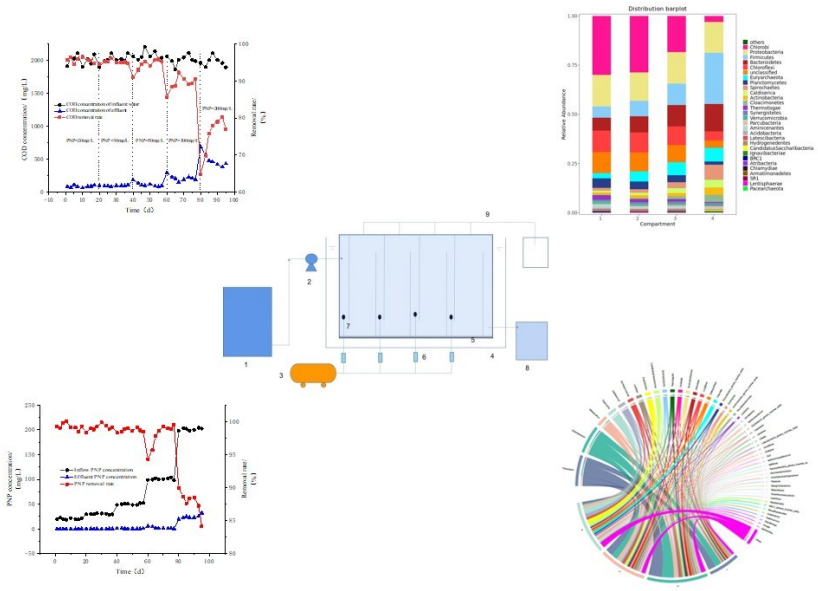
Efficiency and Microbial Community Characteristics of a micro Oxygen Baffled Aeactor for Treating Nitrophenol Wastewater

Qinhua Sun [a]，Jiankun Zhang * [a]

a.School of Environmental Engineering, Xuzhou university of technology, Xuzhou 221018, China.

Email: xzsunqinhua@163.com（Q H Sun), zhangjiankun@xzit.edu.cn（JK Zhang）

Graphical Abstract



Abstract: Using a baffle reactor to degrade nitrophenol wastewater, with p-nitrophenol as the target pollutant, investigate the effects of nitrophenol concentration, external carbon source concentration, hydraulic retention time, and dissolved oxygen changes on the degradation of p-nitrophenol in the micro oxygen baffle reactor. The results showed that when the HRT of the micro oxygen baffle reactor was maintained for 24 hours and the PNP concentration was below 100 mg/L, the COD removal rate remained stable at 93.68%. After the HRT was reduced, the contribution rate of the subsequent compartment to the degradation of p-nitrophenol and COD removal increased, becoming a beneficial supplement for the system to cope with load shocks. When the DO concentration is between 0.3-0.5mg/L, the removal rate of COD is the highest, indicating that micro aeration is beneficial for removing pollutants. Through high-throughput sequencing analysis, it was found that the microbial community composition in each compartment of the reactor was high. The species abundance in the first compartment and the third compartment was increased. The microbial groups in the reactor mainly included Bacteroidetes, Firmicutes, Proteobacteria, Spirochaetes, and Actinobacteria.

Keywords: Micro oxygen baffled reactor; p-nitrophenol; microbial community characteristics

0. Introduction

Phenolic wastewater, typical industrial wastewater, mainly comes from industries such as coal chemical, petrochemical, plastic, pesticide, pharmaceutical synthesis, printing, and dyeing. The wastewater discharged from these industries contains phenolic substances [Varjani et al.2020;Motamedi et al.2022]. Various phenolic compounds include nitrophenols, chlorophenols, phenols, and other types. Nitrophenol, an essential raw material and intermediate, is widely used in the production of the above industries. More and more nitrophenol compounds enter the environment through various channels. Due to their benzene ring structure and containing nitro groups, they have strong chemical stability and can

remain in the background for a long time without being biodegradable [Wang et al.2019;Wang et al. 2021;Bhat et al.2021;Zhang et al.2021]. In the list of priority controlled pollutants listed by the National Environmental Protection Agency of the United States, 2-nitrophenol, 4-nitrophenol, and 2,4-dinitrophenol are among them [Yang et al.2019;Tiwari et al.2019], and phenolic pollutants are also listed as priority controlled pollutants in water bodies in China [Sun et al. 2018].

