase II: nitrogen loading phase, phase III: wastewater introduction phase, and phase IV: recovery phase).

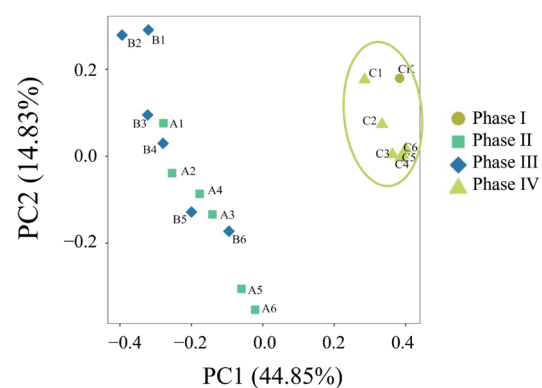


Fig. 8. (Color online) PCoA diagram of microbial community structures in the ABR during different operational phases.

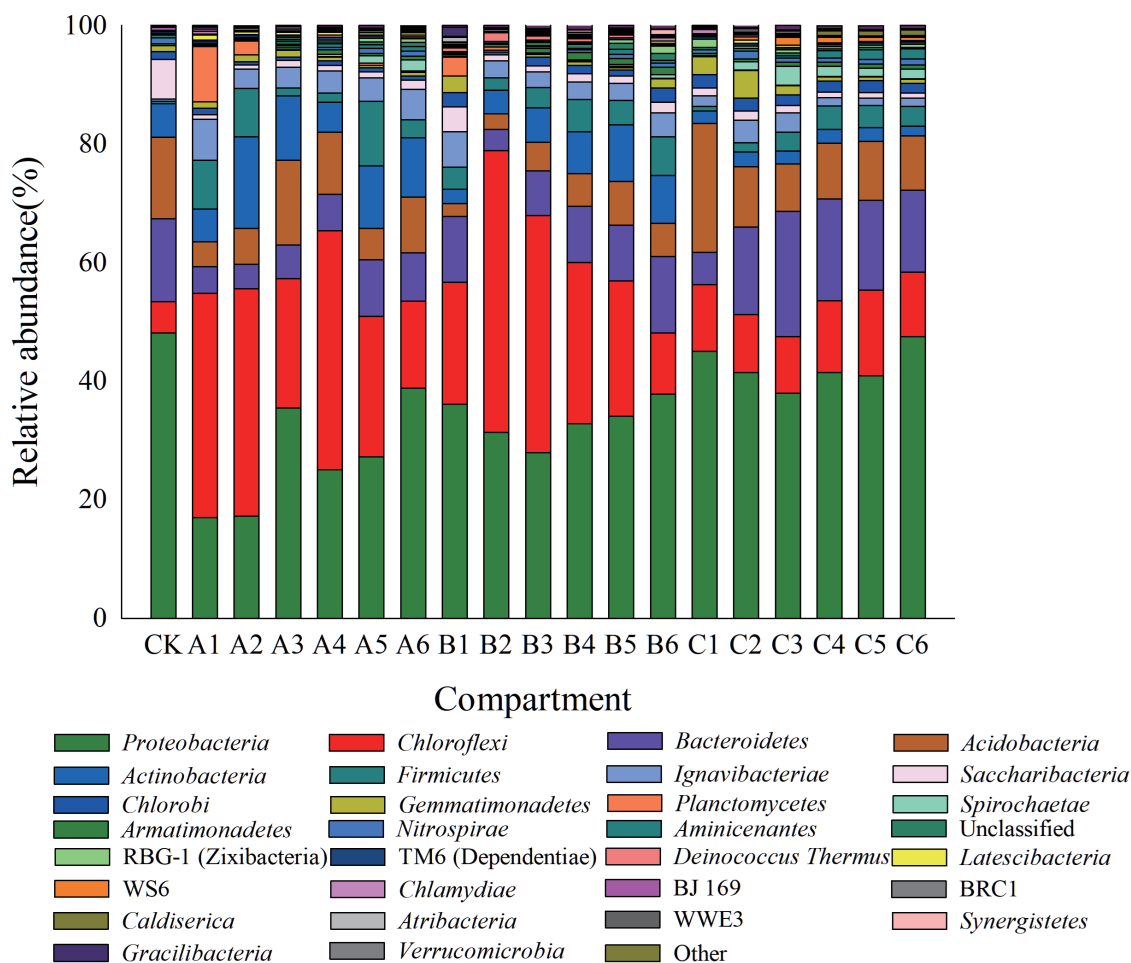


Fig. 9. (Color online) Relative percentage composition of bacterial communities at the phylum level in each ABR compartment during different operational phases.

