

ORIGINAL RESEARCH

Remediation and Treatment

New insights into nonylphenol's impact on pharmaceutical wastewater treatment by aerobic granular sludge

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Abstract

Pharmaceutical wastewater has complex components, containing emerging pollutants such as nonylphenol (NP). Aerobic granular sludge (AGS) has application potential in wastewater treatment due to its unique structure and functions, but the mechanism of NP on AGS remains unclear. This study systematically investigated the effects of different NP concentrations on AGS's pollutant removal efficiency, sludge characteristics, microbial community, and metabolic activity. The results showed that NP exerted a threshold effect of low promotion and high inhibition on AGS: 1 mg/L NP enriched functional bacteria such as ammonia-oxidizing bacteria (*Nitrosomonas*) and denitrifying bacteria (*Pseudomonas*), promoted the secretion of tightly bound extracellular polymeric substances (TB-EPS), enhanced granule aggregation and settleability, and simultaneously improved COD (average 94.2%) and ammonia nitrogen removal rates (average 94.5%). However, total nitrogen (TN) removal is more sensitive to NP toxicity: at 5 mg/L, the TN removal rate dropped sharply by 10.7% because core nitrogen cycle bacteria are more vulnerable to NP toxicity. At 10 mg/L, the abundance of *Nitrospira* plummeted to 0.7%, and pollutant-tolerant bacteria (e.g., *Bacillus*) expanded to occupy ecological niches, exacerbating the decline in community diversity and metabolic respiration inhibition. High-concentration NP further destroyed the granule structure, inducing granule fragmentation, imbalance of EPS components, and deterioration of settleability, forming a negative feedback loop of “structural disintegration - functional decline—microbial imbalance—metabolic inhibition.” This work clarifies the critical transition range and multi-dimensional action mechanism of NP on AGS, providing key theoretical support for the optimization of AGS processes in NP-containing pharmaceutical wastewater treatment.

KEYWORDS

AGS, microbial community, nonylphenol, pharmaceutical wastewater, treatment efficiency

1 | INTRODUCTION

As a typical type of highly recalcitrant industrial wastewater, pharmaceutical wastewater has complex components and an extremely high pollution load, posing severe challenges to environmental governance.¹ Such wastewater not only contains high concentrations of

organic pollutants, with chemical oxygen demand (COD) often reaching tens of thousands of mg per liter, far exceeding that of ordinary industrial wastewater, but also commonly contains various residual antibiotics.² Even at low concentrations, these substances may induce microorganisms to produce antibiotic resistance genes, threatening ecosystems and human health through the food chain. In addition, a

large number of refractory components, such as heterocyclic compounds and aromatic derivatives, in the wastewater, due to their stable chemical structures and biological toxicity, are difficult to completely decompose by traditional microorganisms. Meanwhile, affected by fluctuations in production processes, the water quality and quantity of pharmaceutical wastewater show significant instability, further increasing the operational difficulty of treatment processes.³ Given its potential risks to the water environment and the difficulty of governance, developing efficient, stable, and economical pharmaceutical wastewater treatment technologies has become a research hotspot in the field of environmental engineering, attracting widespread attention from academia and industry.

Aerobic granular sludge (AGS), a novel biological treatment technology, has shown promising application prospects in treating pharmaceutical wastewater due to its unique structural and functional characteristics.⁴ Compared with traditional activated sludge, AGS consists of dense granules formed by the self-aggregation of microorganisms. This structure not only significantly increases the number of microorganisms per unit volume but also creates diverse microenvironments, providing suitable habitats for functional microbial groups such as nitrifiers, denitrifiers, and phosphorus-accumulating organisms.⁵ As a result, it can efficiently remove nutrients like nitrogen and phosphorus from pharmaceutical wastewater simultaneously, alleviating the issue of poor nitrogen and phosphorus removal efficiency in traditional processes.⁶ More importantly, AGS can withstand sudden changes in water quality and quantity. It is less susceptible to inhibition by high-concentration organic matter, antibiotics, and other toxic substances in pharmaceutical wastewater, ensuring stable operation of the treatment system. Additionally, AGS exhibits excellent sludge settling performance, producing only about 60% of the sludge volume compared to traditional processes.⁶ This greatly reduces sludge treatment costs and lowers the risk of secondary pollution, aligning with the current pursuit of green and low-carbon development in environmental protection. Recent studies have found that AGS can also effectively treat refractory organic compounds such as heterocyclic compounds in pharmaceutical wastewater.^{7,8} Its diverse microorganisms and extracellular polymeric substances (EPS) secreted by microorganisms cooperate with each other, enhancing the removal efficiency of these pollutants through methods like adsorption and biotransformation.⁹ Due to these characteristics, AGS technology has gradually become a key approach to overcoming the challenges in pharmaceutical wastewater treatment, providing practical technical support for the up-to-standard discharge and reuse of such refractory wastewater.

Nonylphenol (NP), a common endocrine disruptor, has attracted widespread global attention due to its environmental hazards.¹⁰ In 30 industrial wastewater treatment plants (IWWTPs), the detection rates of NP and octylphenol (OP) were 37% and 50%, respectively. In 12 of these plants, the concentrations of NP or OP in effluents were higher than those in influents.¹¹ NP is not uncommon in pharmaceutical wastewater, with actual detection often showing its concentrations in the microgram to milligram range—studies have indicated that NP levels in certain antibiotic production wastewater can reach

0.5–8 mg/L, which is often associated with the degradation of surfactants used in pharmaceutical processes or residual raw materials.¹² The environmental concern regarding NP stems from its potential for significant impact even at trace concentrations. At nanogram levels, NP can mimic estrogenic activity and disrupt the endocrine systems of organisms. Yuan et al.¹³ investigated the impact of NP on key microorganisms involved in biological nitrogen removal in sequencing batch reactors, and the results showed that NP interference increased microbial diversity, while all nitrogen-removing functional bacteria were inhibited by NP; among them, *Proteobacteria* and *Actinobacteria* were more sensitive to NP. For aquatic organisms, NP exposure can lead to abnormal sex differentiation and impaired reproductive capacity. In fish, for instance, male individuals may develop feminized traits. In invertebrates, NP accumulation can disrupt growth and developmental cycles, ultimately threatening the stability of aquatic food webs. More alarmingly, NP has strong bioaccumulation potential and can biomagnify through the food chain, eventually entering the human body via drinking water or aquatic products, increasing the risk of reproductive system diseases and endocrine disorders.¹⁴ In terms of sludge resource utilization, as NP dosage increases, the main methanogenic pathways change, and metagenomic analysis has shown that the gene expression of substrate transporters, biotransformation proteins, and product transporters is upregulated.¹⁵ Due to its significant ecotoxicity and health risks, the United Nations Environment Programme has included it in the list of priority control pollutants, and the effective removal of NP from pharmaceutical wastewater has thus become one of the key indicators for evaluating whether treatment processes meet standards. While the general inhibitory effect of NP on microbial communities, including those in wastewater treatment systems, has been reported,¹³ its specific impact on the intricate ecosystem of AGS remains poorly understood. Crucially, the coupled responses of AGS structure, function, microbial community, and metabolism under NP stress, along with quantitative thresholds for operational failure, have not been systematically elucidated. This knowledge gap hinders the reliable application of AGS for treating NP-containing pharmaceutical wastewater.

To address this knowledge gap, this study systematically investigated the multifaceted impact of NP on AGS treating pharmaceutical wastewater. By assessing the effects of different NP concentrations (0–10 mg/L) on pollutant removal, sludge characteristics, and microbial activity, this work aims to elucidate the coupled “structure–function–microbiome” mechanisms and define critical toxicity thresholds, thereby providing a theoretical basis for enhancing AGS process resilience against NP stress.

2 | MATERIALS AND METHODS

2.1 | Sources and properties of materials

The AGS used in this experiment was collected from a sequencing batch reactor (SBR) of a pharmaceutical wastewater treatment plant. The sludge was brownish in appearance, with a dense structure and

good settleability. After pretreatment, its basic characteristics were as follows: The particle size distribution, measured by a laser particle size analyzer (Model: Mastersizer3000), ranged from 200 to 1000 μm . The Total Suspended Solids (TSS) concentration was $3.8 \pm 0.2 \text{ g/L}$, and the Volatile Suspended Solids (VSS) concentration was $3.23 \pm 0.18 \text{ mg/L}$, with a VSS/TSS ratio of 0.85, indicating high microbial activity in the sludge. The 30-min sludge volume index (SVI) was $85 \pm 5 \text{ mL/g}$.

