

Multichannel Sensor System for Real-time Monitoring of Up-flow Anaerobic Sludge Bed Anaerobic Ammonium Oxidation Bioreactors

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(Received July 9, 2026; accepted October 8, 2026)

Keywords: anammox bioreactor, multichannel sensor network, real-time water quality sensing, hydrazine dehydrogenase biosensing, instrumentation sampling bias

We investigated the dynamics and microbial pathways of three up-flow anaerobic sludge bed (UASB) anaerobic ammonium oxidation (anammox) bioreactors using an integrated multichannel sensor system: Reactor 1 (control), Reactor 2 (yarn and hollow-ball matrix), and Reactor 3 (vinylon filament-loaded polyethylene). Electrochemical electrodes, optical spectrophotometers, and thermal digestion sensors were used for continuous monitoring across cellular autolysis (Days 1–12), activity lag (Days 13–22), and activity acceleration (Days 23–132). Sensor data showed that filler matrices enhanced biomass retention and stabilized reactor performance. Under increased nitrogen loading, Reactors 2 and 3 showed a near complete removal of ammonium and nitrite, with efficiencies of 99.88 and 99.99%, respectively. Specific anammox activity increased to 2.17 ± 0.12 mg total nitrogen (TN)/g volatile suspended solids (VSS)/h in Reactor 2 and 2.70 ± 0.44 mg TN/g VSS/h in Reactor 3, representing 72 and 66% growth accelerations. Cytochrome c content increased by 153% in Reactor 3, and extracellular polymeric substances (EPS) increased to 51.12 ± 1.48 mg/g VSS in Reactor 2 and 57.89 ± 2.17 mg/g VSS in Reactor 3, supporting granule maturation. Hydrazine dehydrogenase (HDH) activity peaked at 754.69 ± 58.78 μ mol/g protein/min in Reactor 2. The developed sensor system with filler-assisted biomass stabilization enables the nondestructive, high-resolution monitoring of rapid metabolic transitions. To mitigate sensor drift from ion interference and optical biofouling, automated calibration and adaptive sensor design are required. This necessitates filler matrix optimization for sustained granulation and developing self-correcting sensor algorithms.

1. Introduction

Intensive, large-scale livestock and poultry farming has brought significant environmental challenges due to untreated manure and wastewater discharge. Ammonium nitrogen ($\text{NH}_4^+\text{-N}$)

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<https://doi.org/10.18494/SAM6519>

is the most abundant nitrogenous compound in aquatic environments, and its concentration is used as an important indicator of water pollution. Excessive nitrogen loading causes the depletion of dissolved oxygen, promoting eutrophication and uncontrolled algal blooms, which destabilize aquatic ecosystems and reduce biodiversity.⁽¹⁾ Therefore, the effective control of nitrogen pollution in wastewater is vital in environmental management.

Current livestock wastewater treatment strategies include physical–chemical pretreatment, land application, natural purification, and industrialized biological processes. Among these, anaerobic bioreactors have been widely used owing to their strong treatment capacity, small spatial footprint, and cost-effectiveness.^(2,3) To effectively remove ammonium nitrogen, anaerobic ammonium oxidation (anammox) is conducted in an anaerobic bioreactor. Specialized bacteria are used to oxidize NH_4^+ to NO_2^- , producing dinitrogen gas without requiring aeration or external carbon sources. Compared with conventional nitrification–denitrification methods, the anammox process in an anaerobic bioreactor significantly reduces aeration demand, minimizes sludge production, and presents high volumetric efficiency, making it well suited to wastewater containing a high concentration of ammonium.^(4,5) However, a large-scale application of anammox with an anaerobic bioreactor is limited by slow microbial growth (doubling times of 10–12 days) and extended reactor start-up periods exceeding 120 days.^(5,6)

To overcome the limitation, an up-flow anaerobic sludge bed (UASB) reactor is introduced for effective anammox. Owing to its simple design, low operational cost, and ability to treat high-ammonium-containing wastewater, the UASB reactor is regarded as ideal for livestock effluent treatment.⁽⁷⁾ Although polyethylene beads, nonwoven fabrics, and activated carbon are used to enhance bacterial retention and accelerate reactor stabilization in the UASB reactor, complete biomass washout and unstable start-up are challenges to address when using a UASB reactor.^(8,9)

To address these limitations and enhance operational stability, sensor technology, including solid-state ion-selective electrodes (ISEs), optical ultraviolet-visible (UV–Vis) spectrophotometry, and electrochemical probes, is integrated into anaerobic bioreactors. This integration enables the high-resolution continuous monitoring of nitrogen and metabolic changes. However, complex wet-chemical calibrations and offline assays limit the application of real-time multisensor platforms in environmental monitoring.⁽¹⁰⁾ In this study, we developed a multichannel sensor integration framework and applied it to three up-flow UASB bioreactors. Using standard wet-chemical and physiological measurements as reference methods, we calibrated the sensors, and their monitoring capability, signal stability, and anti-fouling performance were evaluated under dynamic nitrogen loading conditions.

2. Materials and Methods

2.1 Bioreactor and sensors

Three identical up-flow UASB reactors (16 L total working volume) were constructed from high-density polyethylene. The lower reaction zone (6.67 L) contained four vertically aligned sampling ports (1–4) and a baseline fluid distributor, and the upper reaction zone (9.4 L) functioned as a three-phase separator equipped with an effluent overflow weir and a gas

collection dome maintaining a strict anaerobic headspace. The ascending gas-liquid-solid mixture was separated by the upper three-phase separator, and the collected biogas was channeled into a gas collection pocket. To protect the autotrophic anammox consortia from light-induced inactivation and prevent thermal fluctuations, all reactor columns were wrapped in 1-cm-thick black polyurethane thermal insulation jackets. The internal liquid temperature of the reaction zone was sustained at 32 ± 1 °C using a closed-loop external water circulation jacket governed by platinum resistance temperature detectors (PT100). To evaluate the effect of biomass immobilization on reactor performance and sensor dynamics, three filling matrices were used.

