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Partial Nitrification Performance of Gel-Immobilized Fillers and Application to Leather Wastewater Treatment

Chenguang Wu, Hong Yang*

Key Laboratory of Beijing for Water Quality Science and Water Environmental Recovery Engineering, College of Architectural Engineering, Beijing University of Technology, Beijing, China
Email: chenguangW@163.com, *yhong@bjut.edu.cn

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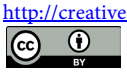

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Abstract

In this study, gel-immobilized fillers were used to conduct partial nitrification experiments, including a small-scale experiment in the laboratory and a pilot experiment in a leather factory. The performance of the filler for partial nitrification was investigated by the small-scale experiment under the reactor start-up condition with low initial ammonia oxidizing bacteria (AOB) relative abundance. Then pilot experiment was carried out for the application of the filler in leather wastewater treatment. Broadening the direction for the application of gel-immobilized fillers. Provide technical parameter reference and optimization basis for the application of gel-immobilized fillers in leather wastewater treatment. The partial nitrification in the small-scale reactor (SR) achieved an ammonia nitrogen oxidation rate (AOR) of 27 - 29 mg·(L·h)$^{-1}$ and a nitrite nitrogen accumulation rate (NAR) of more than 94%. After an 82-day shutdown period, SR recovered its performance and continued to improve. High-throughput sequencing confirmed that AOB was enriched with a relative abundance of 32% and the percentage of nitrite oxidizing bacteria (NOB) was less than 0.01%. A pilot reactor (PR) was built using real leather wastewater as raw water. The experimental results showed that the normal performance of gel-immobilized fillers for partial nitrification was affected against the background of an average influent water quality of 537 mg·L^{-1} ammonia nitrogen concentration and 2990 mg·L^{-1} chemical oxygen demand (COD). Leather wastewater organics persistently affected PR nitrification. The growth of AOB was inhibited. Organics removal should be emphasized prior to nitrification, and the addition of an advanced oxidation process for organics is essential.

Subject Areas

Biological Engineering, Biotechnology

Keywords

Leather Wastewater, Gel-Immobilized Fillers, Partial Nitrification, Organics

1. Introduction

The gel-immobilized technique uses gel material to encapsulate microorganisms to make bioactive fillers. The technology provides a stable growth environment for functional bacteria and has a low sludge yield, improved shock resistance and localized spatial bio-density [1] [2]. Several studies have been carried out on the use of gel-immobilized fillers to achieve partial nitrification. Research has been applied to municipal and industrial wastewater treatment, with good results [3]-[5]. The value of this filler for partial nitrification has been demonstrated. However, research related to leather wastewater treatment is rare. Moreover, the prior experiments used cultured activated sludge to make the filler, which is a special raw material and requires additional culture costs. Further research is needed to determine whether the filler is more applicable and has a wider range of application scenarios.

Leather production consumes a large amount of water, of which about 90% is discharged as wastewater, and each ton of leather generally produces 30 - 34 m^3 of highly polluting wastewater [6] [7]. In the leather production process, wastewater can be mixed with animal hair, fat, ammonium salts, etc. Leather wastewater tends to have high chemical oxygen demand (COD) and ammonia nitrogen concentration. Wastewater Treatment Plants (WWTPs) treat leather wastewater extensively with a combination of biological treatment processes and advanced oxidation processes [8]. However, biological reactions are generally non-linear and complex, while the activated sludge process has to take into account the high sludge yield and the treatment of residual sludge. Sludge management costs account for 30% - 40% of the total WWTP budget or 50% - 55% of process and maintenance costs [9]. Membrane bioreactors have the disadvantage of severe fouling due to contaminant clogging and adsorption on the membrane. The leather industry is still under pressure to find cleaner and more economical wastewater treatment technologies [10].

This study was carried out by combining the advantages of gel-immobilized fillers, research needs and the development of leather wastewater treatment technology. In this study, reactors were built using gel-immobilized fillers. Small-scale reactor was first built in the laboratory. To study the effect of partial nitrification of gel-immobilized fillers at low initial ammonia oxidizing bacteria (AOB) content and the causes. Then a pilot reactor was set up in a sheep fur tanning enterprise to treat the actual leather wastewater. To research the effectiveness of gel-immobilized fillers for partial nitrification in the treatment process of leather wastewater, a highly polluting wastewater, and to analyse the effects affecting the filler. High-throughput sequencing has been used in both

small-scale and pilot experiments to classify microorganisms. The use of high-throughput sequencing to study bacterial community structure provided a bacterial-level basis for the study.