phase (9%), confirming successful anammox start-up, but was suppressed in Phase B and absent in Phase C owing to slow recovery. Figure 10 shows a direct link between sensor-detected nitrogen removal rates and genus-level functional changes. The results showed how the bacterial community of the ABR changes its dominant species to survive different biochemical conditions, swapping out sensitive native strains for resilient ones to maintain nitrogen removal when under stress. This also proves that real-time data from physical sensors directly mirror and predict these invisible, microscale shifts in the microbial population.

The strong continuity of OTUs across different phases was observed despite organic material introduction (Fig. 11). Table 4 shows OTU7 of *Limnobacter* as the dominant denitrifier in the nitrogen loading phase, and OTU5 of *Burkholderia* and *Paraburkholderia* as the dominant species in the baseline phase. These OTU results align with sensor-detected changes, confirming that sensor signals can serve as proxies for microbial role changes. Although the bacterial

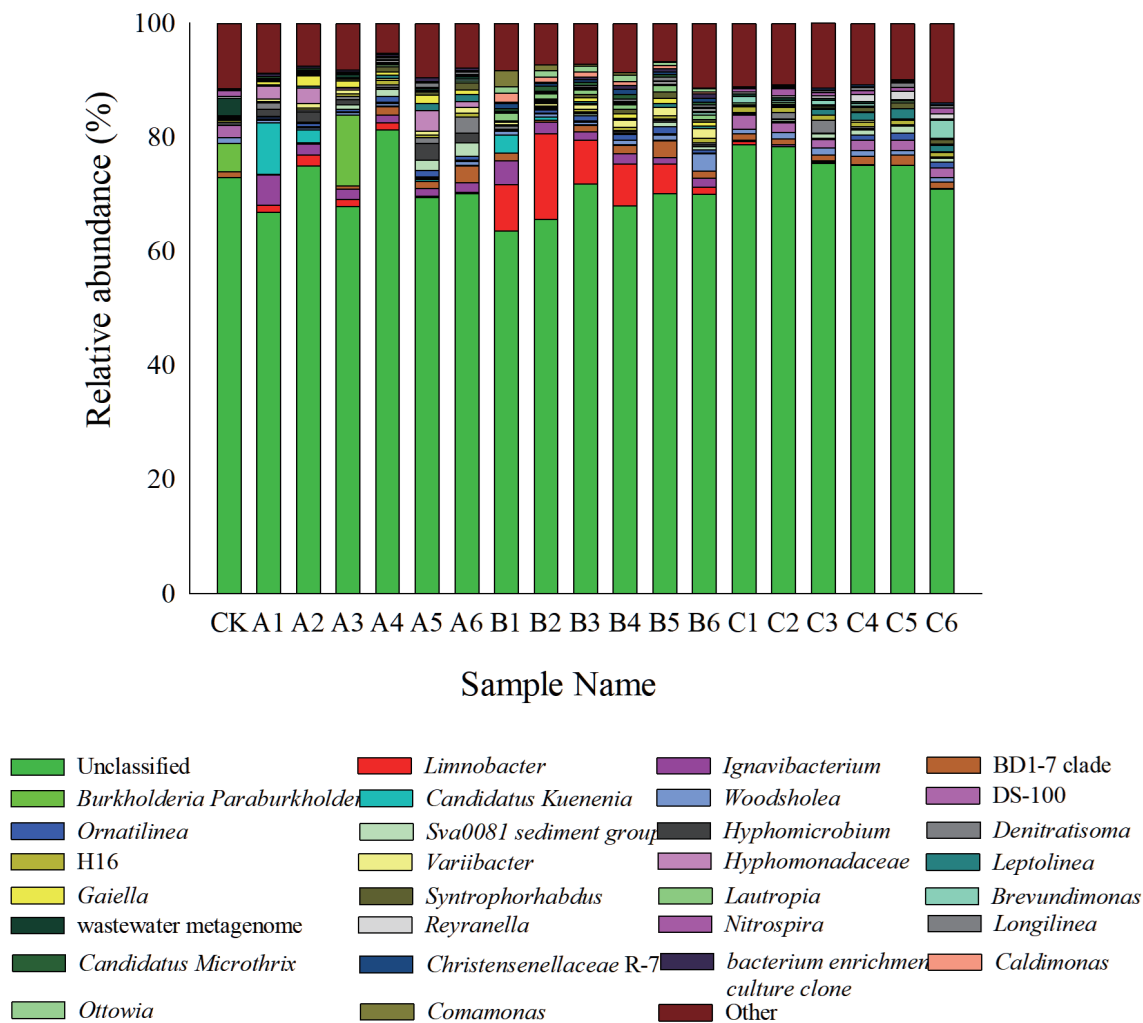


Fig. 10. (Color online) Composition of bacterial communities at genus level in ABR compartments in different operational phases (phase I: baseline phase, phase II: nitrogen loading phase, phase III: wastewater introduction phase, and phase IV: recovery phase).

compositions of the ABR remained stable and continuous across all phases, the specific individual strains (OTUs) changed their dominance to match changing biochemical conditions. Macroscale sensor data can serve as a reliable source to track and predict these microscopic population transitions.

4. Discussion