- Reactor 1 (filler-free control) without matrices
- Reactor 2 (yarn and hollow-ball matrix): equipped with 67 mL of a combined packing matrix consisting of yarn and hollow balls suspended within the reaction zone. This material was chosen to combine macrostructural hydraulic shielding with high-surface-area microfibers. The outer hollow-ball framework attenuates hydraulic shear stress from the up-flow liquid stream, whereas the internal yarn and hollow-ball matrix filaments provide flexible attachment sites that stimulate extracellular polymeric substance secretion and accelerate sludge granulation.⁽¹¹⁾
- Reactor 3 (vinylon filament-loaded polyethylene): contained 67 mL of a vinylon filament-loaded polyethylene matrix. This structural matrix was selected to create a rigid-flexible hybrid packing configuration. The outer polyethylene provides structural integrity against bed compaction under hydraulic loading, whereas the hydrophilic vinylon filaments (OH-functionalized) offer high biomass affinity, ensuring uniform biomass distribution across the reactor height and preventing localized sludge settling.⁽¹²⁾

Synthetic influent was injected continuously using digital peristaltic pumps at a fixed hydraulic retention time of 48 h. The influent pH was maintained between 7.7 and 8.2 through an automated feedback dosing loop using KHCO_3 solution. For high-resolution continuous monitoring, an online sensor array-integrated flow cell was constructed in the effluent recirculation line of each reactor (Fig. 1). The treated effluent passed through the sensor array before final discharge. The sensor array comprised solid-state ISEs for the potentiometric monitoring of NH_2^+ and NO_2^- ion activities, a glass combination electrode for continuous pH feedback monitoring, and a localized UV-Vis spectrophotometric absorption cell operating at 214 nm for the real-time tracking of nitrite kinetics. To calibrate and validate the inline sensor data, influent and effluent samples were collected daily at a fixed time (09:00 AM) before wet-chemical analysis.

2.2 Sensor calibration

The laboratory instruments used in this study are shown in Table 1. These included precision balances and ovens for sample preparation, incubators and sterilizers for microbial cultivation, and spectrophotometric systems for chemical and physiological assays. High-resolution imaging was performed using transmission and scanning electron microscopes, whereas nutrient and nitrogen species were quantified by UV-Vis spectrophotometry, and chemical oxygen demand (COD) values were measured using COD digestion units. COD digestion units are instruments

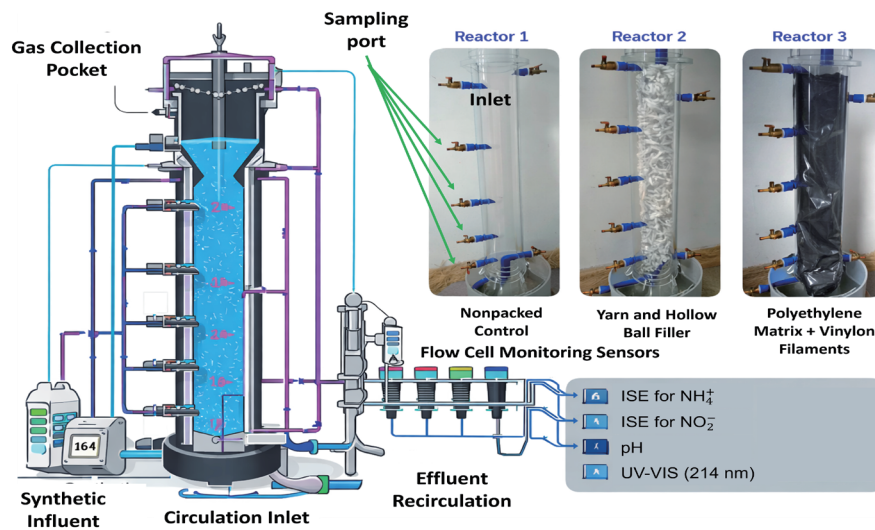


Fig. 1. (Color online) Integrated UASB reactor with filler matrices and sensors (partly created using AI).

Table 1
Instruments used in this study.

Instrument	Model	Manufacturer	Specifications/application
Electronic balance	AL204	Mettler-Toledo Instruments (Shanghai) Co., Ltd.	Analytical balance, readability 0.1 mg, max capacity 200 g
Drying oven	DGG-9240B	Shanghai Senxin Laboratory Instruments Co., Ltd.	Temperature range: +10–250 °C, chamber volume 240 L
Water bath	DK-S24	Shanghai Jinghong Laboratory Equipment Co., Ltd.	Temperature range: RT+5–100 °C, 24 L capacity
Pressure sterilizer	YXQ-LS-30 SII	Shanghai Boxun Industrial Co., Ltd.	Chamber volume 30 L, operating pressure 0.14–0.16 MPa
Refrigerator	BCD-272 (KK28F88TT)	BSH Home Appliances Co., Ltd.	Dual-compartment, 272 L capacity
Incubator	SHP-250	Shanghai Senxin Laboratory Instruments Co., Ltd.	Temperature range: 0–60 °C, chamber volume 250 L
Furnace	DL-I-15	Mettler-Toledo Instruments (Shanghai) Co., Ltd.	Laboratory heating furnace, max temperature 1000 °C
Water-jacketed incubator	GRP-9160	Shanghai Senxin Laboratory Instruments Co., Ltd.	Temperature range: +5–65 °C, chamber volume 160 L
Vacuum drying oven	D2F-6020	Shanghai Senxin Laboratory Instruments Co., Ltd.	Temperature range: +10–200 °C, chamber volume 20 L
Shaker	SPH-100B	Taicang Hongyi Equipment Co., Ltd.	Shaking speed 50–300 rpm, chamber volume 100 L
UV–Vis spectrophotometer	UV-800	Shanghai Mapada Instruments Co., Ltd.	Wavelength range 190–1100 nm, bandwidth 2 nm
Peristaltic pump	UB-7	Sartorius Group	Flow rate range 0.1–700 mL/min, digital control
pH meter	—	Sartorius Scientific Instruments (Beijing) Co., Ltd.	Measurement range: pH 0–14, accuracy ± 0.01
Electron microscope	E-1045	Hitachi High-Tech Corporation, Japan	Resolution 0.2 nm, accelerating voltage 80–120 kV
Scanning electron microscope	SX-60	Hitachi High-Tech Corporation, Japan	Resolution 3 nm, accelerating voltage 0.5–30 kV
NanoDrop MicroVolume Spectrophotometer	1510	Thermo Fisher Scientific Oy	Sample volume 1–2 μL , wavelength range 190–850 nm

that chemically oxidize organic and inorganic matter under controlled heating for the quantification of COD. Peristaltic pumps and pH meters ensured accurate influent dosing and process control. Collectively, this instrumentation provided the analytical accuracy, environmental stability, and reproducibility required for reactor operation, sludge characterization, and sensor calibration.