2. Materials and Methods

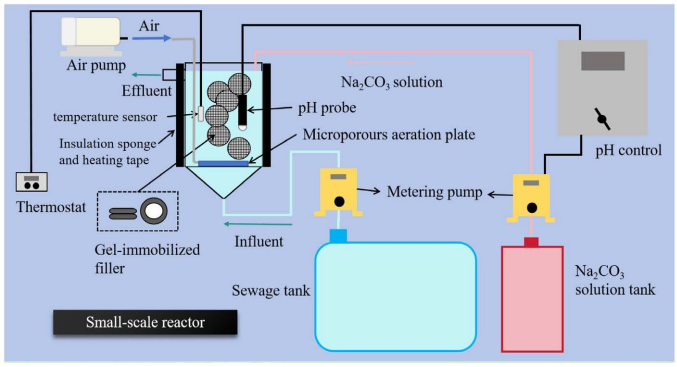
2.1. Production of Gel-Immobilized Fillers

Polyvinyl alcohol (PVA) was used as a material to encapsulate immobilized microorganisms. Subsequently, bioactive fillers were manufactured utilizing the bespoke laboratory equipment. The packing profile is a double-ring particle with an inner diameter of 6 mm, an outer diameter of 10 mm, and a thickness of 5 mm. The filler is loaded into the plastic suspension balls. Place the suspension balls into the reactor for use. Small-scale reactor fillers: relative abundance of AOB less than 1%; Pilot reactor fillers: relative abundance of AOB was 12%.

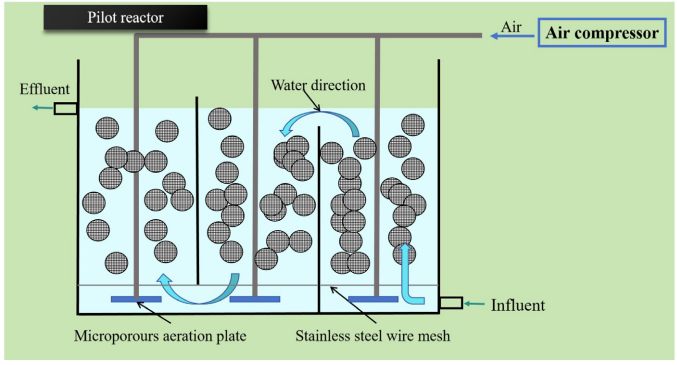
2.2. The Small-Scale Experiment

2.2.1. Experimental Equipment

The small reactor (SR) has an effective volume of 24.3 L and was made of plexiglass, as shown in Figure 1(a). The fill rate was 7.8%. Air pump as air source. Use the valve to control the air intake. A thermostat and heating tape were used to control the temperature. Na_2CO_3 solution was used as an alkaline solution to adjust the pH of SR. Use a PLC control cabinet to control pH.



(a)



(b)

Figure 1. Experimental equipment: (a) Small-scale reactor; (b) Pilot reactor.

2.2.2. Experimental Wastewater

The experimental wastewater was a manually prepared NH_4Cl solution. Ammonia nitrogen concentrations (NH_4^+-N) were 145 - 155 mg·L^{-1}. KH_2PO_4 and trace element concentrates were added to the experimental wastewater. N: P was 5:1. Trace element concentrates were added at 0.1% of the volume of the NH_4Cl solution, and the composition table is shown in Table 1.

Table 1. Composition of trace element concentrate.

Component	Concentration (mg·L^{-1})	Component	Concentration (mg·L^{-1})
EDTA·2Na	5000	$NiCl_2·6H_2O$	700
$ZnSO_4·7H_2O$	500	$MgSO_4·7H_2O$	3000
$Na_2MoO_4·2H_2O$	100	$CaCl_2$	2000
$CoCl_2·6H_2O$	200	Na_2SeO_4	210
$FeCl_3·6H_2O$	800	$MnCl_2·4H_2O$	500
$CuSO_4·5H_2O$	600		

2.2.3. Reactor Operation

SR operation was divided into two periods. In the first period, dissolved oxygen (DO) was 4.0 - 4.5 mg·L^{-1} for the first eleven days. After SR had significant ammonia removal, DO was controlled to be 3.5 - 4.0 mg·L^{-1}. At 28 d because of COVID-19, SR experienced an 82-day shutdown. During the shutdown period, the fillers are kept in the reactor and immersed in water. It then enters the second period, counting from 29 d. The temperature was 24˚C - 26˚C and pH was 7.8 - 8.2 throughout the operational time.