In traditional research on wastewater biological treatment, it is generally believed that anaerobic and aerobic bacteria cannot coexist in the same culture or environment. Anaerobic and Aerobic organisms' biological treatments are developed and selected as mutually independent and complementary technologies. In recent years, research has shown that anaerobic and aerobic bacteria can coexist in the same culture environment, and this co-cultivation can achieve simultaneous degradation of multiple pollutants, including refractory toxic pollutants, nitrogen, phosphorus, sulfur, Etc. [Kumwimba et al.2019;Harb et al.2021;Xi et al.2022]. Microoxygen technology (DO generally ranges from 0 to 1.0mg/L) is a biotechnology that allows anaerobic and aerobic microorganisms to coexist in the same reactor and interact synergistically [Shi et al.2023;Li et al.2023]. The spatial coexistence and simultaneous collaboration of anaerobic, facultative, and aerobic microorganisms in microaerobic biological treatment determine its many technological advantages, such as higher oxygen transfer efficiency, ability to remove difficult-to-biodegrade substances, improved COD removal rate, and strong impact load resistance [Chan et al.2009;Fan et al.2020;Cai et al.2019;Van et al.2023;Wang et al.2023].

This study is based on a baffled reactor, which investigates its degradation of nitrophenols under microoxygen aeration conditions. The physical and chemical indicators during its operation are monitored, and the microbial community structure is analyzed using a high-throughput sequencing method to provide a reference basis for the study of the pollutant removal mechanism in the micro oxygen operation of the baffled reactor.

1. Material and Methods

1.1. Experimental device

Micro oxygen baffled reactor (mOBR) length × wide × Height: 524 × 170 × 460mm, divided into four compartments, with a ratio of 4:1 between the upstream and downstream chambers. An aeration head is installed in the Downward flow chamber, which is connected to an air compressor and controls the aeration rate through a flow meter to achieve a micro oxygen state in the reactor. The exterior of the mOBR is heated by a water bath in a water tank, which facilitates the control of the temperature inside the reactor at (33 ± 1) ℃. The inlet water is pumped from the raw water tank by the Peristaltic pump and pressed into the mOBR.

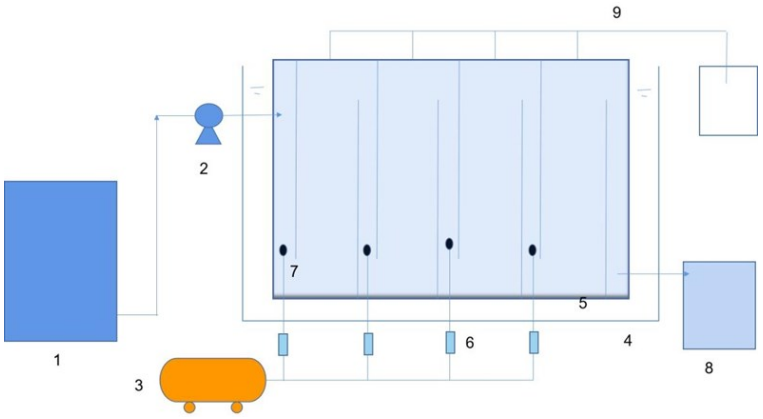


Figure 1. Experimental equipment.

1.Raw water tank, 2.Peristaltic pump, 3.Air pump, 4.Thermostatic water tank, 5.Micro oxygen baffle reactor, 6.Flowmeter, 7.Aerator, 8.Water tank, 9.Gas outlet

1.2. Inoculated sludge

Seed sludge is taken from anaerobic granular sludge in the internal circulation reactor (IC) of a stable citric acid plant, and the amount of sludge inoculated in each compartment of the reactor accounts for about one-third of the volume of each compartment. The micro oxygen baffled reactor (mOBR) first domesticates granular sludge under anaerobic conditions. When the COD concentration in the influent reaches 3000mg/L, and the removal rate reaches over 90%, the downstream compartments of the reactor are aerated, and the dissolved oxygen concentration is controlled to be around 0.3mg/L. When the COD concentration in the influent reaches 3000mg/L or above, the removal rate stabilizes at over 90% and enters the micro oxygen stable operation stage.

1.3 Experiment water

Using glucose as the organic carbon source (COD), NH_4HCO_3 as the nitrogen source, $K_2HPO_4 \cdot 3H_2O$ as the phosphorus source, and maintaining COD: N: P=200:5:1, while adding trace elements such as Fe, Cu, Mn, Ni, etc. PNP concentration 20-200mg/L.