Traditionally, bioreactor sensors have been used as passive instruments, recording physical–chemical fluctuations without providing biological context. The results of this study show that

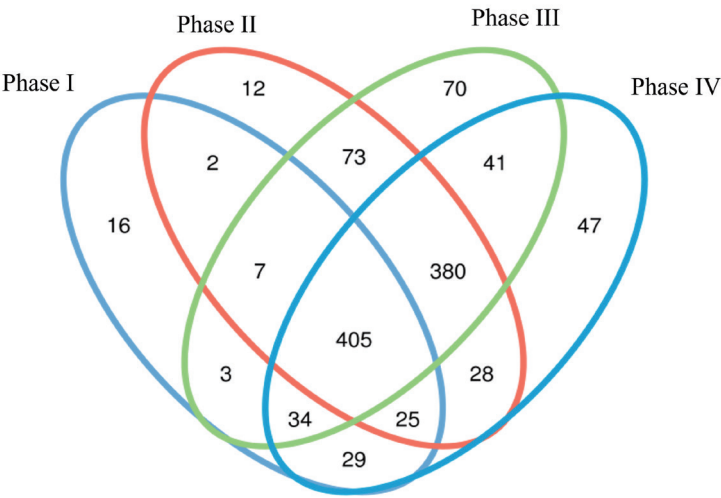


Fig. 11. (Color online) Overlap of microbial communities in ABR in different operational phases (phase I: baseline phase, phase II: nitrogen loading phase, phase III: wastewater introduction phase, and phase IV: recovery phase).

Table 4
OTU relative abundance and classification of 20 important phyla in each compartment in different operation stages of ABR.

OTU identification	Baseline phase	Nitrogen loading phase						Taxonomy (phylum)
	CK	A1	A2	A3	A4	A5	A6	
1	0.52	21.84	20.55	10.53	27.86	6.00	0.28	<i>Chloroflexi</i>
3	0.02	1.76	2.83	3.94	3.98	6.76	4.15	<i>Bacteroidetes</i>
7	0.01	1.22	1.94	1.11	1.11	0.13	0.21	<i>Proteobacteria</i>
2	5.39	0.05	0.02	0.01	0.06	0.02	0.04	<i>Proteobacteria</i>
4	1.15	0.51	1.52	4.34	2.65	1.18	1.96	<i>Acidobacteria</i>
9	0.64	1.80	2.64	1.20	0.90	1.35	0.99	<i>Chloroflexi</i>
5	5.01	0.03	0.03	12.39	0.00	0.01	0.03	<i>Proteobacteria</i>
8	2.35	0.37	0.31	0.65	0.67	0.34	0.72	<i>Proteobacteria</i>
36	1.01	0.05	0.15	0.61	1.47	1.24	2.28	<i>Proteobacteria</i>
13	0.00	4.09	1.61	1.55	1.14	1.18	1.64	<i>Ignavibacteriae</i>
20	1.05	0.65	3.90	2.72	0.82	1.30	1.34	<i>Actinobacteria</i>
10	3.46	0.01	0.00	0.02	0.02	0.03	0.05	<i>Proteobacteria</i>
15	1.89	0.01	0.02	0.08	0.23	0.08	0.26	<i>Proteobacteria</i>
22	0.01	0.00	0.03	0.64	1.41	1.62	3.66	<i>Proteobacteria</i>
19	0.00	0.38	0.50	1.49	1.62	0.65	2.36	<i>Acidobacteria</i>
45	0.00	9.00	2.29	0.05	0.07	0.27	0.19	<i>Planctomycetes</i>
6	0.00	0.40	0.63	0.40	1.02	1.17	0.63	<i>Chloroflexi</i>
23	0.84	0.20	0.23	0.50	0.46	0.45	0.72	<i>Proteobacteria</i>