2.3. The Pilot Experiment

2.3.1. Experimental Equipment

The pilot reactor (PR) dimension: L × W × H = 1500 mm × 1100 mm × 1700 mm, shown in Figure 1(b). The material is stainless steel. The effective volume of the PR is 2000 L. The interior of the PR is equally divided into three compartments, PR1, PR2, and PR3, according to the direction of water flow. The reactor's base is equipped with microporous aeration plates. Stainless steel wire mesh is placed over the aeration disc to separate it from the plastic suspension balls. The fill rate was 6.1%. An air compressor was used as the air source and a valve was used to control the air intake.

2.3.2. Experimental Wastewater

The experimental wastewater was pre-treated leather wastewater. Pre-treatment included coagulation and sedimentation, hydrolytic acidification and bio-aerobic oxidation. The pH, ammonia and COD of the PR influent are shown in Table 2.

Table 2. PR influent water quality.

	pH	NH_4^+-N (mg·L^{-1})	COD (mg·L^{-1})
Range	7.85 - 8.15	279 - 740	865 - 5563
Average value	8.05	537	2990

2.3.3. Reactor Operation

The PR was a continuous flow reactor. DO was controlled to 5.7 - 6.2 mg·L^{-1}. The P1 stage was a short start-up period. The P2 stage had a low HRT and unstable reactor operation. The HRT was continuously adjusted during the P3 stage. The final HRT was 20 h.

2.4. Analytical Methods and Calculation Formulas

NH_4^+-N, nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N): APHA standard methods [11]. COD: Lianhua Technology COD Rapid Determination Instrument. DO: Measured by fluorescence method using HACH HQ30d portable dissolved oxygen meter. Ammonia oxidation rate (AOR), nitrite nitrogen accumulation rate (NAR) and nitrogen removal effect (NRE) were calculated according to Equations (1) to (3) as follows:

$$\mathrm{AOR}\left(\mathrm{mg}\cdot\left(\mathrm{L}\cdot\mathrm{h}\right)^{-1}\right)=\frac{\left(\mathrm{NH_4^+\text{-}N}\right)_{\mathrm{inf}}-\left(\mathrm{NH_4^+\text{-}N}\right)_{\mathrm{eff}}}{\mathrm{HRT}} \quad (1)$$

$$\mathrm{NAR}\left(\%\right)=\frac{\left(\mathrm{NO_2^-\text{-}N}\right)_{\mathrm{eff}}-\left(\mathrm{NO_2^-\text{-}N}\right)_{\mathrm{inf}}}{\left(\mathrm{NO_2^-\text{-}N}\right)_{\mathrm{eff}}-\left(\mathrm{NO_2^-\text{-}N}\right)_{\mathrm{inf}}+\left(\mathrm{NO_3^-\text{-}N}\right)_{\mathrm{eff}}-\left(\mathrm{NO_3^-\text{-}N}\right)_{\mathrm{inf}}} \quad (2)$$

$$\mathrm{NRE}\left(\%\right)=\frac{\left(\mathrm{NH_4^+\text{-}N+NO_2^-\text{-}N+NO_3^-\text{-}N}\right)_{\mathrm{inf}}-\left(\mathrm{NH_4^+\text{-}N+NO_2^-\text{-}N+NO_3^-\text{-}N}\right)_{\mathrm{eff}}}{\left(\mathrm{NH_4^+\text{-}N+NO_2^-\text{-}N+NO_3^-\text{-}N}\right)_{\mathrm{inf}}} \quad (3)$$

where HRT is hydraulic residence time (h); Inf is influent; Eff is effluent.