The wastewater used in the experiment was synthetic pharmaceutical wastewater. The composition of typical antibiotic production wastewater was formulated according to the composition of glucose and protein ketone as carbon sources, NH_4Cl as nitrogen source, and KH_2PO_4 as phosphorus source. It is noteworthy that the COD level of this synthetic wastewater was intentionally set lower than that of typical real pharmaceutical wastewater. This design serves to establish a stable and reproducible microbial baseline, allowing for the precise isolation and attribution of observed effects specifically to NP toxicity, rather than to the complex and variable background of recalcitrant organics and inhibitors present in actual wastewaters. Typical antibiotics (amoxicillin, ciprofloxacin) were added to simulate actual residues, and a small amount of heterocyclic compounds (10 mg/L) was added to represent refractory ingredients. The initial water quality indicators of the synthetic wastewater were detected as: COD $280 \pm 20 \text{ mg/L}$, $\text{NH}_4^+\text{-N}$ $40 \pm 0.5 \text{ mg/L}$, TP $15 \pm 0.3 \text{ mg/L}$, and pH 7.2 ± 0.2 . NP was of analytical grade (purity $\geq 99\%$) and purchased from Sigma-Aldrich. Its chemical formula is $\text{C}_{15}\text{H}_{24}\text{O}$, with a molecular weight of 220.35 g/mol (25°C). A 1000 mg/L NP stock solution was prepared by dissolving analytical-grade NP in methanol (chromatographic grade), followed by sonication at 40 kHz for 30 min (Model: KQ-500DE ultrasonic cleaner) to ensure complete dissolution of NP aggregates. During use, the stock solution was added dropwise to synthetic wastewater under magnetic stirring (300 rpm) for 1 h to avoid local precipitation, especially for high-concentration groups (5–10 mg/L). To verify the actual NP concentration in the wastewater, HPLC analysis was performed before each batch of experiments (detection method as described in 2.3), and the measured concentration deviated from the target value by $\leq 5\%$, confirming uniform dissolution without significant precipitation.

2.2 | Effect of NP on the operational performance of AGS in treating pharmaceutical wastewater

AGS was cultivated and operated in SBRs made of acrylic material, with an effective volume of 5 L, a diameter of 15 cm, and a height of 30 cm. Sampling ports were installed on the outer wall (5, 15, and 25 cm from the bottom) to monitor the characteristics of mixed liquor at different heights. The operation cycle was set to 8 h, consisting of specific phases: influent filling for 5 min (synthetic pharmaceutical wastewater was pumped in via a peristaltic pump, with a filling degree of 80%), aeration for 420 min (core reaction phase), settling for 30 min, and effluent discharge for 5 min (discharge ratio of 50%). Three cycles were operated daily to ensure sufficient pollutant

degradation and stable growth of granular sludge. DO concentration was controlled via linkage between an aeration device (silicone aeration disc) and an online DO monitor (Model: HQ30d), maintaining DO at $2.5 \pm 0.5 \text{ mg/L}$ during the aeration phase. Magnetic stirrers were activated during non-aeration phases (influent filling and the first 10 min of settling) with a stirring speed of 150 rpm, aiming to ensure uniform mixing of the mixed liquor without damaging the granular structure.

NP exposure concentrations were set at 4 gradients: 0 (control group), 1, 5, and 10 mg/L. The concentration selection was based on the following: (1) Referring to actual pharmaceutical wastewater monitoring data (NP concentrations are mostly 0.5–8 mg/L), 1 mg/L and 5 mg/L are close to typical pollution levels, used to simulate actual environmental exposure.¹⁶ (2) Combining preliminary pre-experiments and literature reports, the half-maximal inhibitory concentration (IC50) of NP on microorganisms is mostly 10–30 mg/L, so 10 mg/L and 20 mg/L were set to investigate high-concentration stress effects, with 20 mg/L allowing observation of the extreme response of granular structure and function.¹⁷ (3) The control group (0 mg/L) was used to exclude interference from other factors and clarify the independent effect of NP.

Other operational parameters were controlled as follows: Reaction temperature was maintained at $25 \pm 1^\circ\text{C}$ via a constant temperature water bath jacket. pH was monitored via an online pH meter (Model: PHS-3C), and 0.1 mol/L HCl or NaOH was added immediately for adjustment when pH deviated from 7.0 to 7.5, avoiding inhibition of microbial activity by extreme pH. Hydraulic retention time (HRT) was 16 h (calculated from reactor volume and daily effluent volume), and sludge retention time (SRT) was controlled at 18 ± 2 days by sludge discharge twice a week. Each NP concentration group was tested in three independent, parallel SBRs as biological replicates. These reactors were operated simultaneously but independently for 100 days, ensuring that the results reflect variability between distinct experimental units. To verify the stability of NP exposure doses during the reaction cycle, water samples were collected at 4 key stages of each 8-h SBR cycle: (1) immediately after influent filling (T0), (2) 1 h into aeration (T1), (3) at the end of aeration (T2), and (4) immediately before effluent discharge (T3). NP concentrations in these samples were determined via the HPLC method described in Section 2.3, with three parallel measurements per sample.

2.3 | Analytical methods

Detection of water quality indicators: COD and $\text{NH}_4^+\text{-N}$ were determined by the potassium dichromate method and Nessler's reagent method, respectively.¹⁸ TN was determined by alkaline potassium persulfate digestion-ultraviolet spectrophotometry, with detection at wavelengths of 220 nm and 275 nm after digestion.¹⁹ NP concentration was analyzed by high-performance liquid chromatography (HPLC, Agilent 1260) with a ZORBAX Eclipse XDB-C18 column ($4.6 \times 250 \text{ mm}$, $5 \mu\text{m}$). The mobile phase was methanol–water (85:15, vol/vol) at a flow rate of 1.0 mL/min, column temperature was 30°C ,

detection wavelength was 224 nm, injection volume was 20 μL , and the method detection limit was 0.01 mg/L.^{20–22}

Analysis of sludge indicators: TSS and VSS were determined by gravimetric method. SVI was measured according to standard methods: 100 mL mixed liquor was allowed to settle for 30 min, and the sludge volume was recorded. Granule size was determined using a laser particle size analyzer (Mastersizer 3000) with a detection range of 0.02–2000 μm , and each sample was measured in triplicate.²³

SEM measurements of AGS samples were carried out using a Hitachi SU8010 microscope at 5.0 kV. Prior to imaging, the samples were fixed with 2.5% glutaraldehyde, subjected to gradient dehydration, freeze-dried, and sputter-coated with gold. Measurements of the overall structure were performed at 500 $\times$ magnification, and higher-resolution measurements of surface details were performed at $\times 2000$ magnification.

The EPS fractions, including soluble EPS (S-EPS), loosely bound EPS (LB-EPS), and tightly bound EPS (TB-EPS), were extracted using a modified heat extraction method. Briefly, 50 mL of sludge sample was centrifuged (4000 $\times$ g, 10 min, 4°C), and the supernatant was collected as S-EPS. The pellet was resuspended in 0.05% NaCl and centrifuged (5000 $\times$ g, 15 min, 4°C) to obtain the LB-EPS fraction. For TB-EPS extraction, the remaining pellet was resuspended in 0.05% NaCl, heated at 60°C for 30 min, and then centrifuged (12,000 $\times$ g, 20 min, 4°C). The protein and polysaccharide contents in all EPS fractions were determined by the modified Lowry method and the anthrone-sulfuric acid method, respectively.^{24,25} Specific Oxygen Uptake Rate (SOUR) is measured by taking a sludge sample, monitoring the rate of oxygen consumption under aerobic conditions, and calculating it based on the oxygen consumed per unit mass of sludge over time.

Determination of microbial indicators: Microbial community structure was analyzed by high-throughput sequencing. Total DNA was extracted from 50 mL sludge samples using the FastDNA Spin Kit for Soil. PCR amplification was performed targeting the V3-V4 region

of the 16S rRNA gene with primers 338F (5'-ACTCCTACGGGAGG-CAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Purified amplification products were sequenced on the Illumina MiSeq platform.²⁶ After quality control using QIIME2 software and OTU clustering (97% similarity), species annotation and diversity analysis were conducted. After sequencing, raw reads were quality-filtered using QIIME2 (version 2022.8), and effective sequences per sample ranged from 45,238 to 52,610, with an average of 48,925. Rarefaction curves showed that OTU richness plateaued when sequencing depth exceeded 30,000 reads, and Good's coverage index for all samples was >0.99, indicating that sequencing depth sufficiently covered >99% of the OTUs in each sample.

2.4 | Statistical analysis

All experiments were carried out in three independent replicates, with results expressed as the mean $\pm$ standard deviation (SD). Variations between groups were assessed via one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons. A *p*-value less than 0.05 was deemed statistically significant, while *p* < 0.01 denoted a highly significant difference. All statistical evaluations were conducted using SPSS 22.0.

3 | RESULTS AND DISCUSSION

3.1 | Effects of NP on the performance of AGS in pharmaceutical wastewater treatment

Long-term exposure to NP can significantly affect the removal of COD in pharmaceutical wastewater treated by AGS. As shown in Figure 1, the COD removal rate in the 1 mg/L NP group (e.g., with