To ensure the accuracy of the integrated online sensors and understand sludge physiology, we performed periodic wetchemical assays and enzymatic validations.⁽¹³⁾ The offline test results were used as references for the calibration of sensors and the data.

2.2.1 Specific anammox activity (SAA) verification

Potentiometric sensors for ammonium (NH_4^+) and nitrite (NO_2^-) were cross-checked with SAA.⁽⁹⁾ SAA refers to the rate at which anammox bacteria convert ammonium and nitrite into nitrogen gas. Nitrogen conversions were quantified spectrophotometrically. Ammonium was measured using Nessler's reagent (a chemical that produces a yellow color proportional to ammonium concentration), and nitrite was measured using a diazotization reaction with 4-aminobenzenesulfonamide and N-(1-naphthyl)ethylenediamine dihydrochloride.⁽¹⁴⁾

2.2.2 Cytochrome c quantification

Cytochrome *c* content was used as a proxy for anammox bacterial density.⁽¹⁵⁾ Cytochrome *c* is a heme protein involved in electron transfer. Sludge pellets were lysed in sodium hydroxide, and absorption spectra (500–600 nm) were recorded. Oxidized samples (treated with potassium ferricyanide) were compared with reduced samples (treated with sodium dithionite) in phosphate buffer to determine relative concentrations.

2.2.3 Extracellular polymeric substances (EPS) and protein-to-polysaccharide ratio (PN/PS) ratio

EPS are sticky biopolymers secreted by microbes that help form stable sludge granules. Their composition was analyzed to assess granule integrity.⁽¹⁶⁾ Polysaccharides (sugar polymers) were analyzed using the anthrone colorimetric method, whereas proteins were measured using a modified Lowry assay.⁽¹⁴⁾ The PN/PS ratio was used to examine the balance between structural proteins and carbohydrates in the sludge matrix. A higher PN/PS ratio reflects a higher granule stability.

2.2.4 Hydrazine dehydrogenase (HDH)

HDH is an enzyme in the anammox pathway, catalyzing the oxidation of hydrazine to nitrogen gas. Its activity was monitored to validate sensor-derived kinetic trends.^(17,18) Cell suspensions were stabilized in phosphate buffer (pH 8.0), and enzyme activity was determined spectrophotometrically by tracking the reduction of cytochrome *c* at 550 nm in the presence of hydrazine or hydroxylamine. These calibration procedures were conducted to ensure that

subsequent sensor data could be directly compared with conventional wet-chemical assay results.

3. Results

After calibration, the multichannel sensor system enabled the continuous monitoring of changes in nitrogen species, sludge physiology, and reactor stability in the UASB bioreactors. The reactors were operated in two stages: the preloading phase (Days 1–61) and the load-increment phase (Days 62–132). In these stages, three operational phases were identified: cellular autolysis, activity lag, and activity acceleration.

- Cellular autolysis phase (Days 1–12)

During the initial days, effluent ammonium ($\text{NH}_4^+\text{-N}$) and nitrite ($\text{NO}_2^-\text{-N}$) concentrations increased in all reactors (Figs. 2 and 3). These concentrations exceeded influent baselines,

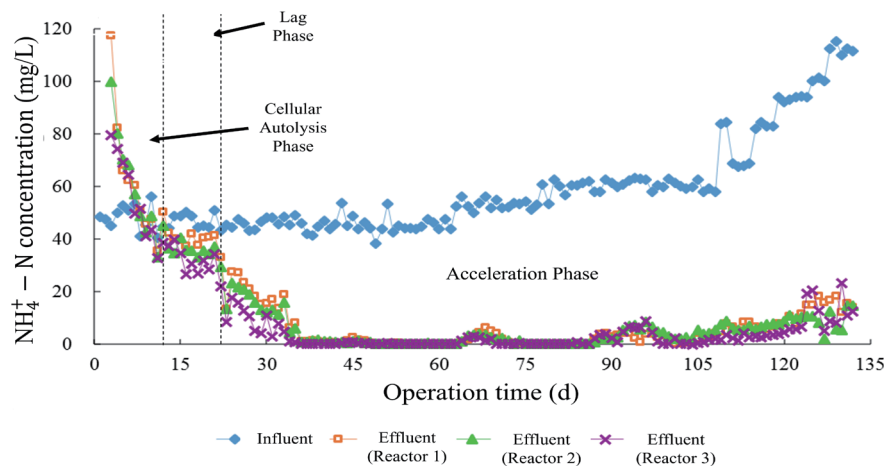


Fig. 2. (Color online) $\text{NH}_4^+\text{-N}$ concentrations in Reactors 1, 2, and 3.

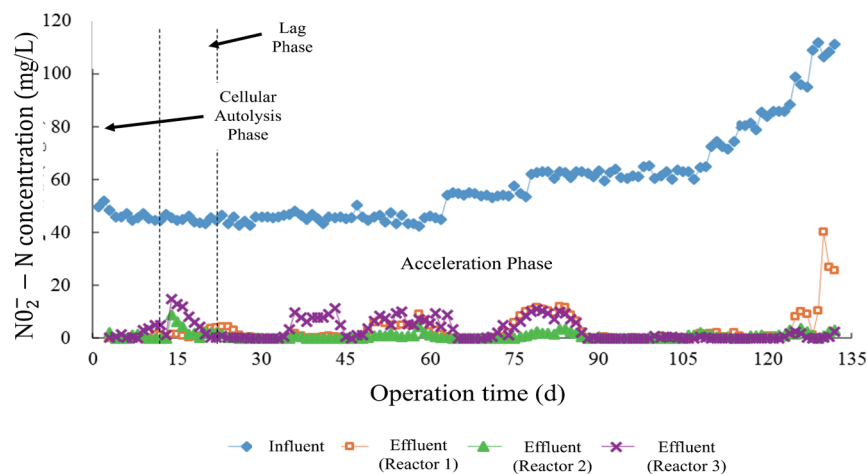


Fig. 3. (Color online) $\text{NO}_2^-\text{-N}$ concentrations in Reactors 1, 2, and 3.

resulting in negative removal efficiencies (Figs. 4 and 5). The results indicated that biomass was undergoing autolysis, releasing intracellular nitrogen compounds into the liquid phase. The sensors captured this instability with high temporal resolution, confirming that cell lysis and organic matter release dominated the early stage of reactor operation.⁽⁶⁾

- Lag phase (Days 13–22)

As microbial communities adapted to the anaerobic environment, electrochemical sensor data showed effluent concentrations falling below influent levels. Removal efficiencies turned positive but remained below 50%, as shown in Figs. 2 and 5. The fluctuations in sensor signals reflected the gradual establishment of anammox activity, with microbial populations adjusting to the reactor's electrochemical conditions.⁽¹³⁾ This transitional state was characterized by unstable but improving nitrogen removal performance.