2.5. Microbial Taxonomic Sequencing

During the latter period of the experiments, microbial taxonomic sequencing of SR and PR1, PR2, and PR3 fillers was performed using high-throughput sequencing. The fillers were frozen in liquid nitrogen and then ground. Total DNA was extracted from the samples using the E. Z. N. A™ Mag-Bind Soil DNA Kit. PCR amplification was performed using universal primers. The DNA concentrations were determined using a Qubit® 4.0 DNA assay kit, with quality control performed using a bioanalyzer. DNA sequencing was performed using the Illumina MiSeq sequencing platform. The sequencing results were processed and compared with the RDP database to classify the bacterial OTU sequences.

3. Results and Discussion

3.1. The Small-Scale Experiment

3.1.1. The First Period of SR and Recovery

Figure 2 shows the operating effect of SR. At the beginning of operation,

NH_4^+-N removal efficiency was low, with AOR below 5 mg·(L·h)$^{-1}$. But by this time the NAR had reached over 80%. At 11 d, the AOR was greater than 5 mg·(L·h)$^{-1}$. The AOR rose rapidly after that. At 14 d, the AOR was 15.49 mg·(L·h)$^{-1}$. The AOR stabilized at 15 - 17 mg·(L·h)$^{-1}$ at the end of the first period. The effect of nitrite accumulation was more prominent. NAR stabilized above 90% after 13 d. At 28 d, because of COVID-19, SR experienced an 82-day shutdown. However, in the second period, the activity of the filler was successfully restored.

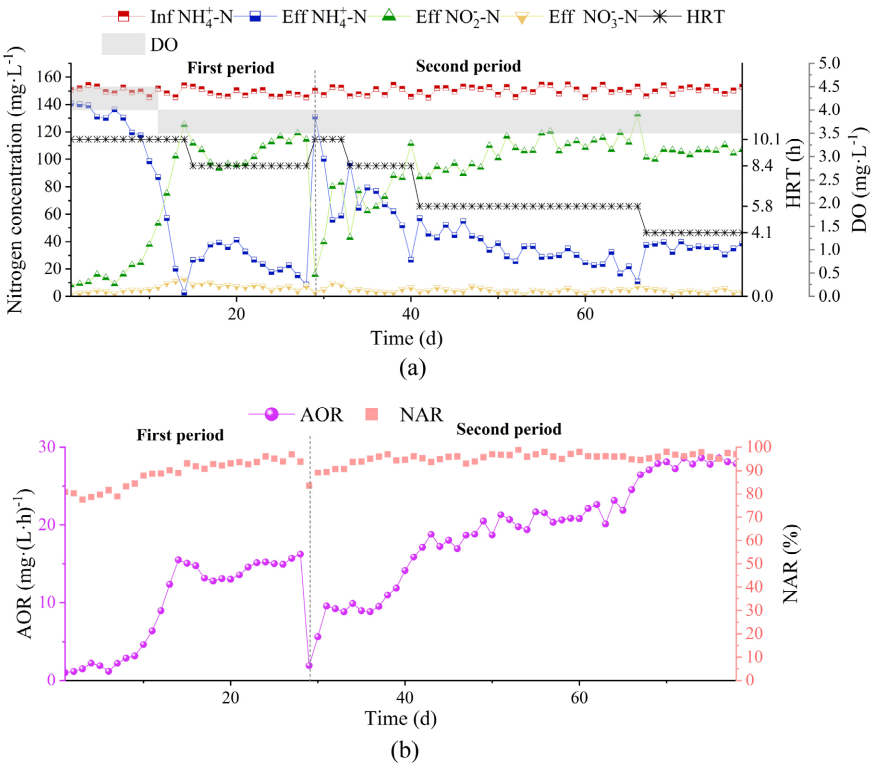


Figure 2. Operational effects of SR: (a) Changes in NH_4^+-N, NO_2^--N, NO_3^--N, HRT and DO; (b) AOR and NAR.

On the second day of the second period (30 d), the filler regained obvious partial nitrification performance. The AOR was 5.62 mg·(L·h)$^{-1}$ and the NAR was 89.18%. On the 31 d, the AOR recovered to 9.56 mg·(L·h)$^{-1}$. The filler already had good performance for partial nitrification in the first period. Based on this, the initial performance of the filler in the second period was higher than the initial period of the first period. After twelve days, the AOR recovered to its level obtained at the end of the first period. The NAR recovered more quickly. After seven days of recovery, NAR re-stabilized at over 94%. After an 82-day shutdown of SR, the filler was able to rapidly restart the effect of partial nitrification and recover to its pre-shutdown performance and then continue to grow. This showed that the gel-immobilization technology has good retention and recovery of biological activity. Gel-immobilized fillers have excellent resistance to envi-

ronmental changes. The findings of similar studies with a long shutdown period yielded comparable results [1] [3].