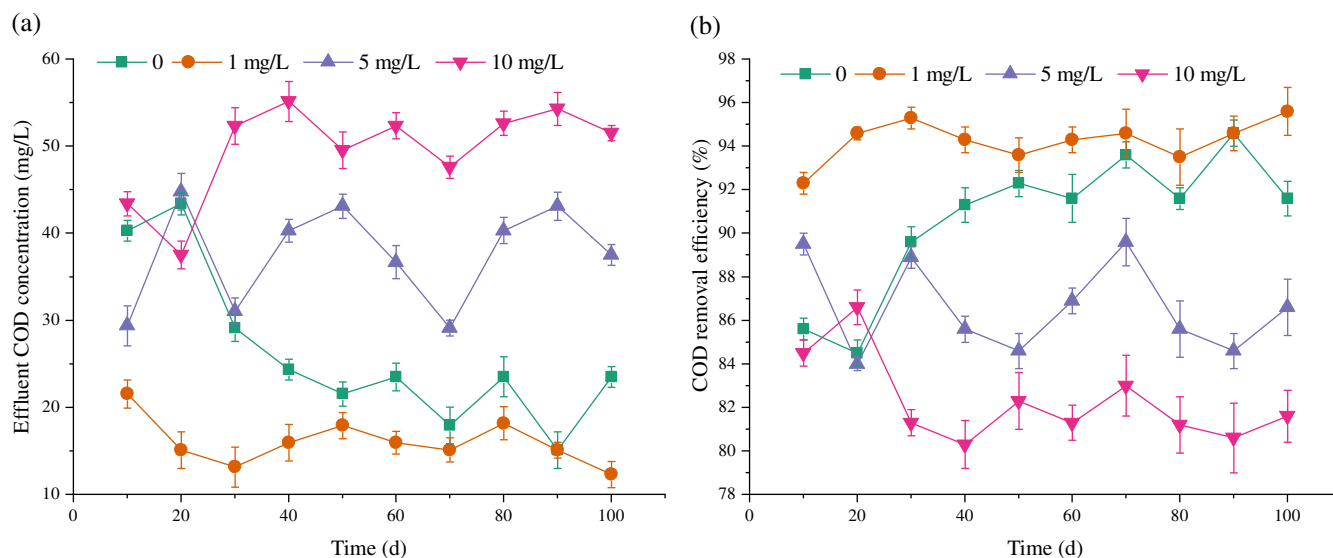


FIGURE 1 Effects of NP on effluent COD concentration (a) and removal efficiency (b) in the AGS system. Error bars represent the standard deviation of triplicate measurements. The influent COD was maintained at 280 ± 20 mg/L throughout the experiment.

influent COD ranging from 10 to 100 mg/L, the average removal rate reached 94.2%) was significantly higher than that in the control group (average 89.6%), with the corresponding effluent COD concentration being the lowest (average 16.8 mg/L) ($p < 0.01$). It is speculated that low-concentration NP enhances COD metabolic capacity by inducing microorganisms to synthesize degrading enzymes (such as extracellular oxidoreductases).²¹ The removal rate in the 5 mg/L NP group (average 88.7%) decreased slightly but remained higher than that in the control group, indicating a weakening of the stimulatory effect at moderate concentrations. The removal rate in the 10 mg/L NP group (average 83.5%) decreased significantly ($p < 0.01$), with effluent COD increasing (average 38.6 mg/L), reflecting that high-concentration NP interferes with cell membrane fluidity, impedes substrate transport and electron transfer, and inhibits the metabolism of heterotrophic microorganisms.²⁷ This trend is consistent with the hormesis effect: low-concentration NP triggers stress responses to optimize metabolism, while high concentrations exceed the tolerance threshold, damage cell functions, and ultimately reduce COD degradation efficiency. Low-concentration NP stress activates the expression of key enzymes (such as hexokinase and citrate synthase) in pathways like glycolysis and the tricarboxylic acid cycle, accelerating the decomposition of organic matter. When NP concentration is ≥ 10 mg/L, high-concentration NP triggers lipid peroxidation, damages the cell

membrane structure, reduces the activity of substrate transporters, and restricts the entry of organic matter into cells.^{28,29} NP concentrations remained stable throughout the SBR cycle across all exposure groups (Table 1). Measured values deviated by less than 5% from target concentrations (1, 5, and 10 mg/L), indicating consistent dosing with only minor loss, likely due to marginal adsorption or slow biodegradation. No NP was detected in the control group. These results confirm a reliable exposure basis for the dose-effect analysis.

Figure 2 shows the impact of NP on the removal efficiency of $\text{NH}_4^+\text{-N}$ and TN in pharmaceutical wastewater treated by AGS. The effect of NP on nitrogen removal by AGS is characterized by a higher tolerance threshold for ammonia oxidation than for complete nitrification-denitrification. At 5 mg/L NP, although $\text{NH}_4^+\text{-N}$ removal remained relatively high (90.2%), a severe nitrite accumulation was observed (effluent $\text{NO}_2^-\text{-N}$: 7.2 mg/L vs. 0.2 mg/L in the control, Table S1), indicating specific inhibition of the nitrite oxidation step. This blockage led to a sharp decline in TN removal (70.3%). At 10 mg/L NP, both ammonia nitrogen (88.7%) and TN (63.1%) decreased simultaneously, with the decline in TN being more prominent. NP affects $\text{NH}_4^+\text{-N}$ and TN removal primarily through its impact on functional microbial communities and metabolic processes. The regulatory effects of NP concentrations on nitrogen metabolism and electron transfer efficiency of AGS are directly evidenced by enzyme

TABLE 1 Dynamic NP concentrations during the 8-hour SBR cycle (mean $\pm$ SD, $n = 3$, mg/L).

NP set concentration (mg/L)	Influent NP (mg/L)	1 h post-aeration	Aeration end	Pre-effluent	Max deviation (%)
0	ND	ND	ND	ND	/
1	1.02 $\pm$ 0.05	1.00 $\pm$ 0.02	0.98 $\pm$ 0.02	0.95 $\pm$ 0.03	4.9
5	5.15 $\pm$ 0.12	5.05 $\pm$ 0.12	4.98 $\pm$ 0.10	4.90 $\pm$ 0.13	4.0
10	10.3 $\pm$ 0.21	10.1 $\pm$ 0.19	9.9 $\pm$ 0.18	9.7 $\pm$ 0.20	4.9

Note: ND = Not detected (below HPLC detection limit of 0.01 mg/L). Deviation = Actual concentration–Set concentration/Set concentration $\times$ 100%.

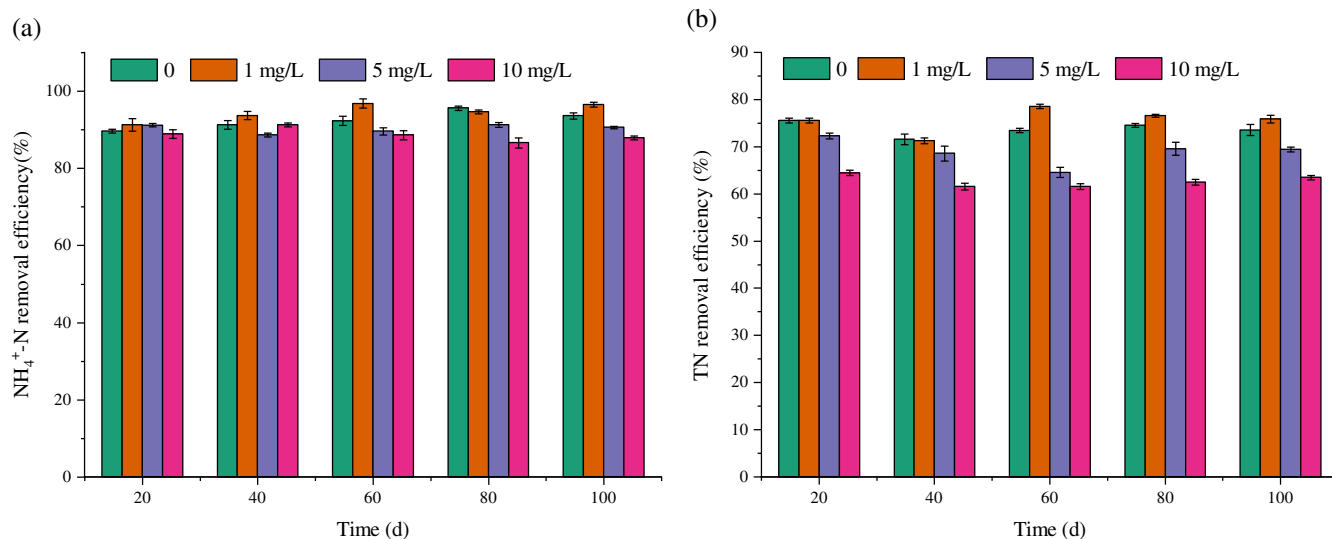


FIGURE 2 Effects of NP on $\text{NH}_4^+\text{-N}$ (a) and TN (b) removal efficiency in the AGS system. Error bars represent the standard deviation of triplicate measurements. The influent $\text{NH}_4^+\text{-N}$ and TN concentrations were maintained at 40 ± 0.5 mg/L and 50 ± 0.5 mg/L, respectively.

activities and functional gene expression. The impact of NP on key enzymes and functional genes in the AGS system is presented in Table S2. At 1 mg/L NP, AMO activity (12.8 U/mg VSS) and *amoA* expression (1.32-fold) show slight increases while NOR and Cyt c oxidase activities remain stable, which correlates positively with the improved NH_4^+ -N removal rate (94.2%) at this concentration. At 5 mg/L NP, NOR activity drops sharply by 38.8% (5.2 U/mg VSS) and *nrxA* is downregulated to 0.61-fold, whereas AMO remains stable. This mismatch directly induces nitrite accumulation (7.2 mg/L) and reduces the TN removal rate to 70.3%. At 10 mg/L NP, AMO (36.2% reduction), NOR (75.3% reduction) and Cyt c oxidase (45.5% reduction) activities are comprehensively inhibited, and the expressions of *amoA*, *nrxA*, and *cyt c ox* are significantly downregulated. These changes not only disrupt the function of key enzymes in nitrogen metabolism but also block the electron transfer chain, ultimately causing a sharp decline in NH_4^+ -N (88.7%) and TN (63.1%) removal rates and the complete deterioration of AGS nitrogen removal efficiency.^{30,31} These mechanistic insights are further supported by

microbial community analysis (Section 3.4), where the abundance of *Nitrospira* decreased by 62% at 10 mg/L NP, consistent with the downregulation of *nrxA* and NOR activity.