macroscale electrochemical sensor data can be linked to microscale ecological transformations. For example, the sharp decline in microbial diversity (Shannon diversity index) and the collapse of the autotrophic core (*Candidatus Kuenenia*) during high-strength organic loading were mirrored by sensor signals, specifically negative voltage shifts in ORP and nitrite accumulation

Table 4 (continued)

OTU relative abundance and classification of 20 important phyla in each compartment in different operation stages of ABR.

OTU ID	Wastewater introduction phase						Recovery phase						Taxonomy (phylum)
	B1	B2	B3	B4	B5	B6	C1	C2	C3	C4	C5	C6	
1	11.28	39.79	32.40	19.40	11.41	1.15	4.74	2.52	1.72	2.02	2.46	1.87	<i>Chloroflexi</i>
3	1.74	0.94	4.09	4.78	5.61	3.57	2.41	8.64	12.75	10.11	8.47	7.42	<i>Bacteroidetes</i>
7	7.46	13.97	7.10	6.89	4.75	1.05	0.55	0.04	0.05	0.02	0.01	0.00	<i>Proteobacteria</i>
2	0.06	0.06	0.03	0.05	0.10	0.06	3.80	2.99	2.62	5.13	5.07	5.40	<i>Proteobacteria</i>
4	0.17	0.23	0.67	0.79	1.07	0.90	3.37	0.85	0.63	0.73	1.03	0.72	<i>Acidobacteria</i>
9	0.55	0.37	0.31	0.25	0.57	0.48	0.55	1.28	1.91	2.79	3.44	2.36	<i>Chloroflexi</i>
5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	<i>Proteobacteria</i>
8	0.50	0.17	0.25	0.21	0.45	0.55	1.24	0.80	1.51	3.14	3.60	3.17	<i>Proteobacteria</i>
36	0.27	0.27	0.96	1.38	2.83	1.17	1.08	0.99	1.09	1.56	1.73	1.19	<i>Proteobacteria</i>
13	3.25	1.99	1.25	1.56	0.97	1.38	0.24	0.30	0.19	0.06	0.02	0.05	<i>Ignavibacteriae</i>
20	0.06	0.18	1.05	1.17	1.99	1.40	0.14	0.12	0.19	0.15	0.11	0.12	<i>Actinobacteria</i>
10	0.00	0.03	0.00	0.00	0.04	0.02	2.62	1.53	1.39	1.98	2.54	2.41	<i>Proteobacteria</i>
15	0.04	0.04	0.11	0.17	0.16	0.21	1.11	0.82	1.04	2.31	2.04	2.77	<i>Proteobacteria</i>
22	0.02	0.03	0.37	0.64	1.65	0.99	0.00	0.12	0.54	1.08	1.13	1.34	<i>Proteobacteria</i>
19	0.46	0.78	1.11	1.41	2.35	1.51	0.07	0.04	0.01	0.01	0.01	0.02	<i>Acidobacteria</i>
45	3.15	0.52	0.12	0.14	0.16	0.05	0.00	0.00	0.00	0.00	0.00	0.00	<i>Planctomycetes</i>
6	0.20	0.61	0.94	0.96	1.24	0.61	0.10	0.21	0.32	0.97	1.21	0.98	<i>Chloroflexi</i>
23	1.28	0.27	0.60	0.61	0.61	0.69	1.17	0.88	0.55	0.88	0.78	0.73	<i>Proteobacteria</i>

detected by ISEs. This coupling shows that sensor data are not only indicators of chemical imbalance but also reliable proxies for microbial succession events.