3.1.2. Performance of Gel-Immobilized Fillers for Partial Nitrification

SR at the later part of the second period, AOR was 27 - 29 mg·(L·h)$^{-1}$ and NAR reached 95% - 98%. Gel-immobilized fillers enable excellent and stable partial nitrification. In other studies where gel-immobilized fillers were used for partial nitrification, excellent partial nitrification effect was also achieved at influent NH_4^+-N concentrations of 550 mg·L^{-1} and 300 mg·L^{-1} [12] [13]. These studies achieved an AOR of 21 - 23 mg·(L·h)$^{-1}$ with a NAR of more than 92% and a maximum of 46.25 mg·(L·h)$^{-1}$ with a NAR of more than 97%. In previous studies, cultured activated sludge was used to make fillers and the sludge had a high relative abundance of AOB [1] [3] [12]-[14]. In contrast, the fillers used in SR were made using uncultured sludge. Its initial AOB relative abundance was less than 1%, but the SR achieved equally good partial nitrification. Approximate or even better results were obtained under similar pH, temperature and DO conditions. The gel-immobilization technology itself can facilitate the achievement of partial nitrification. The filler was applied in municipal wastewater treatment and industrial wastewater treatment to study and meet the wastewater discharge requirements [4] [5]. The performance of gel-immobilized fillers for partial nitrification was proven.

3.1.3. Reason Analysis: AOB Enrichment and DO Partitioning

The species taxonomic sequencing result of the filler at the end of the SR operation is shown in **Figure 3** (genus level). The AOB in the filler was *Nitrosomonas*, which accounted for 32%. Meanwhile, the percentage of nitrite oxidizing bacteria (NOB) was below 0.01%. Similar experimental results have also been found in other studies. In the experiment of Fang *et al.* [15] with partial nitrification gel-immobilized fillers, the relative abundance of Nitrosomonas in the filler was increased from 18.43% to 28.14%, and the percentage of NOB was always kept below 1%. This suggests that gel-immobilized fillers provide a more suitable environment for AOB to survive relative to NOB. Enrichment of AOB while inhibiting and eliminating NOB during operation.

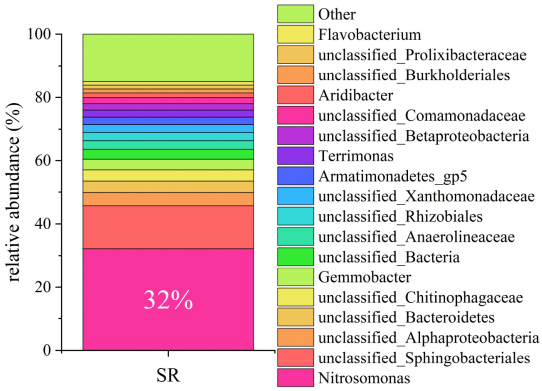


Figure 3. Relative abundance of species in SR fillers (genus level).

In the long-term operation of gel-immobilized fillers reactor, the enrichment of AOB and the suppression of NOB are the key to achieve efficient and stable partial nitrification. The different effects on AOB and NOB may be due to the limitation of DO transfer by the filler. Meanwhile, AOB has a stronger pro-oxidant capacity compared to NOB. [16]. DO control is the usual operational strategy for partial nitrification. The DO condition for achieving partial nitrification was easily achieved with the gel-immobilized filler. PVA was used as a material to encapsulate the bacteria and a DO gradient was formed in the three-dimensional structure of the fillers [17]. DO decreases gradually from the surface of the filler to the interior [18]. The limitation to DO was key to achieve and maintain this bacterial community structure. High-throughput sequencing results also provided evidence for the presence of regions with different DO in the filler. **Figure 5** shows the microbiological classification results of PR1, PR2 and PR3 at the end of the operation. Strictly anaerobic bacteria occupied significant numbers with relative abundances of 17% - 27%. The gel-immobilization technology conveniently enabled the restriction of DO. DO-restricted regions existed within the gel-immobilized fillers. In this DO environment, AOB obtained better growth conditions relative to NOB. Thus gel-immobilized fillers conveniently enabled partial nitrification.