3.2 | Effects of NP on the physicochemical properties of AGS

The sludge characteristics of AGS are the core foundation for maintaining its functional integrity. They directly affect the metabolic activity and synergistic effects of microbial communities, determine pollutant removal efficiency, and are crucial for ensuring the stable and efficient operation of AGS systems. Figure 3 shows the impact of long-term NP exposure on the sludge characteristics of AGS. As shown in Figure 3a,b, the TSS concentration of AGS and the proportion of organic matter (VSS/TSS) exhibit a structural-functional synergistic response under NP exposure. The TSS in the 1 mg/L NP group (reaching 6.28 g/L at 100 days) is higher than that in the control group

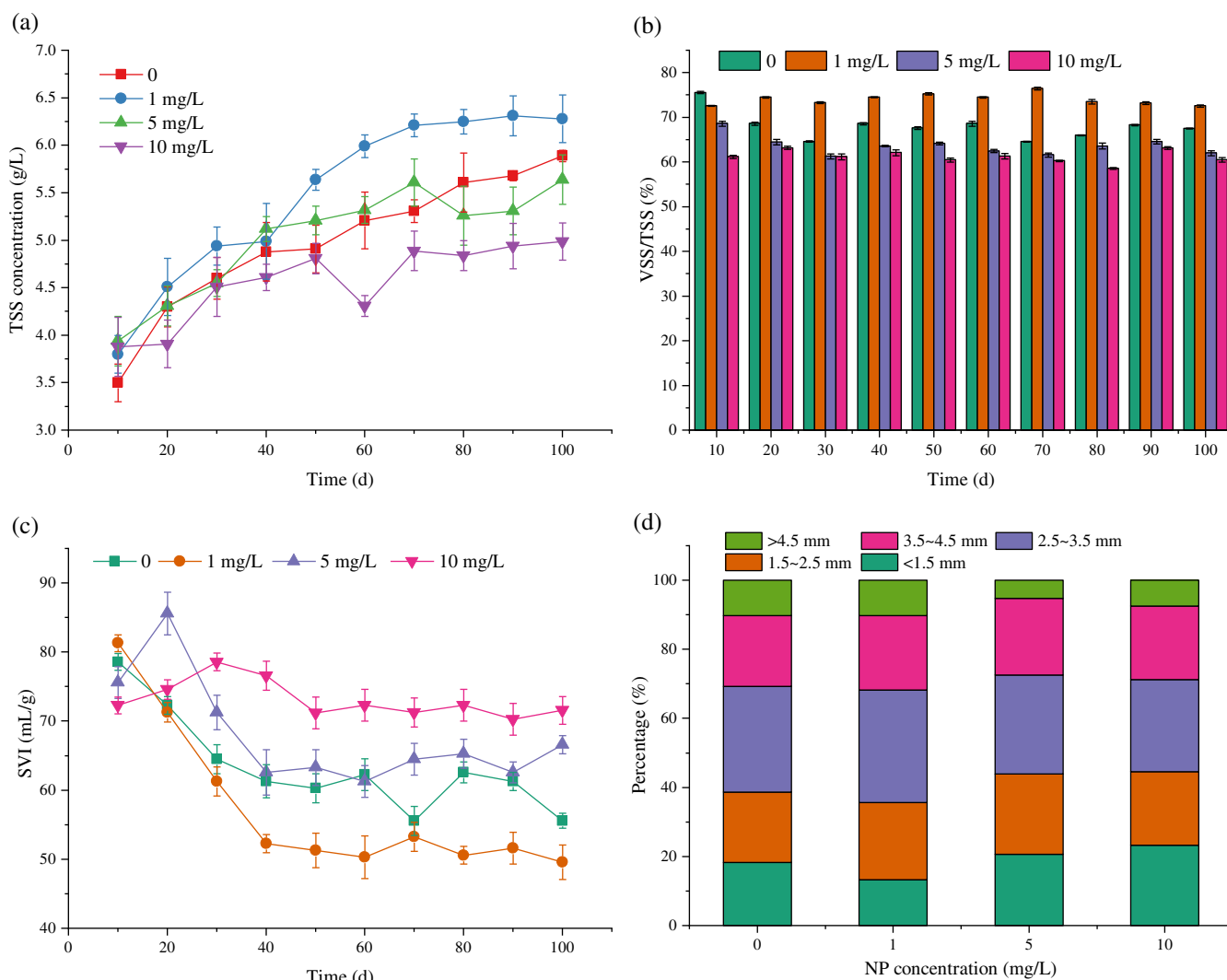


FIGURE 3 Effects of NP on the sludge characteristics of AGS (a) TSS; (b) VSS/TSS; (c) SVI; (d) sludge particle size. Error bars represent the standard deviation of triplicate measurements.

(5.89 g/L), and the average VSS/TSS of 73.5% is also higher than that of the control group (69.8%). The TSS in the 5 mg/L group (5.64 g/L) is close to that of the control group, while the VSS/TSS drops to 65.2%. But, the TSS in the 10 mg/L group (4.99 g/L) decreases significantly, and the VSS/TSS continues to drop to 61.2% ($p < 0.01$). Low-concentration NP (1 mg/L) promotes particle aggregation by inducing microorganisms to secrete protein-based EPS, which not only enhances TSS accumulation but also optimizes substrate interception due to the increased proportion of VSS, corresponding to the “low-promotion effect” of COD and ammonia nitrogen removal in this group—the denser particle structure enhances the synergistic metabolic efficiency of the microbial community. When NP concentration is ≥ 5 mg/L, especially 10 mg/L, high-concentration NP triggers microbial apoptosis, leading to VSS degradation and a decrease in VSS/TSS. At the same time, the particles lose the adhesive support of EPS due to microbial death, resulting in a loose structure, and TSS growth stagnates or even decreases.³² This chain reaction, which progresses from the loss of organic matter to structural disintegration, is fundamentally linked to the observed pattern in which total nitrogen removal declines prior to the inhibition of COD at high NP concentrations. The attenuation of microbial activity begins with the impairment of functional bacteria and extends to the loss of key structural-supporting bacteria, such as phosphorus-accumulating organisms and filamentous bacteria. This cascade ultimately amplifies the inhibition of pollutant removal by compromising granule stability and, consequently, reducing the efficiency of substrate contact. The declining rate of VSS/TSS exhibited a positive correlation with NP concentration, which reflects the progressive attenuation of microbial activity. Meanwhile, the delayed change in TSS relative to VSS indicates a transition in granule state from enhanced aggregation to structural disintegration and loosening. Collectively, these responses elucidate the dynamic shift in AGS treatment capacity from low-concentration promotion to high-concentration inhibition, driven by the coupled degradation of sludge structure and microbial activity that underpins the gradient decline in pollutant removal efficiency.

The impact of NP on the settling property of AGS exhibits a concentration-driven “dynamic game” characteristic (Figure 3c). In the 1 mg/L NP group, the initial SVI (81.3 mL/g at 10 days) was slightly higher than that of the control group (78.6 mL/g), while in the later stage (dropping to 49.6 mL/g at 100 days), it was conversely lower than the control (55.6 mL/g). In the 5 mg/L group, the SVI surged to 85.6 mL/g at 20 days and then fell back to 66.6 mL/g in the later stage. While, the SVI in the 10 mg/L group remained higher than the control throughout the period (average 72.3 vs. 61.8 mL/g in the control). The bidirectional effect observed at low NP concentration (1 mg/L) originates from a process of structural stress repair. Initially, NP stimulation induces microorganisms to secrete EPS; however, uneven distribution of the newly secreted EPS results in transient particle loosening, reflected in a slight increase in the sludge volume index. With prolonged exposure, granules become progressively densified through synergistic EPS restructuring and microbial community adaptation, evidenced by rising total suspended solids and an optimized volatile fraction. This ultimately leads to settling performance

that surpasses that of the control. The enhanced structural stability thereby supports microbial metabolism, corresponding to the observed promotion in COD and ammonia-nitrogen removal.³³ At 5 mg/L NP, the mid-term damage to NOB causes accumulation of nitrite nitrogen, and local gas production from denitrification destroys particle integrity (sharp rise in SVI). In the later stage, microbial apoptosis leads to particle shrinkage, and although SVI decreases, the degree of settling property repair is weaker than that of the low-concentration group due to the decrease in VSS/TSS (organic matter loss), accompanied by a significant decline in TN removal efficiency. At 10 mg/L NP, high-concentration toxicity continuously damages cell membranes and microbial interactions, keeping particles on the edge of “looseness-disintegration” (reduced particle size), resulting in persistently high SVI, and its fluctuations reflect the loss of structural stability, which is deeply coupled with the “high-inhibition effect” of COD and nitrogen removal.¹⁰ The deterioration of settling property therefore stems from a synergistic collapse involving both the loss of structural-supporting bacteria (e.g., filamentous bacteria) and the damage to functional bacteria. This initiates a systematic failure that progresses from the deterioration of the physical sludge structure to the impairment of microbial function, ultimately amplifying the inhibition of pollutant removal. The dynamic variation of SVI thus serves as an intuitive indicator of the interplay between structural repair and functional damage under NP stress. At low NP concentrations, an adaptive reconstruction process is triggered, characterized by structural optimization that leads to improved settleability and consequently enhances treatment efficiency. In contrast, high NP concentrations exceed the toxicity threshold, initiating a sequence of structural collapse, deteriorating settleability, and a sharp decline in efficiency. This pattern fully illustrates the gradient response of AGS to NP stress, ranging from tolerance adjustment to complete collapse and disintegration.