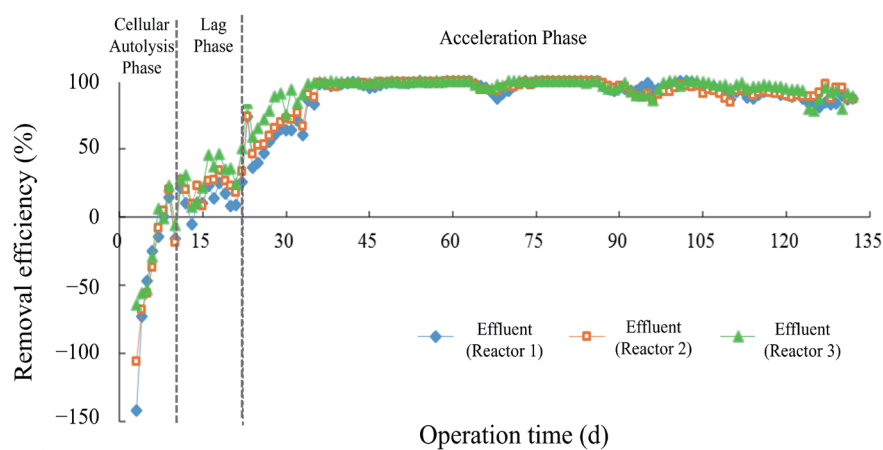


Fig. 4. (Color online) $\text{NH}_4^+\text{-N}$ removal efficiencies in Reactors 1, 2, and 3.

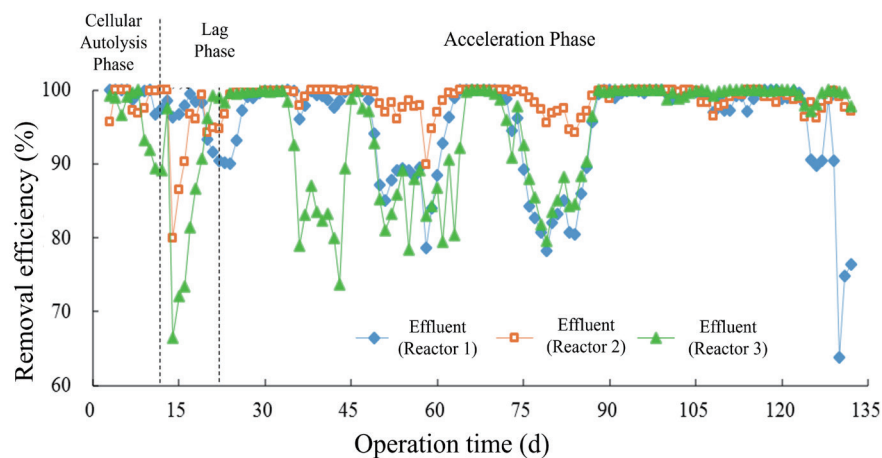


Fig. 5. (Color online) $\text{NO}_2^-\text{-N}$ removal efficiencies in Reactors 1, 2, and 3.

- Acceleration phase (Days 23–132)
During the extended acceleration phase, optical sensors recorded steadily decreasing concentrations of nitrogen species (Fig. 6). Removal efficiencies of nitrate (NO_3^- -N) increased progressively (Fig. 7), and the convergence of sensor signals in the baseline values showed that the reactors had reached higher operational capacity. Reactors 2 and 3 showed the near-complete removal of ammonium and nitrite, whereas Reactor 1 (control) showed divergence by Day 125, with NO_2^- -N concentrations increasing again. Reactor 1 reached its biomass retention limit, whereas the filler matrices in Reactors 2 and 3 provided structural support that stabilized microbial activity and enhanced nitrogen removal.⁽⁹⁾
- pH
Effluent pH increased rapidly from 7.3 to 8.4 during autolysis (Fig. 8), reflecting the release of ammonia and buffering ions from lysed biomass. In the lag phase, pH declined

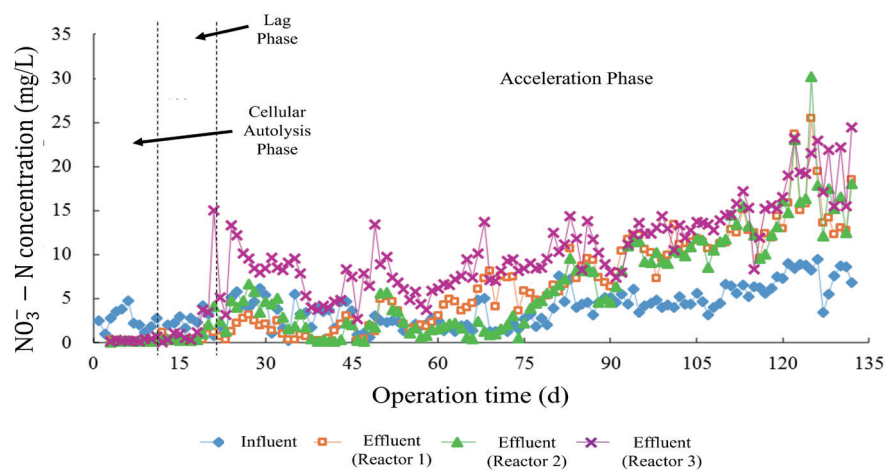


Fig. 6. (Color online) NO_3^- -N concentrations in Reactors 1, 2, and 3.