3.2. Pilot Experiment: Leather Wastewater Treatment

3.2.1. Ammonia Nitrogen Removal Effect

The effect of PR on the treatment of NH_4^+-N is shown in **Figure 4(a)** and **Figure 4(b)**. The maximum NH_4^+-N removal was 288.07 mg·L^{-1}. PR was less efficient than SR. During the P1 and P2 stages, the PR operated unstably. The influent NH_4^+-N was sometimes much larger than the effluent NH_4^+-N. This was due to the poor effect of the hydrolytic acidification fillers unit (HAU) in the pre-treatment process. HAU carried out the hydrolysis of complex organics and converted organic nitrogen to inorganic nitrogen [19]. The HRT was small during this period and the initial performance of the HAU was weak. This resulted in insufficient conversion of organic nitrogen. During the P3 phase, HRT increased and HAU performance improved. PR did not repeat this anomaly.

The performance of PR in terms of partial nitrification was severely compromised in the context of highly polluted wastewater leather wastewater compared to SR and other prior experiments. The average value of influent NH_4^+-N for PR was 537 mg·L^{-1}. Leather wastewater also contained large amounts of organics. The average COD value of PR influent was 2990 mg·L^{-1} with a maximum of 5563 mg·L^{-1}. In this context, the AOR of PR was relatively low and eventually stabilized at 5 - 7 mg·(L·h)$^{-1}$. This was quite different from the efficiency of SR.

The weak performance of PR in partial nitrification was attributed to the inhibition of AOB activity and growth in the filler. AOB did not gain a competitive advantage. It was confirmed in high-throughput sequencing results that the AOB of PR was not enriched as in SR. The relative abundance of AOB was less than 1%, shown in **Figure 5**.