By calibrating electrochemical baselines with metagenomic footprints, soft-sensor algorithms such as artificial neural networks or support vector machines can be trained to recognize signature patterns of ecological stress. For instance, synchronized changes in ORP and pH can serve as a digital fingerprint of functional niche substitution, such as the suppression of autotrophic anammox activity by heterotrophic *Limnobacter*. In this way, sensor systems evolve from simple data loggers into intelligent, real-time diagnostic frameworks capable of predicting biological regime shifts before macroscopic system failure occurs.^(24,25)

Continuous multichannel sensor data can be used for the development and operational reliability of advanced nitrogen removal systems such as ABRs. Autotrophic nitrogen pathways, particularly the anammox process, are fragile because of the slow growth of functional strains and their sensitivity to organic overloads and toxic shocks.⁽²⁶⁾ By integrating diverse sensors, wastewater treatment facilities can establish an automated, real-time control layer. In this study, the stabilization of pH and the normalization of ISE data during the recovery phase coincided with the rebound in the number of OTUs and microbial evenness, confirming that sensor-guided management enables ecological recovery.

The workflow of the sensor network can be summarized as real-time sensor data → diagnostic interpretation → automated control action. This workflow enables the precise regulation of influent composition, dilution, or carbon dosing, stabilizing denitrification zones and maintaining balanced nitrogen removal. In addition, the sensor network enables early warning-triggered bypass or dilution strategies to protect reactor performance against sudden

agricultural organic load. Such integration transforms bioreactors from fragile laboratory prototypes into resilient, industrialscale automated systems capable of self-optimization, rapid response, and longterm stability.^(27,28)

The integrated multisensor monitoring method developed in this study successfully monitored microbial succession in real time, but limitations must be addressed. The long-term operation of ISEs and ORP probes in high-strength wastewater environments is prone to membrane biofouling and signal drift.⁽¹⁹⁾ In the experiment, periodic manual calibration and cleaning were required to maintain signal fidelity. For industrial-scale deployments, automated self-cleaning mechanisms such as physical wipers or ultrasonic antifouling systems will be essential to ensure stable sensor performance.⁽¹⁹⁾ Although sensor data are significantly correlated with microbial diversity metrics, offline 16S ribosomal RNA gene sequencing is necessary for validation to match the data to biological data. To overcome this limitation, physics-informed machine learning and deep learning approaches must be applied to real-time ORP and pH data to predict microbial community stability without delays associated with genomic assays.^(11,29)

The continuous real-time data collected from sensors provide a multidimensional feature matrix, which is wellsuited for machine learning. By combining these electrochemical parameters with models such as long short-term memory networks or random forest regressors, the sensor array functions as an AI-driven soft sensor. This framework can be used to reconstruct compartment-specific bacterial profiles and predict functional group transitions, such as changes between autotrophic and heterotrophic communities without delayed, labor-intensive sequencing. Such AI-enhanced virtual sensing converts physical measurements into proactive operational indicators, enabling predictive automated feedback for multistage anaerobic baffled reactors.

5. Conclusions

We explored how sensors can be applied to monitor and predict microbial succession in ABRs treating high-strength agricultural wastewater. A laboratory-scale ABR equipped with pH, ORP, SEs, and DO sensors was operated through four phases: baseline, nitrogen loading, wastewater introduction, and recovery. Sensor data showed that nitrogen removal rates increased from 90.2% ($\text{NH}_4^+\text{-N}$) and 99.8% ($\text{NO}_2^-\text{-N}$) at moderate loading to 96.1 and 100% under peak organic material introduction ($0.289 \text{ kg/m}^3\cdot\text{d}$). During organic material introduction, removal rates decreased to 73.4% ($\text{NH}_4^+\text{-N}$), 66.1% ($\text{NO}_2^-\text{-N}$), and 60.3% (COD), aligning with negative ORP changes and NO_2^- accumulation. Recovery was captured in real time, with efficiencies recovering to 94.2, 100, and 94.1% for $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and COD, respectively.