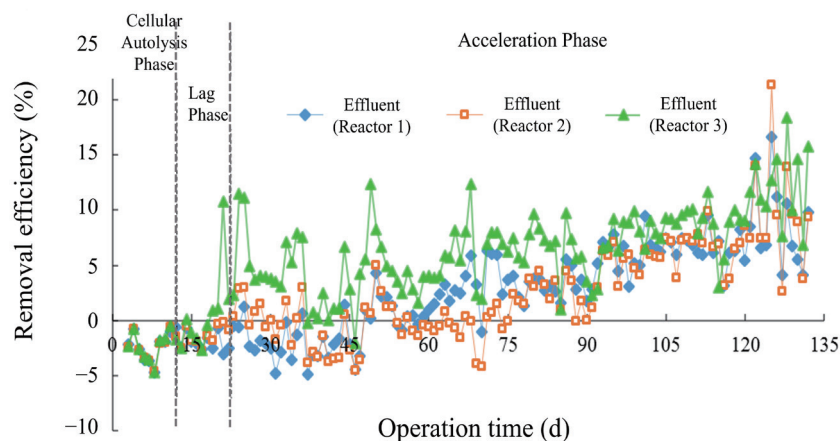


Fig. 7. (Color online) NO_3^- -N removal efficiencies in Reactors 1, 2, and 3.

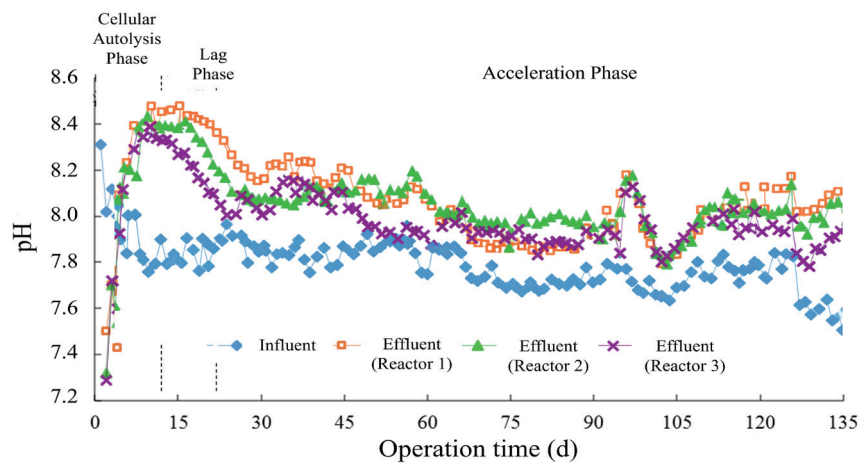


Fig. 8. (Color online) pH changes in Reactors 1, 2, and 3.

and stabilized near 8.0, showing the recovery of the system's internal buffering capacity. During acceleration, pH remained between 7.9 and 8.1, indicating electrochemical equilibrium. Reactors 2 and 3 showed more stable pH profiles than Reactor 1, indicating that filler matrices improved hydraulic uniformity and reduced localized fluctuations.⁽¹³⁾

- COD

COD values increased rapidly during autolysis owing to soluble organics released from decaying cells (Fig. 9). In the lag phase, COD values decreased as microbial metabolism recovered. In the acceleration phase, COD values stabilized below 50 mg/L, indicating the efficient removal of organic matter. Removal efficiencies exceeded 90% and remained stable throughout the acceleration phase (Fig. 10). Reactors 2 and 3 showed smoother COD profiles than Reactor 1, showing that filler matrices minimized turbulence and improved flow distribution. These results demonstrate that COD monitoring, when combined with nitrogen species data, provides a reliable indicator of reactor stability and organic degradation efficiency.⁽¹⁹⁾

- Sludge compaction and granulation

Sludge settling and granulation were evaluated using sludge volume index (SVI_{30}/SVI_5) ratios (Table 2). SVI is used to measure the volume occupied by sludge after settling, normalized to suspended solids concentration. A ratio close to 1.0 indicates well-granulated, compact sludge with stable settling properties.⁽¹⁶⁾ Reactor 2 achieved perfect granulation (ratio = 1.0), and Reactor 3 showed a ratio increasing from 0.76 to 0.94. Reactor 1 showed declining ratios, reflecting poor granulation without filler support. These findings were consistent with the stable pH and COD values observed in Reactors 2 and 3, confirming that filler matrices accelerated sludge pelletization and improved reactor stability.⁽⁹⁾ Reactors 2 and 3 equipped with filler matrices outperformed the control Reactor 1. Reactors 2 and 3 showed higher nitrogen removal efficiencies, more stable pH values, smoother COD profiles, and better sludge granulation than Reactor 1. Continuous multichannel sensor monitoring results

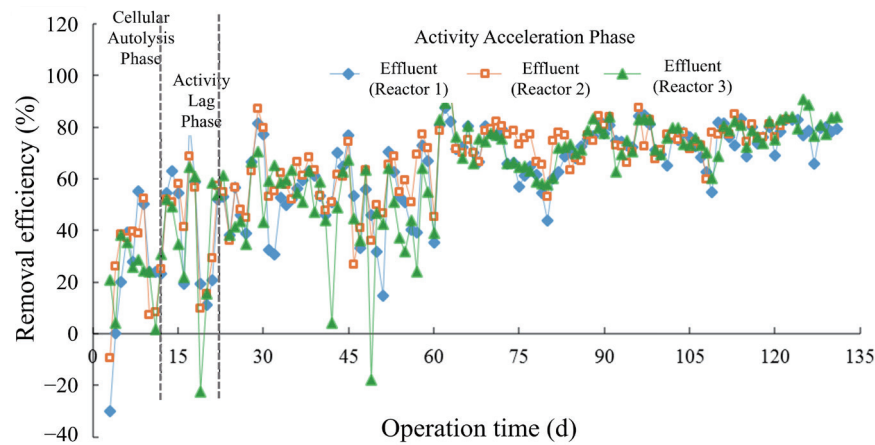


Fig. 9. (Color online) COD values (mg/L) in Reactors 1, 2, and 3.

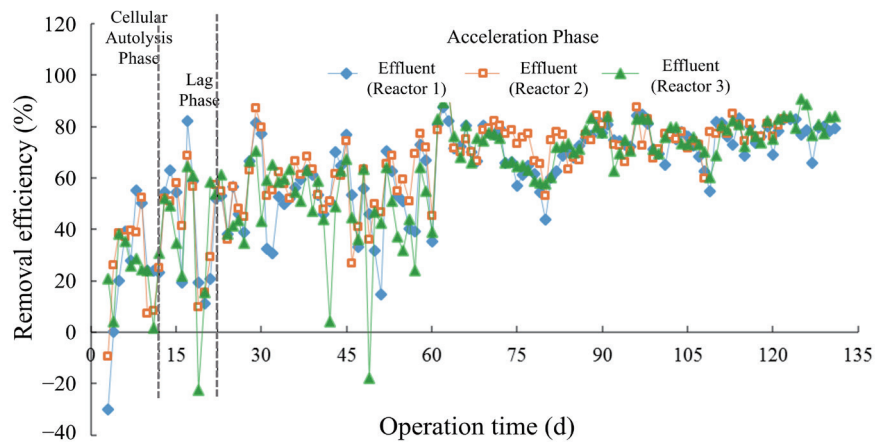


Fig. 10. (Color online) Reduction in COD values in Reactors 1, 2, and 3.