Figure 3d shows the effect of NP on the particle size of particulate sludge during the stable period of AGS operation. It can be found that NP exerts low-concentration-promoted aggregation and high-concentration-promoted fragmentation effects on AGS particle size distribution. At 1 mg/L NP, the proportion of particles <1.5 mm decreased from 18.3% to 13.3%, while the proportions of 2.5–4.5 mm particles increased (30.6% $\rightarrow$ 32.6%, 20.5% $\rightarrow$ 21.6%). Low-concentration NP induces EPS secretion to enhance particle adhesion, leading to the aggregation of small particles into medium and large ones, optimizing the mass transfer structure. At 5–10 mg/L, the proportion of particles <1.5 mm rose to 20.6%–23.3%, and the proportion of particles >4.5 mm decreased from 10.3% to 5.2%–7.5%. High-concentration NP damages microbial membrane structures, causes failure of EPS adhesion, and leads to the disintegration of large particles into small ones. The gradient change of particle size is coupled with pollutant removal: particle size optimization at low concentrations facilitates degradation, while fragmentation at high concentrations amplifies efficiency inhibition due to reduced specific surface area and hindered microbial synergy, revealing the internal logic of NP mediating AGS function through “structural regulation.”

SEM images (Figure S1) further confirm this key difference between the control and high NP groups. The control group (0 mg/L)

presents intact and dense large aggregates with smooth surfaces and no cracks. Microbial cells are tightly packed, which matches the high proportion of medium-large and large particles in the control. In contrast, the 10 mg/L NP group shows severe fragmentation. Originally intact large granules break into numerous loose small clusters, most of which are smaller than 3.0 mm. The surface is covered with obvious cracks and scattered single cells can be observed. High-magnification images also show disrupted cell aggregation in the 10 mg/L group, which is in sharp contrast to the compact cell arrangement in the control.

3.3 | Effects of NP on the content and composition of EPS in AGS

EPS plays a crucial role in maintaining the structural stability of AGS, facilitating microbial aggregation, protecting against environmental stressors, and regulating substrate transport, thereby being vital for

sustaining the functional integrity and pollutant removal efficiency of AGS systems.^{34,35} Figure 4 shows the effects of long-term NP exposure on the content of EPS and the content of stratified EPS in AGS. NP exerts a concentration-dependent regulatory effect on the EPS components in AGS: it promotes active secretion at low concentrations, whereas it induces passive release at high concentrations. The total EPS content in the 1 mg/L NP group (average 94.5 mg/g) was higher than that in the control group (92.8 mg/g), with a significant increase in TB-EPS (tightly bound EPS, average 35.2 mg/g). This reflects that microorganisms actively secrete TB-EPS to strengthen granule structure under low-concentration stress, corresponding to the “low-promotion effect” on granule size aggregation, settling optimization, and pollutant removal in this group. SB-EPS (slightly bound EPS, average 70.3 mg/g) also increased simultaneously, enhancing substrate adsorption and assisting in COD degradation. When NP concentration was ≥ 5 mg/L, TB-EPS in the 10 mg/L group surged to 44.4 mg/g in the later stage, which actually resulted from the leakage of intracellular substances due to cell apoptosis (passive release)

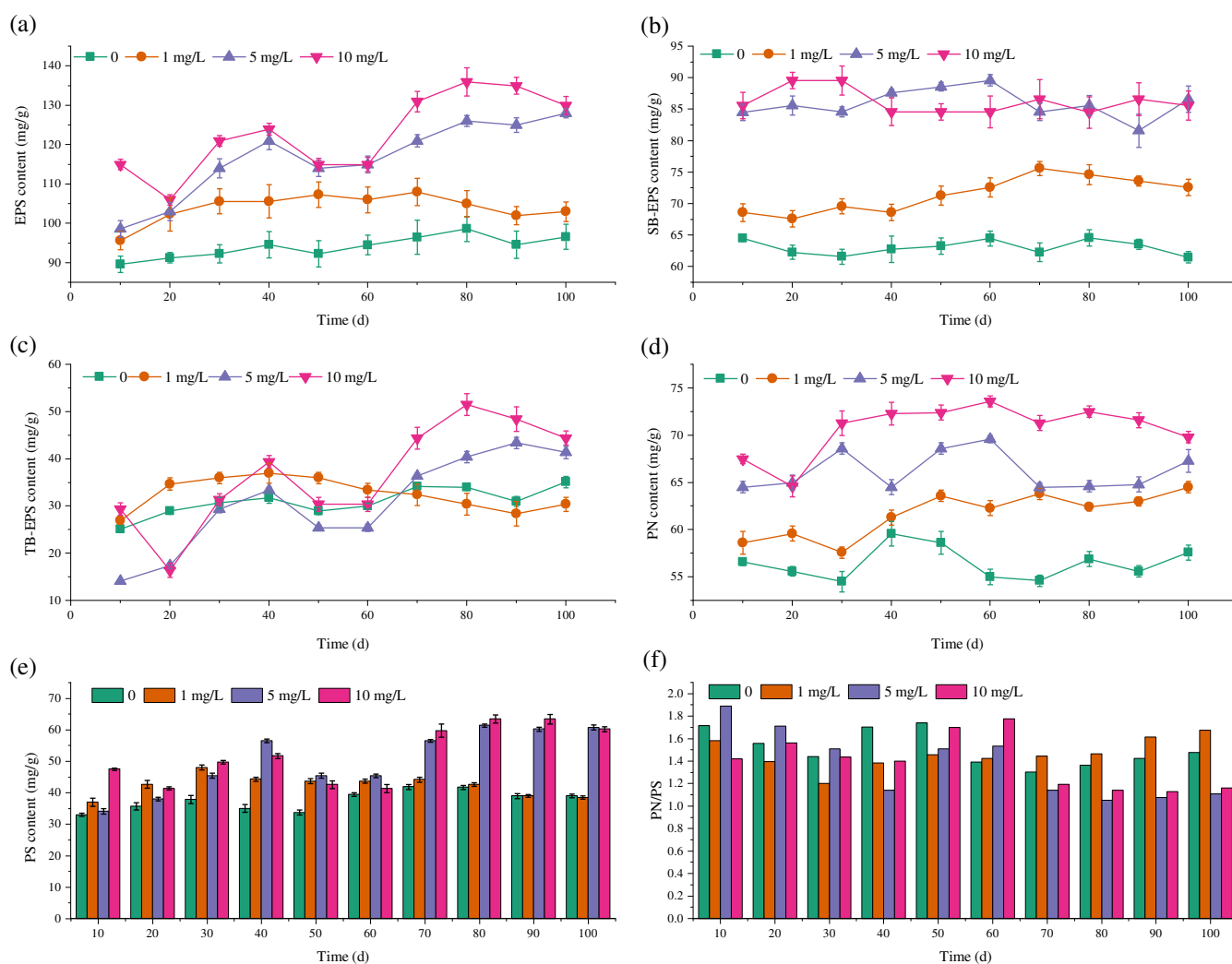


FIGURE 4 Effects of NP on the content and components of EPS in the AGS system (a) Total EPS; (b) SB-EPS; (c) TB-EPS; (d) PN; (e) PS; (f) PN/PS. Error bars represent the standard deviation of triplicate measurements.

rather than functional enhancement.³⁶ SB-EPS fluctuated due to granule disintegration (average 85.2 mg/g in the 5 mg/L group and 84.8 mg/g in the 10 mg/L group), with its adhesive function failing. The transition from active EPS enhancement to passive disintegration is closely linked to both AGS structure (e.g., particle size, settleability) and function (e.g., pollutant removal). At low NP concentrations, active TB-EPS secretion helps build a stable granular structure that supports microbial metabolism. In contrast, high NP concentrations cause cell damage, triggering passive TB-EPS release and the failure of SB-EPS adhesion, which accelerates granule disintegration and reduces treatment efficiency. These dynamics highlight the central role of EPS in mediating the coordinated response of AGS structure and function under NP stress.³⁷ NP exerts characteristics of “low-concentration-promoted synergistic regulation and high-concentration-induced imbalance and disintegration” on EPS components and PN/PS ratio in AGS: at 1 mg/L NP, PN (average 62.3 mg/g) and PS (average 42.5 mg/g) increased moderately synchronously, with a slight adjustment in the PN/PS ratio (e.g., 1.58 at 10 days vs. 1.71 in the control). This reflects that microorganisms actively synthesize proteins to enhance electron transfer (supporting enzyme activity for COD degradation) and polysaccharides to optimize granule adhesion (corresponding to the increased proportion of medium and large particles). Through the “metabolism-structure” synergy of EPS, they maintain granule compactness (particle size aggregation) and settling stability (later decrease in SVI), supporting the “promotion effect” on COD and ammonia nitrogen removal under low concentrations. When NP concentration was ≥ 5 mg/L, although PN continued to increase (reaching 69.8 mg/g at 100 days in the 10 mg/L group), it was actually due to the leakage of intracellular proteins caused by cell apoptosis; PS surged in the later stage of high concentrations (reaching 60.2 mg/g at 100 days in the 10 mg/L group vs. 39 mg/g in the control), resulting from passive release of polysaccharides induced by membrane damage. Together, they drove a fluctuating decrease in the PN/PS ratio (dropping to 1.108 at 100 days in the 5 mg/L group vs. 1.476 in the control), disrupting the functional balance of EPS–protein-mediated electron transport chains were blocked, and polysaccharide adhesion failed (corresponding to granule disintegration and increased proportion of small particles). This cascade, initiating with structural disintegration and leading to disrupted microbial synergy and insufficient metabolic drive, ultimately amplifies the inhibitory effect of high-concentration NP on COD and nitrogen removal. This illustrates that the imbalance in EPS component proportions serves as the core molecular mechanism driving the transition of AGS from a state of low-concentration adaptation to high-concentration functional collapse.