Microbial sequencing results showed sensor-detected transitions. The number of OTUs decreased to 31159 in the wastewater introduction phase but increased to 38564 in the recovery phase, with the Shannon diversity index stabilizing at more than 7.4. The autotrophic core (*Candidatus Kuenenia*) enriched in the nitrogen loading phase (up to 9% in A1) was suppressed under organic shock, and the count of heterotrophic *Limnobacter* increased to 14.0%, aligning with sensor-detected nitrite accumulation. These results demonstrate that sensor signals can serve as reliable proxies for microbial role substitutions and ecological regime changes.

The operational workflow of the sensor network can be conceptualized as real-time sensor data → diagnostics → automated actuators. By using real-time sensor data, the traditional 3–5-day latency of manual assays can be eliminated, and automated feedback loops enable precision carbon dosing, the dynamic adjustment of HRT, and bypass actuation. This transforms ABRs from fragile laboratory prototypes into resilient, industrial-scale systems capable of self-optimization and toxic shock mitigation.

The results of this study showed that sensor networks can transform wastewater treatment from simple passive data logging to predictive ecological diagnostics within bioreactors. By integrating electrochemical sensor data with microbial ecology and calibrating sensors with metagenomic footprints, machine learning can be employed in wastewater treatment systems to mitigate ecological stress. A new paradigm for smart wastewater treatment can be established, where advanced sensors are used to provide intelligent, real-time diagnostics and automated resilience, enabling bioreactors to perform stable nitrogen removal under variable organic loads.

Multichannel sensors and data can be used to monitor and predict internal microbial community changes in a multicompartment ABR. Despite limitations, such as potential long-term sensor fouling and the current dependence on offline sequencing for verification, the results of this study provide a framework for smart bioreactor monitoring. By incorporating automated anti-fouling systems and predictive soft-sensor algorithms, passive wastewater treatment systems can be advanced to self-optimizing, automated industrial bioreactors. AI predictive algorithms can be integrated with multichannel sensors for inferring compartment-specific bacterial profiles, enabling the automated, predictive, and intelligent operational control of anaerobic baffled reactors.

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About the Authors



Limei Jiang received her M.S. degree from the College of Bioscience and Bioengineering, Jiangxi Agricultural University, in 2014. Since 2018, she has been a lecturer at Guangdong University of Petrochemical Technology. Her research interests include environmental microbial remediation and microbial electrosynthesis systems. (Limeijiang@gdupt.edu.cn)



Wei Sun received her B.S., M.S., and Ph.D. degrees from Changchun University of Technology (2004), Jiangxi Agricultural University (2006), and South China University of Technology (2014), respectively. From 2014 to 2019, she was a lecturer at Longyan University, China. Since 2019, she has been an associate professor at Guangdong University of Petrochemical Technology. Her research interests include bioengineering and sensor technology. (sunwei@gdupt.edu.cn)



Yinbo Xiang received his B.E. degree from Hunan Normal University in 2010, his M.S. degree from Jiangxi Agricultural University in 2014, and his Ph.D. degree from Sun Yat-sen University in 2017. He has been a lecturer at Guangdong University of Petrochemical Technology since 2018. His research interests include environmental microbial remediation and microbial electrosynthesis systems. (xiangyinbo@gdupt.edu.cn)



Zihan Chen received his B.E. degree from Guangdong University of Petrochemical Technology in 2025, where he is currently pursuing his master's degree. His research interests focus on environmental microbial ecology. (xiaochenv587@gdupt.edu.cn)



Lifeng Zheng obtained his bachelor's degree from China University of Petroleum (East China) in 2019 and his master's degree from Hong Kong Baptist University in 2024. His research includes AI communication, computational media, and artificial intelligence applications.

(810275673@qq.com)



Zhongzhen Li received her B.S. degree from Guangdong University of Technology in 2013 and her Ph.D. degree from Shantou University in 2019 through an integrated M.S.–Ph.D. program. She was a postdoctoral fellow at the First Affiliated Hospital of Jinan University (2019–2021) and Zhuhai People's Hospital (2021–2024) in China. Since October 2024, she has been a faculty member at Guangdong University of Petrochemical Technology. Her research interests include microbiology, probiotics, and aquaculture.

(lizhongzhen@gdupt.edu.cn)