Table 2
*SVI*₃₀/*SVI*₅ ratios.

Reactor	Cellular autolysis phase	Lag phase	Acceleration phase
Reactor 1	0.98	0.97	0.96
Reactor 2 (yarn and hollow-ball matrix)	0.98	0.98	1
Reactor 3 (vinylon filament-loaded polyethylene)	0.76	0.86	0.94

provided high-resolution data on reactor transitions, enabling the early detection of autolysis, microbial adaptation, and acceleration phases. The integration of filler matrices enhanced biomass retention and protected sensor fidelity by reducing fouling and turbulence. The results show that a sensor network can be used as a powerful diagnostic tool for real-time process control, indicating the importance of reactor design in ensuring reliable sensor performance.⁽¹⁰⁾

Table 3
MLSS and MLVSS measurements.

Reactor	Parameter	Cellular autolysis phase	Lag phase	Acceleration phase
Reactor 1	SS (mg/L)	44752	34291	38566
	VSS (mg/L)	19455	14354	16653
	VSS/SS Ratio	0.43	0.42	0.43
Reactor 2	SS (mg/L)	38894	33522	43739
	VSS (mg/L)	16918	14306	17951
	VSS/SS Ratio	0.43	0.43	0.41
Reactor 3	SS (mg/L)	28173	34406	26635
	VSS (mg/L)	11818	16650	10137
	VSS/SS Ratio	0.42	0.48	0.38

The sensor data were used to estimate biomass density using the values of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) (Table 3). MLSS represents the total suspended solids in the mixed liquor, whereas MLVSS indicates the organic fraction of those solids, primarily microbial biomass. The ratio of volatile to total solids (VSS/SS) is therefore a useful indicator of the proportion of active biomass relative to inert material.

In Reactor 3, the VSS/SS ratio increased during the activity lag phase, indicating rapid biomass proliferation supported by vinylon filament-loaded polyethylene. However, the ratio declined in the activity acceleration phase owing to the sampling bias caused by heavy mineral particles settling near the lower valves. Reactor 2 maintained stable ratios across phases, whereas Reactor 1 showed little change, presenting limited biomass retention without filler materials. Despite these localized gravimetric fluctuations, the optical sensor data showed stable reductions in COD values (Figs. 10). MLSS and MLVSS were used to estimate biomass quantity and composition, indicating that optical sensors enable the monitoring of reactor performance under dynamic loading conditions.

Reactors containing filler matrices showed much faster nitrogen conversion than the control reactor. In the acceleration phase, Reactor 2 showed 2.17 ± 0.12 mg total nitrogen (mg-TN)/g-VSS/h, representing a 72% increase compared with its autolysis phase. Reactor 3 showed the highest value of 2.70 ± 0.44 mg-TN/g-VSS/h, corresponding to a 66% growth acceleration (Table 4). These results show that filler matrices are structural supports for microbial communities, enabling them to process nitrogen more efficiently. The results align with the nitrogen attenuation curves shown in Figs. 4–9, indicating that filler matrices enhanced catalytic velocity, stabilized sensor signals, and reduced optical noise in the monitoring system that includes the UV–Vis spectrophotometer.

The redox sensor data showed different trends in cytochrome *c* content (Table 5). Reactors 1 and 2 showed decreases to 0.13 ± 0.03 and 0.14 ± 0.08 mgTN/gVSS/h, respectively, in the acceleration phase. The increase in cytochrome *c* content for Reactor 3 was calculated by comparing the acceleration phase value (0.48 ± 0.28 mgTN/gVSS/h) with its baseline value in the cellular autolysis phase (0.19 ± 0.02 mgTN/gVSS/h) (Table 5). Evaluating this net change of 0.29 mgTN/gVSS/h relative to the initial concentration showed a 153% increase (from 0.19 to 0.48 mgTN/gVSS/h). Cytochrome *c* is a protein involved in electron transfer, so higher values

Table 4
SAA using spectrophotometric sensing.

Reactor	Cellular autolysis phase	Acceleration phase (mg-TN/g-VSS/h)	Kinetic growth acceleration (%) (mg-TN/g-VSS/h)
Reactor 1	0.55 ± 0.08	1.12 ± 0.33	51
Reactor 2 (yarn and hollow-ball matrix)	0.61 ± 0.03	2.17 ± 0.12	72
Reactor 3 (vinylon filament-loaded polyethylene)	0.92 ± 0.09	2.70 ± 0.44	66

Table 5
Cytochrome c content.

Reactor	Cellular autolysis phase (mg-TN/g-VSS/h)	Acceleration phase (mg-TN/g-VSS/h)
Reactor 1	0.29 ± 0.02	0.13 ± 0.03
Reactor 2 (yarn and hollow-ball matrix)	0.29 ± 0.05	0.14 ± 0.08
Reactor 3 (vinylon filament-loaded polyethylene)	0.19 ± 0.02	0.48 ± 0.28

indicate stronger metabolic activity. The increase in Reactor 3 suggests that the polyethylene filler prevented biomass settling and localized sludge compaction, eliminating sampling bias and allowing sensors to capture the true metabolic increase. This observation complemented the stable pH data in Fig. 10 (7.9–8.1), which reflected balanced electronacceptor use in Reactor 3.⁽¹³⁾

Colorimetric sensor measurements of EPS in Reactors 2 and 3 showed significant increases in Reactors 2 and 3, whereas those in Reactor 1 remained nearly unchanged (Table 6). EPS values in Reactor 2 increased from 38.19 ± 0.75 to 51.12 ± 1.48 mg/gVSS, and those in Reactor 3 increased from 33.18 ± 1.08 to 57.89 ± 2.17 mg/gVSS. EPS are sticky biopolymers secreted by microbes that help form stable sludge granules. PN/PS ratios decreased across all reactors, decreasing to 13.61 ± 17 mg/gVSS in Reactor 2 and 16.28 ± 17 mg/gVSS in Reactor 3, indicating that the sludge was maturing into compact granules.⁽¹⁶⁾ These extracellular bonding layers stabilized COD removal efficiency curves (Fig. 12) and entrapped fine particulates, preventing optical flow-cell fouling and protecting electrochemical membranes from abrasion.