3.4 | Effects of NP on microbial metabolic activity in AGS

Exogenous pollutants can affect the microbial metabolic activity in AGS, and SOUR serves as an effective indicator to assess such metabolic activity.³⁸ As shown in Figure 5, NP induces a metabolic

response of low promotion and high inhibition on the SOUR of AGS: The SOUR in the 1 mg/L NP group (average 48.7 mg O₂/g VSS-h) was consistently higher than that in the control group (45.3 mg O₂/g VSS-h), reflecting that low-concentration stress activates microbial respiratory metabolism. This is achieved by upregulating the activity of electron transport chain enzymes (e.g., cytochrome c oxidase) and promoting the proliferation of functional bacteria (phylum Proteobacteria), which provides energy for COD degradation (enzyme synthesis) and EPS secretion (energy supply). This corresponds to the characteristics of compact granule structure (particle size aggregation) and enhanced pollutant removal in the “low-promotion” stage. In the 5 mg/L NP group, SOUR was equal to the control in the early stage (both 48.5 at 20 days) but gradually decreased in the later stage (42.6 vs. 43.6 in the control at 100 days), suggesting that the respiratory function of sensitive bacteria such as nitrite-oxidizing bacteria (*Nitrospira*) began to be impaired, but initially still relied on compensation from ammonia-oxidizing bacteria (*Nitrosomonas*) and others. The SOUR in the 10 mg/L NP group was consistently lower than the control. High-concentration NP damages cell membrane integrity and chelates respiratory enzyme cofactors, leading to the collapse of microbial metabolic drive.^{39–41} This is directly associated with the sharp decline in the abundance of core nitrogen cycle bacteria and blocked denitrification electron transport, forming a negative feedback loop of metabolism-structure-function together with the sharp drop in TN removal rate and granule structure disintegration. The dynamic changes in SOUR essentially reflect the gradient response of microbial metabolic activity to NP toxicity: low concentrations trigger “metabolic compensation,” while high concentrations exceed the “respiratory threshold.” This explains the intrinsic mechanism of AGS treatment efficiency transitioning from “enhancement” to “collapse” from the perspective of energy metabolism.

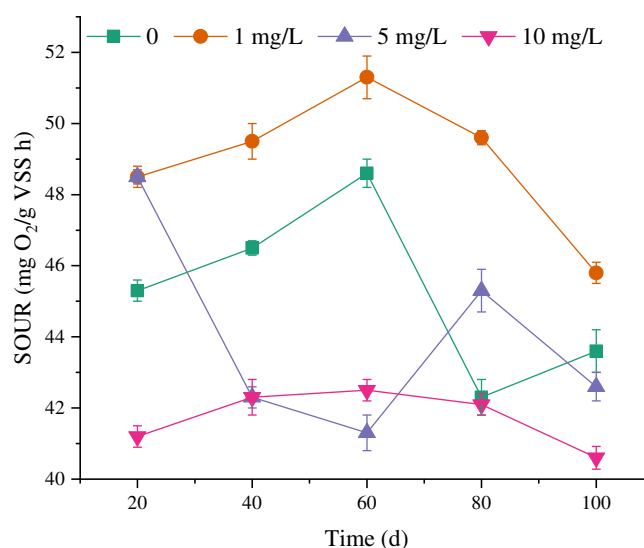


FIGURE 5 Effects of NP on SOUR activity in the AGS system. Error bars represent the standard deviation of triplicate measurements.

3.5 | Effects of NP on microbial diversity and community structure in AGS

The microbial community structure is the core foundation for maintaining the operational efficiency of AGS, and its composition and functions directly determine the pollutant removal efficiency.⁴² Therefore, exploring the impact of NP on microbial diversity is crucial for analyzing the response mechanism of AGS systems. Microbial Alpha diversity was analyzed via high-throughput sequencing (Table 2, with Good's coverage all >97%). In the 1 mg/L NP group, Chao1 (2412 ± 38), Ace (2405 ± 36), and PD whole tree (130.2 ± 4.8) increased slightly synchronously, and the Shannon index (7.95 ± 0.12) was also higher than that of the control group. This reflects that low-concentration NP activates stress-tolerant rare bacteria (e.g., specific clades of *Pseudomonas*), supplements metabolic potential through the expansion of phylogenetic diversity, and supports the “low-promotion effect” on COD and ammonia nitrogen removal—the microbial community not only maintains core clades of nitrification/denitrification but also incorporates new functional bacteria to enhance stress metabolism. When NP concentration was ≥5 mg/L, Chao1 and Ace dropped sharply (decreasing by 7.1% and 7.3% respectively in the 5 mg/L group), PD whole tree shrank (decreasing by 10.6%), and the Shannon index plummeted (decreasing by 10.7% in the 5 mg/L group). This reveals that NP toxicity damages phylogenetic barriers: *Nitrospira* and denitrifying *Vibrio* (core of denitrification) were lost due

to membrane damage and enzyme inhibition, and the microbial community degenerated from a “synergistic network” to “monotony of pollutant-tolerant bacteria,” directly cutting off the functional chain of nitrogen cycle (sharp drop in TN removal rate) and simultaneously weakening the synergy of COD degradation.^{43–45} This diversity collapse forms a vicious cycle with granule disintegration, confirming that diversity is the cornerstone of AGS “structure–function” stability. By destroying this cornerstone, NP drives the qualitative transition of the system from “low-promotion adaptation” to “high-inhibition collapse” at the microbial community level.

The effect of NP on the microbial community at the phylum level in AGS presents a bifurcated differentiation characterized by the attenuation of functional phyla and the enrichment of pollution-tolerant phyla (Figure 6). As shown in Figure 6a, the abundance of *Proteobacteria* (containing functional bacteria such as AOB and denitrifying bacteria) slightly increased (45.1%) under 1 mg/L NP, consistent with its “low-concentration promoting effect” of enhancing ammonia oxidation by upregulating the *amoA* gene. Meanwhile, the abundance of *Bacteroidetes* (involved in organic matter degradation) remained stable, supporting efficient COD removal. *Nitrospirae* slightly increased (5.9%) at 1 mg/L, ensuring smooth nitrite oxidation, which was consistent with stable TN removal. When NP concentration was ≥5 mg/L, the abundance of *Proteobacteria* dropped sharply (32.8% in the 10 mg/L group), and *Nitrospirae* nearly collapsed (only 1.5% in the 10 mg/L group), directly leading to insufficient ammonia oxidation

NP concentrations (mg/L)	Chao1	Ace	PD whole tree	Shannon
0	2356 ± 42	2348 ± 39	125.6 ± 5.2	7.82 ± 0.15
1	2412 ± 38	2405 ± 36	130.2 ± 4.8	7.95 ± 0.12
5	2189 ± 35	2176 ± 34	112.3 ± 6.1	6.98 ± 0.18
10	1965 ± 45	1952 ± 43	105.5 ± 5.7	6.23 ± 0.21

TABLE 2 Alpha diversity indices of the microbial community in AGS under different NP concentrations.

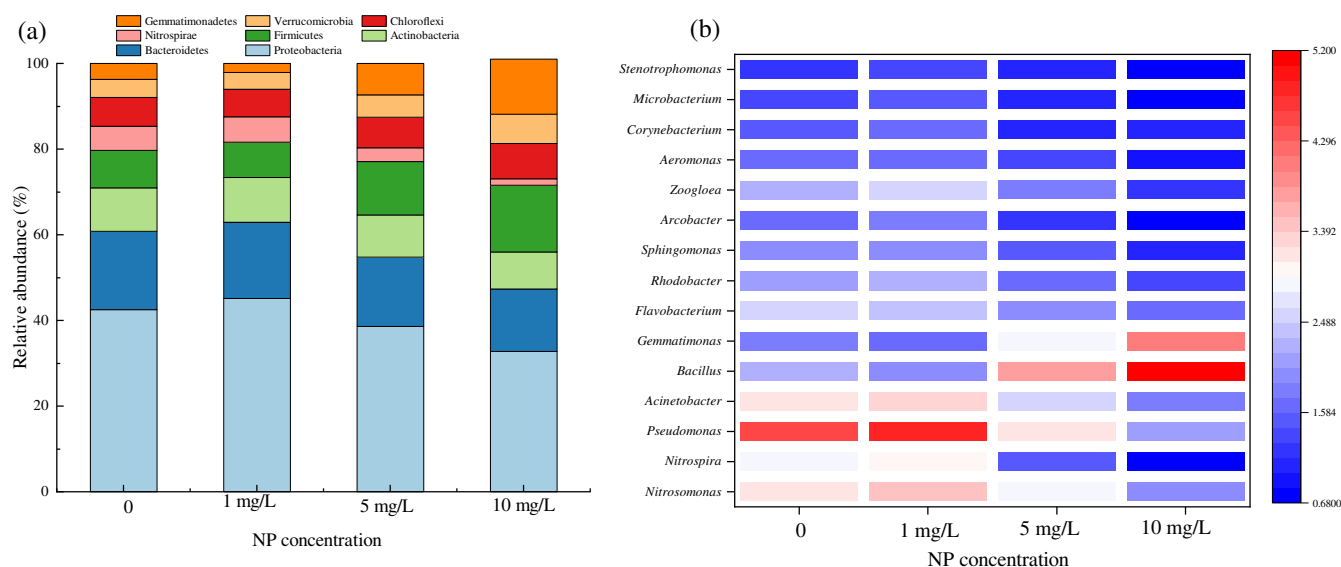


FIGURE 6 Effects of NP on the relative abundance of microorganisms in AGS at the phylum level (a) and genus level (b).