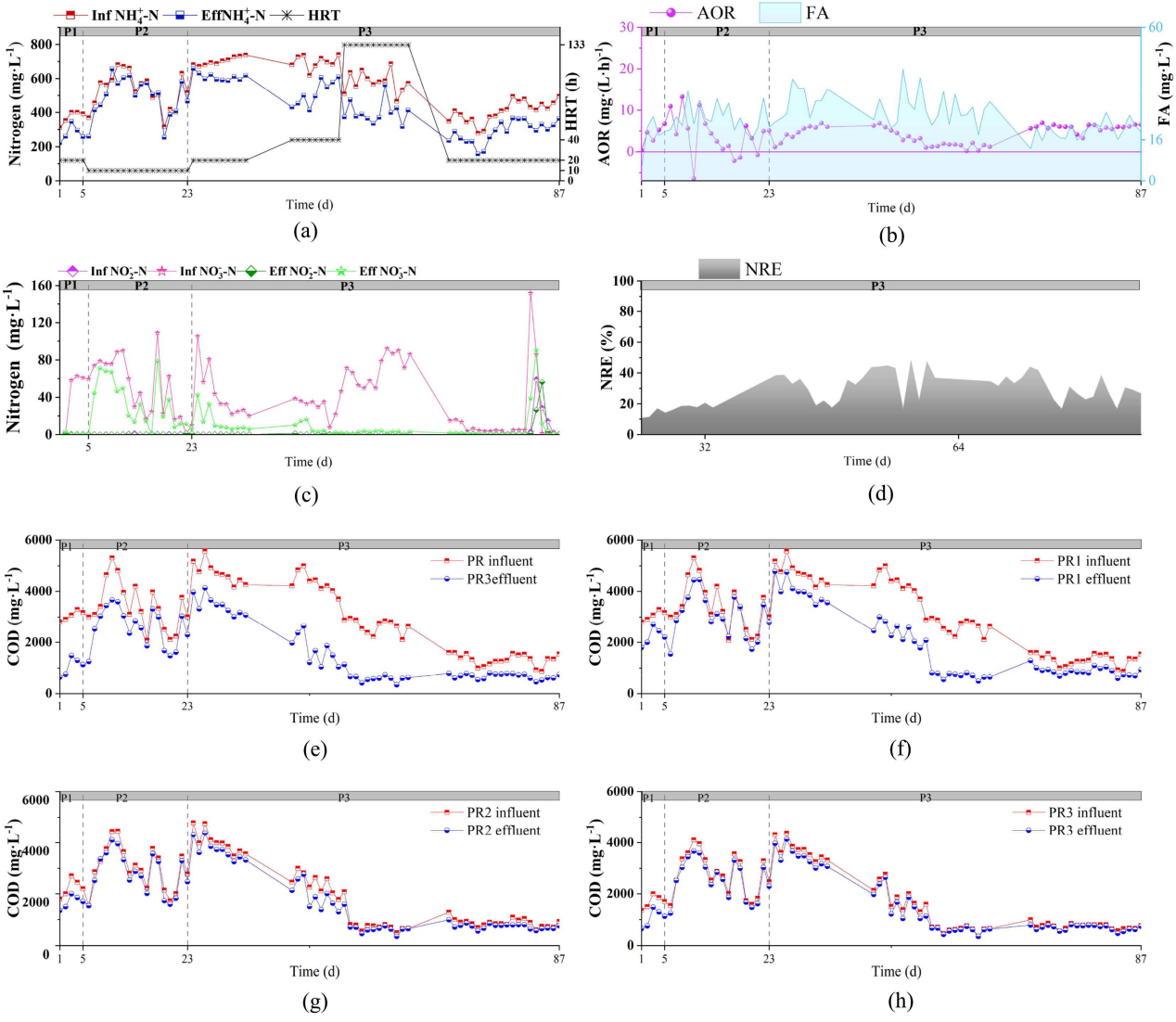


Figure 4. Operational effects of PR: (a) Change in NH_4^+-N and HRT; (b) Change in AOR and influent FA; (c) Changes in NO_2^--N and NO_3^--N; (d) NRE of PR; (e) Change in COD of PR; (f) Change in COD of PR1; (g) Change in COD of PR2; (h) Change in COD of PR3.

3.2.2. FA and COD Effects of Binding Bacterial Flora

Free ammonia (FA) inhibits AOB [20]. Although good partial nitrification performance of gel-immobilized fillers has been demonstrated at NH_4^+-N of 550 mg·L^{-1} [12]. However, under high HRT conditions, the PR filler showed that the activity was affected. The HRT changes in the P3 stage were: 20 h → 40 h → 133 h → 20 h. AOR decreased with increasing HRT. During the period of maximal HRT (133 h), the AOR decreased significantly to 1 - 2 mg·(L·h)$^{-1}$.

The high COD was an important factor affecting the effectiveness of the partial nitrification of the filler. On the one hand, the growth of AOB was affected, and on the other hand, the normal functioning of the nitrification reactor was affected. **Figure 5** shows the relative abundance of species at the genus level for each of the PR1 - PR3 fillers at the end of the PR operation. The relative abundance

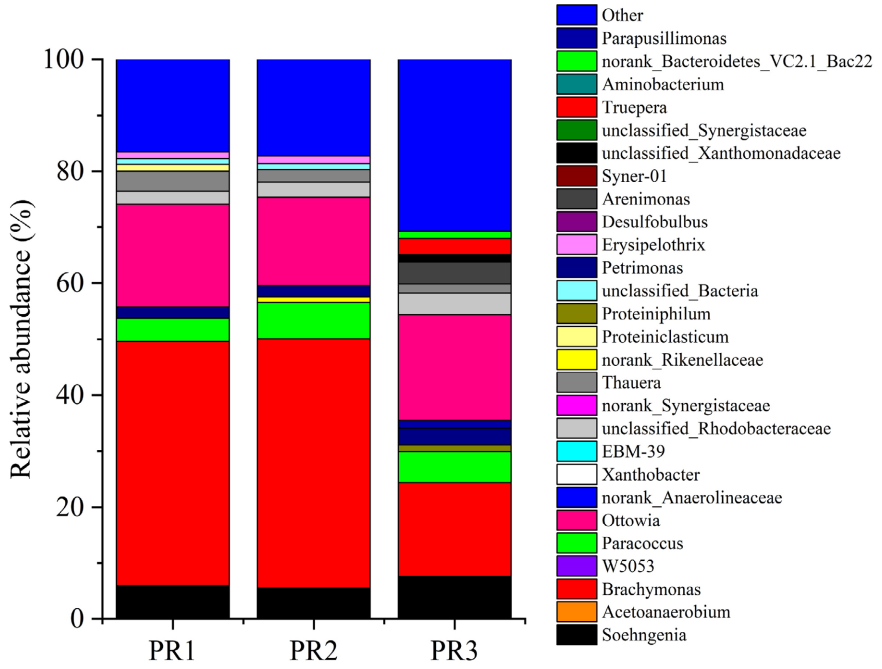


Figure 5. Relative abundance of species in PR fillers (genus level).