Photometric sensor measurements of HDH activity confirmed the strong stimulation of metabolic pathways in filler-supported reactors (Table 7). Reactor 2 showed the highest HDH activity at 754.69 ± 58.78 $\mu\text{mol/gprotein/min}$, Reactor 3 also showed high activity at 621.12 ± 48.84 $\mu\text{mol/gprotein/min}$, and Reactor 1 showed the HDH activity at 579.24 ± 29.36 $\mu\text{mol/gprotein/min}$. HDH is a key enzyme in the anammox pathway, and higher activity directly supports faster nitrogen removal. These intracellular data demonstrate that filler materials promoted enzyme activity, improving reactor performance. They also show that HDH multiheme complexes serve as promising targets for immobilization onto functionalized nanomaterials, which can be used as reagentless amperometric biosensors for smart monitoring networks.^(17,18)

Table 6
EPS chemical components and PN/PS ratios.

Reactor	Cellular autolysis phase	Acceleration phase
Reactor 1	PS: 0.83	PS: 1.97
	PN: 39.01	PN: 37.77
	EPS: 39.84 ± 2.59	EPS: 39.74 ± 1.11
	PN/PS: 46.74	PN/PS: 19.17
Reactor 2 (yarn and hollow-ball matrix)	PS: 0.91	PS: 3.50
	PN: 37.28	PN: 47.62
	EPS: 38.19 ± 0.75	EPS: 51.12 ± 1.48
	PN/PS: 40.94	PN/PS: 13.61
Reactor 3 (vinylon filament-loaded polyethylene)	PS: 0.92	PS: 3.35
	PN: 32.25	PN: 54.54
	EPS: 33.18 ± 1.08	EPS: 57.89 ± 2.17
	PN/PS: 34.90	PN/PS: 16.28

Table 7
Intracellular HDH activity measured by photometric sensing.

Reactor	Cellular autolysis phase	Acceleration phase
Reactor 1	9.03 ± 0.00	579.24 ± 29.36
Reactor 2 (yarn and hollow-ball matrix)	366.23 ± 14.22	754.69 ± 58.78
Reactor 3 (vinylon filament-loaded polyethylene)	59.46 ± 65.05	621.12 ± 48.84

4. Discussion

4.1 Biofouling mitigation and biomass immobilization using sensor data

The initialization and maintenance of anammox systems are constrained by the low growth rates and chemical sensitivity of the functional *Planctomycete* consortia.⁽⁶⁾ Conventional bioreactors depend on discrete, manual ex situ sampling, which introduces delays in feedback and increases the risk of toxic loading overshoots. In this study, the integration of a synchronized multichannel sensor system, including electrochemical potentiometric electrodes, optical spectrophotometer arrays, and thermal digestion sensing units, enabled the continuous monitoring of UASB reactors. This approach provided the real-time tracking of metabolic transitions and uninterrupted feedback during high-load operations.

Traditional offline monitoring methods show sampling latency and do not enable rapid intervention during shock-loading events. In contrast, continuous sensor signals allowed the nondestructive, immediate detection of early cellular autolysis (Days 1–12) and the transition into activity acceleration phases (Days 23–132) without relying on labor-intensive wet-chemistry assays.⁽¹⁰⁾ Filler-mediated biogranulation played a critical role in protecting sensor surfaces. For example, Reactor 2 promoted dense sludge granulation and enhanced EPS formation, which reduced suspended mineral debris and mitigated optical biofouling and signal drift.

The sensor data were used to redefine the macroscale start-up sequence into three distinct stages: cellular autolysis, activity lag, and activity acceleration. During autolysis, increases in

NH_4^+ -N and NO_2^- -N concentrations were immediate indicators of biomass lysis and transient denitrification (Figs. 4–7). These optical signals provided real-time alerts of functional transitions, offering a faster and less destructive alternative to conventional chemical assays. As the system progressed into the acceleration phase, sensors captured thresholds of the microfluidic environment, enabling controlled stepwise increases in nitrogen loading without inducing substrate inhibition.

Cross-validation results of sensor data showed reproducible stabilization patterns across reactor configurations. For instance, by Day 125, NO_2^- -N concentrations in Reactor 1 increased, whereas those in Reactors 2 and 3 remained stable (Fig. 6). This divergence demonstrated that three-dimensional packing matrices, particularly the yarn and hollow-ball filler in Reactor 2, significantly enhanced biomass retention and conferred resilience against hydraulic shock loads.⁽⁹⁾

The results also present a reciprocal relationship. Cytochrome *c* data showed that Reactor 3, equipped with the vinylon filament-loaded polyethylene matrix, retained functional biomass evenly throughout the column, preventing localized settling into unsampled zones (Table 5). This structural uniformity eliminated spatial sampling bias and allowed sensors to capture the true 153% increase in cytochrome *c* content.

SEM and electron backscatter imaging revealed that the flexible three-dimensional filaments in Reactor 2 reduced local hydraulic shear stress, promoting the formation of a dense and cohesive EPS layer (Fig. 11). This EPS secretion, presented by colorimetric assays (Table 6), stabilized COD removal efficiency curves (Fig. 10) and physically entrapped fine particulates, preventing optical flow-cell fouling and protecting electrochemical membranes. Finally, photometric measurements of HDH activity showed that filler matrices stimulated metabolic pathways. Reactor 2 achieved the highest HDH activity and Reactor 3 also showed strong performance compared with the control. Since HDH is a key enzyme in the anammox pathway, these results directly support faster nitrogen removal and suggest that HDH multiheme complexes could serve as promising targets for immobilization onto functionalized nanomaterials.^(17,18) Such immobilization would enable the development of next-generation reagentless amperometric biosensors for smart monitoring networks.