power, nitrite accumulation (e.g., effluent NO_2^- -N in the 5 mg/L group reached 7.2 mg/L), and a plummeting TN removal rate. At the same time, the abundances of *Firmicutes* (stress-tolerant) and *Gemmatimonadetes* (toxin-tolerant) increased significantly (reaching 15.6% and 12.9%, respectively in the 10 mg/L group) ($p < 0.01$). *Chloroflexi* and *Verrucomicrobia* increased slowly with the rise of NP concentration (8.2% and 6.8% respectively in the 10 mg/L group), which may obtain nutrients by decomposing EPS residues, indirectly reflecting the disintegration of particle structure.⁴⁶ This community reconstruction of “decline of functional phyla (*Proteobacteria*, *Nitrospirae*)—expansion of pollution-tolerant phyla (*Firmicutes*, *Gemmatimonadetes*)” confirms at the phylum level that NP drives AGS to shift from “synergistic metabolism” to “disordered tolerance” by destroying the core flora of nitrogen cycle and replacing functionally redundant bacteria, ultimately leading to the synchronous deterioration of nitrogen removal efficiency and particle stability.

The effect of NP on microorganisms at the genus level in AGS presents a precise targeting effect (Figure 6b). The decline of functional genera and the enrichment of pollutant-tolerant genera occur. At low NP concentration (1 mg/L), the abundances of ammonia-oxidizing bacteria *Nitrosomonas* (3.5%) and nitrite-oxidizing bacteria *Nitrospira* (3.0%) slightly increased, and denitrifying functional genera *Pseudomonas* (4.8%) and *Acinetobacter* (3.3%) increased synchronously. These bacteria enhance nitrogen metabolism by upregulating functional genes such as *amoA* and *nirS*, which is consistent with the characteristics of improved ammonia nitrogen and TN removal rates, as well as active secretion of PN in EPS (optimizing electron transfer) in this group. Meanwhile, *Zoogloea* (2.5%), a key genus for EPS synthesis, slightly increased in abundance, supporting granule adhesion and particle size aggregation (increased proportion of medium and large particles in the 1 mg/L group). When NP concentration was ≥ 5 mg/L, core nitrogen cycle genera significantly declined ($p < 0.01$). *Nitrospira* was the most sensitive to NP, with its abundance in the 10 mg/L group dropping to only 0.7% (2.8% in the control), directly causing blocked nitrite oxidation (nitrite accumulation) and a sharp drop in TN removal rate. The abundances of *Nitrosomonas* (1.9%) and denitrifying genera *Pseudomonas* (2.1%) and *Arcobacter* (0.8%) decreased sharply, weakening the coupling efficiency of ammonia oxidation and denitrification, corresponding to the “cliff-like decline” of nitrogen removal at high concentrations.⁴⁷ Correspondingly, the abundances of pollutant-tolerant genera *Bacillus* (5.2%) and *Gemmatimonas* (4.1%) increased significantly with rising NP concentration ($p < 0.01$). Although *Bacillus* can include denitrifying species, its marked enrichment under high NP stress was associated with a sharp decline in TN removal, suggesting its potential denitrification function was likely suppressed, or its role was dominated by stress tolerance rather than effective nitrogen metabolism in this specific context. The abundances of organic matter-degrading genera such as *Flavobacterium* and *Sphingomonas* decreased, directly associated with the inhibitory effect on COD removal rate at high concentrations. This community reconstruction of “attenuation of functional genera (*Nitrosomonas*, *Nitrospira*, *Pseudomonas*)—expansion of pollutant-tolerant genera (*Bacillus*, *Gemmatimonas*)” reveals at the micro level how NP drives AGS to shift from

“efficient metabolism-stable structure” to “functional disorder-structure disintegration” by destroying core bacteria involved in the nitrogen cycle and structural support, forming a complete chain of evidence with previous changes in sludge characteristics and treatment efficiency.⁴⁸

3.6 | Implementation significance and prospects

This study systematically reveals the mechanism of NP's influence on the efficiency of AGS in treating pharmaceutical wastewater, sludge characteristics, and microbial communities, clarifies the toxicity threshold of NP and the “low promotion and high inhibition” response pattern, providing quantitative parameter support for optimizing the treatment process of NP-containing industrial wastewater. By analyzing the correlation between EPS component imbalance, functional microbial community decline, and treatment efficiency attenuation, a coupled regulatory model of “pollutant-sludge structure-microbial function” has been established, which provides a theoretical basis for developing toxicity-resistant AGS systems (e.g., by inoculating NP-tolerant functional bacteria or regulating EPS synthesis). While this study employed a synthetic wastewater with a comparatively low COD and readily biodegradable carbon sources to mechanistically elucidate the specific impact of NP on AGS, it is important to consider the implications for real-world applications. The absolute values of the toxicity thresholds and performance responses reported here might be modulated in full-strength pharmaceutical wastewater, where high organic loads, antibiotics, and other co-pollutants are present and could interact with NP. Therefore, the core contribution of this work lies in revealing the fundamental response patterns and mechanisms of AGS to NP stress. Translating these findings to industrial settings should involve careful consideration of these additional factors in future validation studies.

Future research can be further extended to the combined toxicity effects of NP and other typical pharmaceutical and personal care products, exploring the synergistic response mechanisms of AGS under the coexistence of multiple pollutants. Meanwhile, gene editing technology can be combined to enhance the NP tolerance of functional bacteria (e.g., *Nitrospira*, *Pseudomonas*), or the shock resistance of the system can be improved by targeted regulation of microbial interaction networks. In addition, functional recovery strategies of AGS under long-term operation conditions (such as biochar carrier enhancement and carbon source optimization) and cost-benefit analysis in practical engineering applications will be important directions to promote the translation of research results, helping to build a more robust biological treatment technology system for industrial wastewater.^{49,50}

4 | CONCLUSIONS

NP exerts a concentration-dependent threshold effect on AGS, revealing novel system-specific mechanisms beyond general hormesis.

At 1 mg/L, NP induces secretion of TB-EPS, promoting granule aggregation and settleability. It enriches functional bacteria like *Nitrosomonas* and *Pseudomonas*, increasing community diversity (Shannon index 7.95) and metabolic activity (SOUR 48.7 mg O₂/g VSS·h), thereby improving COD (94.2%) and ammonia nitrogen (94.5%) removal. At ≥5 mg/L, synergistic structural-functional decline occurs, with 5 mg/L identified as the critical threshold for specific inhibition of the nitrification link. 5 mg/L damages *Nitrospira*, disrupting nitrification-denitrification links and reducing TN removal by 10.7%. At 10 mg/L, microbial apoptosis causes granule fragmentation (<1.5 mm particles >23%), EPS imbalance, and pollutant-tolerant bacteria dominance, lowering diversity and SOUR, forming a “structural disintegration-functional collapse” loop. This study clarifies the narrow concentration window of 1–5 mg/L and coupled mechanisms, aiding AGS optimization for NP-containing pharmaceutical wastewater.

AUTHOR CONTRIBUTIONS

Shukun Bai: Writing—original draft. **Mo Zhang:** Writing—review and editing; project administration.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are presented in the main text.