of AOB was less than 1%, and the dominant genus were all heterotrophic. AOB didn't gain a competitive advantage. A large number of COD was removed in the PR, with a maximum removal of 3193 mg·L^{-1} (**Figure 4(e)**). Meanwhile, denitrifying bacteria carried out denitrification under the condition of sufficient carbon source. This caused the PR to not perform the NO_2^--N and NO_3^--N accumulation normally. **Figure 4(c)** and **Figure 4(d)** show this denitrification phenomenon. NO_2^--N and NO_3^--N were reduced in PR. Nitrogen removal effect (NRE) of PR was above 20% during most of the P3 stage. High-throughput sequencing confirmed the presence of large numbers of denitrifying bacteria in PR. PR1 - PR3 all had significant numbers of traditional denitrifying bacteria *Brachymonas* (16.8% - 44.57%), *Ottowia* (15.81% - 18.92%) and aerobic denitrifying bacteria *Paracoccu* (4.16% - 6.44%).

The impact of leather wastewater organics was continuous. **Figures 4(e)-(h)** represent COD removal from PR and COD removal from each region. The removal of COD by each zone was reduced one by one. The treatment effect of PR3 shows that COD removal was not obvious when COD was treated below 800 mg·L^{-1}. This is related to the complex composition of organics in leather wastewater. Leather wastewater has a large number of organic matters with low biochemistry [21] [22]. Li *et al.* [23] found that there was always COD residual in the effluent of leather wastewater biological treatment system. However, some of the COD still continues to be slowly degraded in PR and was not completely non-biodegradable. Heterotrophic bacteria always gained a growth advantage. COD persistently affected the normal partial nitrification performance of gel-immobilized fillers.

3.3. Optimization of the Partial Nitrification Filler Process for Leather Wastewater

COD maintained a slow decline in the later part of the PR, which continued to affect the normal nitrification performance of the filler. The accumulation of ammonia-nitrogen oxidation products was affected. The proper operation of the denitrification system would be negatively impacted. However, partial COD will remain in the biological treatment system. Advanced oxidation processes (AOPs) can be set up to remove residual recalcitrant COD or to improve the biochemistry of the wastewater. For poorly biochemical wastewater from leather factories and other industries, AOPs have been used in a large number of practical applications for the treatment of biochemical tailwaters and pre-treatments and have operated reliably [24]-[26]. COD above 500 - 2000 $mg \cdot L^{-1}$ can be removed. AOPs can enhance wastewater biochemistry and PR showed that gel-immobilized fillers have the ability to remove large amounts of organic matter. So, it is possible to remove residual COD only partially. Breaks down organics into readily biodegradable organics. Let it be treated in the subsequent nitrification gel-immobilized fillers process, relying on the aerobic digestion properties of the filler.

4. Conclusions and Future Prospects

1) Under start-up conditions with low initial AOB content, gel-immobilized fillers still conveniently achieved good and stable partial nitrification. The conditions for making gel-immobilized fillers are much simpler. In addition, the filler has excellent bioactive retention and recovery capabilities.

2) The gel-immobilized fillers enriched AOB while inhibiting NOB. This is the key to achieving efficient and stable partial nitrification of the filler. The existence of the DO restriction zone within the filler facilitates this.

3) In the application of treatment of leather wastewater, a highly polluted wastewater, the performance of fillers for partial nitrification was affected by high FA and COD. The effect of FA was shown under the long HRT condition. The COD of the leather wastewater continuously affected the partial nitrification of the filler. Organic removal prior to nitrification fillers should be emphasized.

4) Optimizations of the process for the treatment of leather wastewater using partial nitrification gel-immobilized fillers require further investigation. Installation of AOPs between the nitrification and denitrification units to fully eliminate the effects of organics on nitrification. To study the extent of COD residuals in the biological treatment unit. Specific process options for AOPs should be studied. In order to ensure the proper functioning of subsequent partial nitrification gel-immobilized fillers, it is required to investigate the extent to which COD needs to be treated.

Conflicts of Interest

The authors declare no potential conflict of interest that could have appeared to

influence the work reported in this paper.

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