4.2 Limitations and further development

Compared with recent single-parameter monitoring studies in anammox reactors, the multichannel sensor system resolves problems related to early cellular autolysis and rapid nitrogen kinetics in real time. By integrating electrochemical, optical, and thermal sensors, the system provides a comprehensive view of reactor dynamics that conventional *ex situ* assays or single-parameter monitoring cannot achieve. However, operational limitations must be addressed. ISEs are prone to drift when exposed to ionic strength fluctuations in complex wastewater. Optical interfaces are interfered with by biofouling challenges, as microscale biofilm accumulation on UV–Vis flow-cell windows can offset baseline data under high-solids loading. The use of yarn-ball carrier matrices in Reactor 2 promoted dense sludge granulation, reduced suspended debris, and significantly lowered fouling rates. Long-term deployment

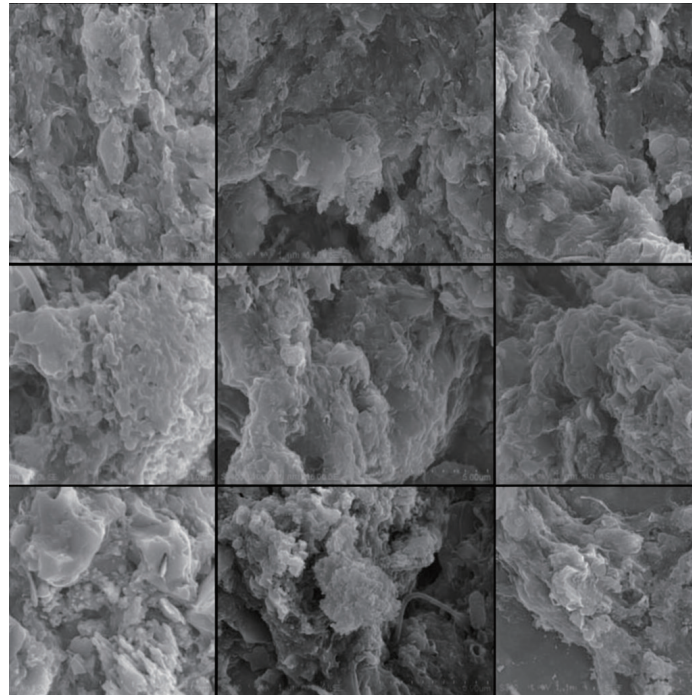


Fig. 11. Scanning electron microscopy (SEM) images of sludges of Reactors 1, 2, and 3 at different phases: (a) cellular autolysis phase (upper layer), (b) lag phase (middle layer), and (c) acceleration phase (lower layer).

requires stabilization even in extreme pH changes and temperature fluctuations. Periodic automated recalibration must be developed to solve such problems. Despite these problems, the multisensor system was able to detect rapid changes in microbial activity and sudden increases in nitrogen loading. This showed that biogranulation, where microbes form compact clusters on carrier materials, protects the sensors from fouling and helps them keep working reliably, extending the overall resilience of the monitoring system.

5. Conclusions

The developed multichannel sensors with filler-assisted biomass stabilization provide a powerful approach for monitoring and improving UASB anammox bioreactors. Electrochemical, optical, and gravimetric sensors enabled the high-resolution tracking of microbial transitions, dividing the start-up sequence into cellular autolysis (Days 1–12), lag (Days 13–22), and acceleration (Days 23–132). Continuous sensor data were used to explore transient substrate inversions during early decay, allowing the identification of indicators that help prevent localized inhibition under highload conditions. The effectiveness of filler matrices was validated in this study. Reactor 3 showed ammonium removal efficiencies up to 95.97%, with a peak performance of 99.88 mg/L, whereas Reactor 2 (yarn and hollow-ball matrix) showed the most effective

nitrate degradation at 98.82%, showing a maximum efficiency of 99.99%. Specific anammox activity increased to 2.17 ± 0.12 mgTN/gVSS/h in Reactor 2 and 2.70 ± 0.44 mgTN/gVSS/h in Reactor 3. Cytochrome c content rose by 153% in Reactor 3, confirming enhanced electron transfer capacity. EPS secretion increased to 51.12 ± 1.48 mg/gVSS in Reactor 2 and 57.89 ± 2.17 mg/gVSS in Reactor 3, supporting granule maturation. Hydrazine dehydrogenase activity peaked at the yarn and hollow-ball matrix in Reactor 2, establishing a clear metabolic hierarchy (Reactor 2 > Reactor 3 > Reactor 1).

At the same time, several challenges must be addressed. The sensor drift caused by ion interference, optical biofouling, and the need for frequent recalibration limit long-term resilience. To resolve these issues, automated calibration protocols, adaptive sensor algorithms, and optimized filler matrices can be applied for sustained granulation. Overall, the results of this study show how reactor architecture and sensor integration can work together to stabilize complex autotrophic systems and provide design strategies for future smart sensing platforms in wastewater treatment.

Acknowledgments

This research was supported by the Wuchuan County Rural Science and Technology Special Envoy Workstation, Zhanjiang City, Guangdong Province (Project No. 2025A0301003), the National Natural Science Foundation of China (Project No. 42006137), the Applied Basic Research Foundation of Guangdong Province (Project Nos. 2023A1515012424, 2023A1515012411, and 2022A1515010539), the Scientific Research Fund of Talent Introduction of Guangdong University of Petrochemical Technology (Project Nos. 2019rc108 and 2019rc115), Maoming Science and Technology Plan Project, (Project No. 2025652), the Guangdong Province University Key Field Special Research and Development, Application and Service of Salt-Tolerant Nitrogen Removal Biologics for Green and Healthy Aquaculture (Project No. 2023ZDZX4035), the 2022 Maonan District Science and Technology, Three Industry-University-Research Cooperation Special Fund Plan Project (Project No. 2022A00102), the Science and Technology Innovation Strategic Special Project of Guangdong Province (Project No. 2022DZXHT024), the 2025 College Student Innovation Training Program Project (Project No. 24C257), the Projects of Talents Recruitment of Guangdong University of Petrochemical Technology (Project No. 2025rcyj1006), the Sci-Tech Innovation Project for Joint Training Base of Professional Degree Graduate of Maoming Green Chemical Industry Research Institute Graduate School and Guangdong University of Petrochemical Technology (Project No. 2025KJCX088), and the Innovation and Entrepreneurship Project for College Students of GDUPT (Project No. 25C257).

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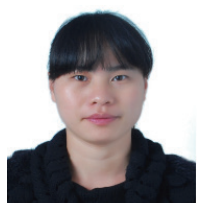
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