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REFERENCES

- Eniola JO, Kumar R, Barakat MA, Rashid J. A review on conventional and advanced hybrid technologies for pharmaceutical wastewater treatment. *J Clean Prod.* 2022;356:131826.
- Li Y, Li C, Wang Z, et al. Navigating the complexity of pharmaceutical wastewater treatment by “effective strategy, emerging technology, and sustainable solution”. *J Water Process Eng.* 2024;63:105404.
- Adeoye JB, Tan YH, Lau SY, et al. Advanced oxidation and biological integrated processes for pharmaceutical wastewater treatment: a review. *J Environ Manag.* 2024;353:120170.
- Tanavarotai K, Kamyab H, Anuar AN, et al. Storage and reactivation of aerobic granular sludge: a review. *Fuel.* 2022;330:125536.
- Peng T, Wang Y, Wang J, Fang F, Yan P, Liu Z. Effect of different forms and components of EPS on sludge aggregation during granulation process of aerobic granular sludge. *Chemosphere.* 2022;303:135116.
- Haaksman VA, Schouteren M, Van Loosdrecht MCM, Pronk M. Impact of the anaerobic feeding mode on substrate distribution in aerobic granular sludge. *Water Res.* 2023;233:119803.
- Jiang Y, Shi X, Ng HY. Aerobic granular sludge systems for treating hypersaline pharmaceutical wastewater: start-up, long-term performances and metabolic function. *J Hazard Mater.* 2021;412:125229.
- Amorim CL, Alves M, Castro PML, Henriques I. Bacterial community dynamics within an aerobic granular sludge reactor treating wastewater loaded with pharmaceuticals. *Ecotoxicol Environ Saf.* 2018;147:905–912.
- Burzio C, Mohammadi AS, Smith S, et al. Sorption of pharmaceuticals to foam and aerobic granular sludge with different morphologies. *Resour Environ Sustain.* 2024;15:100149.
- Hernandez-Raquet G, Soef A, Delgenès N, Balaguer P. Removal of the endocrine disrupter nonylphenol and its estrogenic activity in sludge treatment processes. *Water Res.* 2007;41(12):2643–2651.
- Ryu HD, Han H, Park TJ, Park JH, Kim YS. New findings on the occurrence, removal, and risk assessment of nonylphenol and octylphenol in industrial wastewater treatment plants in Korea. *J Hazard Mater.* 2024;461:132615.
- de Souza Dornelles H, Motteran F, Sakamoto IK, et al. 4-nonylphenol degradation changes microbial community of scale-up anaerobic fluidized bed reactor. *J Environ Manag.* 2020;267:110575.
- Yuan X, Cui K, Chen Y, et al. Response of microbial community and biological nitrogen removal to the accumulation of nonylphenol in sequencing batch reactor. *Int J Environ Sci Technol.* 2023;20(11):12669–12680.
- González MM, Martín J, Santos JL, Aparicio I, Alonso E. Occurrence and risk assessment of nonylphenol and nonylphenol ethoxylates in sewage sludge from different conventional treatment processes. *Sci Total Environ.* 2010;408(3):563–570.
- Duan X, Chen Y, Feng L, Zhou Q. Metagenomic analysis reveals nonylphenol-shaped acidification and methanogenesis during sludge anaerobic digestion. *Water Res.* 2021;196:117004.
- Lara-Moreno A, Aguilar-Romero I, Rubio-Bellido M, et al. Novel nonylphenol-degrading bacterial strains isolated from sewage sludge: application in bioremediation of sludge. *Sci Total Environ.* 2022;847:157647.
- He X, Yan B, Jiang J, et al. Identification of key degraders for controlling toxicity risks of disguised toxic pollutants with division of labor mechanisms in activated sludge microbiomes: using nonylphenol ethoxylate as an example. *J Hazard Mater.* 2023;457:131740.
- Guan D, Zhao J, Wang Y, et al. A critical review on sustainable management and resource utilization of digestate. *Process Saf Environ.* 2024;183:339–354.
- Zhao J, Wang Y, Li B, et al. New insights into microplastics inhibiting kitchen waste dry digestion: digestion efficiency, microbial communities and microplastic aging mechanisms. *Chem Eng J.* 2025;508:160907.
- Hung CM, Chen CW, Huang CP, Shiung Lam S, Yang YY, Dong CD. Performance and bacterial community dynamics of lignin-based biochar-coupled calcium peroxide pretreatment of waste-activated sludge for the removal of 4-nonylphenol. *Bioresour Technol.* 2022;354:127166.
- Guo C, Xu Y, Deng N, et al. Efficient degradation of organophosphorus pesticides and in situ phosphate recovery via NiFe-LDH activated peroxymonosulfate. *Chem Eng J.* 2025;524:169107.
- Ni C, Zhang Q, Xu B, et al. The role of solid electrolytes in suppressing joule heating effect for scalable H₂O₂ electrosynthesis. *ACS Sustain Chem Eng.* 2025;13(42):17958–17968.
- Rong L, Zhang B, Qiu H, et al. Significant generational effects of tetracyclines upon the promoting plasmid-mediated conjugative transfer between typical wastewater bacteria and its mechanisms. *Water Res.* 2025;287:124290.
- Liu H, Fang HHP. Extraction of extracellular polymeric substances (EPS) of sludges. *J Biotechnol.* 2002;95(3):249–256.
- Fu Z, Zhao J, Zhong Z, et al. Novel insights into liquid digestate-derived hydrochar enhances volatile fatty acids production from anaerobic co-digestion of sludge and food waste. *Chem Eng J.* 2025;507:160635.

26. Zhong Z, Ye W, Li B, et al. Phosphate-iron modified Enteromorpha Prolifera Hydrochar enhances dry anaerobic digestion of food waste: synergistic mechanisms of electron transfer network, microbial consortia remodeling, and metagenomic insights. *Environ Res.* 2025;289: 123385.
27. Najim AA, Radeef AY, al-Doori I, et al. Immobilization: the promising technique to protect and increase the efficiency of microorganisms to remove contaminants. *J Chem Technol Biotechnol.* 2024;99(8):1707-1733.
28. Liu Y, Dai X, Wei J. Toxicity of the xenoestrogen nonylphenol and its biodegradation by the alga *Cyclotella caspia*. *J Environ Sci.* 2013;25(8): 1662-1671.
29. Song Y, Li Y, Lu J, et al. Strengthening and toughening mechanisms of martensite-bainite microstructure in 2 GPa ultra-high strength steel during hot stamping. *Sci China Technol Sci.* 2025;68(5):1520201.
30. Qi H, Hu Z, Yang Z, et al. Capacitive aptasensor coupled with microfluidic enrichment for real-time detection of trace SARS-CoV-2 nucleocapsid protein. *Anal Chem.* 2022;94(6):2812-2819.
31. Zhao J, Guan D, Zhong Z, et al. Cobalt-modified digestate-derived biochar enhances kitchen waste anaerobic dry digestion: performance, microbial mechanisms, and metabolic pathways. *Chem Eng J.* 2024;499:155951.
32. Hong Y, Feng C, Yan Z, et al. Nonylphenol occurrence, distribution, toxicity and analytical methods in freshwater. *Environ Chem Lett.* 2020;18(6):2095-2106.
33. Iorhemen OT, Hamza RA, Zaghloul MS, Tay JH. Aerobic granular sludge membrane bioreactor (AGMBR): extracellular polymeric substances (EPS) analysis. *Water Res.* 2019;156:305-314.
34. Zhang H, Fu Z, Guan D, et al. A comprehensive review on food waste anaerobic co-digestion: current situation and research prospect. *Process Saf Environ.* 2023;179:546-558.
35. Al-Majali MR, Zhang M, Al-Majali YT, et al. Impact of raw material on thermo-physical properties of carbon foam. *Can J Chem Eng.* 2025; 103(3):1309-1318.
36. Wan C, Fu L, Li Z, Liu X, Lin L, Wu C. Formation, application, and storage-reactivation of aerobic granular sludge: a review. *J Environ Manag.* 2022;323:116302.
37. Lin H, Ma R, Hu Y, et al. Reviewing bottlenecks in aerobic granular sludge technology: slow granulation and low granular stability. *Environ Pollut.* 2020;263:114638.
38. Jiang Y, Li C, Hou Z, et al. Pollutants removal and connections among sludge properties, metabolism potential and microbial characteristics in aerobic granular sequencing batch reactor for petrochemical wastewater treatment. *J Environ Manag.* 2023;344:118715.
39. Tong Y, Lu P, Zhang W, et al. The shock of benzalkonium chloride on aerobic granular sludge system and its microbiological mechanism. *Sci Total Environ.* 2023;895:165010.
40. Meng T, Liu R, Cai J, Cheng X, He Z, Zhao Z. *Adv Funct Mater.* 2025; e22046.
41. Zhao J, Zhang M, Wang C, et al. First-principles study of CO, NH₃, HCN, CNCl, and Cl₂ gas adsorption behaviors of metal and cyclic C-metal B-and N-site-doped h-BNs. *Electron Mater Lett.* 2025;21(2): 268-288.
42. Pan K, Guo T, Liao H, et al. Adding iron shavings in activated sludge system to enhance removal of refractory organics and nitrogen for textile-dyeing wastewater. *J Environ Chem Eng.* 2023;11(5):110999.
43. Mi YN, Cui YW, Zhou DX, et al. Alkalinity role in recovery of disintegrated halophilic aerobic granular sludge treating high salt-alkali wastewater: insights into community ecology of fungi and bacteria. *Chem Eng J.* 2025;518:164554.
44. He S, Ding L, Xiong Z, et al. A distinctive Eocene Asian monsoon and modern biodiversity resulted from the rise of eastern Tibet. *Sci Bull.* 2022;67(21):2245-2258.
45. Guo C, Zhang L, Zhang Q, et al. Efficient adsorptive removal of phosphonate Antiscalant HEDP by Mg-Al LDH. *Separations.* 2025; 12(10):259.
46. Yang X, Wang W, Liu X, et al. Nitrogen metabolism functional shifts of indigenous bacteria and effect on nitrogen removal in microalgae-based municipal wastewater treatment system across aeration modes. *Bioresour Technol.* 2025;435:132881.
47. Zhang M, Zhang C, Wu Q, et al. Deciphering nitrogen removal performance concerning heterotrophic microorganism's succession by using three typical acid-rich fermentation liquids of food waste as carbon sources in high ammonium and high salt wastewater treatment. *Environ Res.* 2025;268:120763.
48. Semblante GU, Hai FI, Ngo HH, et al. Sludge cycling between aerobic, anoxic and anaerobic regimes to reduce sludge production during wastewater treatment: performance, mechanisms, and implications. *Bioresour Technol.* 2014;155:395-409.
49. Yang J, Qian M, Wu S, et al. Insight into the role of chitosan in rapid recovery and re-stabilization of disintegrated aerobic granular sludge. *J Environ Manag.* 2024;356:120613.
50. Guo H, Zhao Y. New insights into the impact of ribavirin on enhanced biological phosphorus removal process efficiency. *Environ Prog Sustainable.* 2025;44(3):e14605.

SUPPORTING INFORMATION

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