1.4. Analysis methods

COD: Potassium dichromate method, pH value: pH meter, DO: dissolved oxygen meter method, VFA: steam distillation method. High throughput sequencing method for microbial diversity: mOBR took granular sludge from four compartments under stable operating conditions of HRT for 24 hours and commissioned Shanghai Shenggong Biotechnology Co., Ltd. for sequencing. DNA was extracted using OMEGA reagent kit E.Z.N.ATM Mag-Bind Soil DNA Kit, The primers used were V3-V4 universal primers 341F (CCCTACACGCTCTTCCGATCTG (barcode) CTACGGGNGGCGCAG) and 805R (GACTGGAGTTCCTGGCACCCGAATTCCGAGACTCAGACTACHVGGGTAT), the sample was purified, recovered, and sequenced after two rounds of PCR amplification.

2. Results and Discussion

2.1. Impact of influent nitrophenol concentration

Using glucose as the primary carbon source, maintain the COD concentration in the influent of the reactor at around 2000 mg/L and control the HRT at 24 hours to investigate the effect of the influent on nitrophenol concentration. The influent PNP concentration gradually increases in gradients of 20, 30, 50, 100, and 200 mg/L, and various indicators of the effluent are measured. The impact of influent PNP concentration on organic matter is shown in Figure 2, the effect on PNP removal is shown in Figure 3, and the impact on VFA and pH is shown in Figure 4.

The PNP concentration in the influent increased from 20mg/L to 200mg/L, and the COD removal rate decreased from 95.56% to 77.32%, indicating that the PNP concentration in the influent has a particular impact on COD removal. When the PNP concentration is below 100 mg/L, the COD removal rate is relatively stable, with a removal rate regular at 93.68%, and the reactor operates well. When the PNP concentration increases from 100 mg/L to 200 mg/L, the COD removal rate decreases to about 77%, indicating that the increase in PNP concentration inhibits the removal of COD. Under micro oxygen conditions, the intermediate products of PNP degradation and reduction are mainly p-aminophenol (PAP), 1,2,4- Trihydroxybenzenes, and 4-nitro Catechol [Zeng et al.2019]. The toxicity of PNP affects the removal of COD by granular sludge, resulting in a decrease in the removal rate of COD. According to research in the literature, the reduction in COD removal rate is not related to the degradation rate of added glucose but rather to the priority utilization of glucose by microorganisms, which then degrade PNP. The increase in COD effluent concentration is due to the generation of intermediate products.

During the experiment, the variation of PNP concentration in the inlet and outlet water is shown in Figure 3. The PNP concentration in the outlet water gradually increases after the inlet PNP concentration exceeds 100mg/L. When the influent concentration is below 100mg/L, the effluent is relatively stable. This indicates that high concentrations of PNP have a certain degree of inhibition on the reactor. If untamed microorganisms are used, the inhibitory effect on microorganisms is more substantial [Tong et al.2022;Yi et al.2006]. When the concentration of VFA is less than 100mg/L of PNP, the effluent is relatively stable, maintaining around 70-100mg/L. When the PNP concentration reaches 200mg/L, the highest VFA concentration in the effluent is 252mg/L, which exceeds 200mg/L. The change in effluent pH is relatively tiny, basically maintained between 6.3 and 7.5.

The changes in COD concentration and PNP concentration in each compartment are shown in Figures 5 and 6. When the PNP concentration in the influent is less than 100mg/L, there is no significant difference in COD effluent concentration between the first and fourth compartments under different influent concentration conditions. When the influent PNP concentration is 100mg/L, the COD concentration in the effluent of the first compartment is higher than that of 50mg/L. However, the concentration difference in the second compartment is not significant, indicating that the second compartment bears a specific COD degradation load. The final COD concentration in the effluent is similar to that of the influent PNP concentration below 50mg/L. However, when the influent PNP concentration is 200mg/L, the COD concentration in each compartment increases significantly.

The variation of PNP concentration in the effluent of each compartment is similar to that of COD. When the inlet PNP concentration is 100mg/L, the first compartment of mOBR has a significant increase in PNP concentration in the effluent. Still, the second compartment can degrade PNP to the previous level, indicating that the reactor can withstand a specific impact load. The second compartment can play a

buffering and degradation function, ensuring the degradation of pollutants. But when the influent concentration of PNP reaches 200mg/L, the effluent PNP concentration of each reactor compartment significantly increases, and the reactor operation is inhibited. The VFA value of the effluent also increases significantly, indicating that the particle sludge in the reactor can withstand a PNP concentration of 200mg/L. Excessive PNP concentration is not conducive to the stable operation of the reactor.

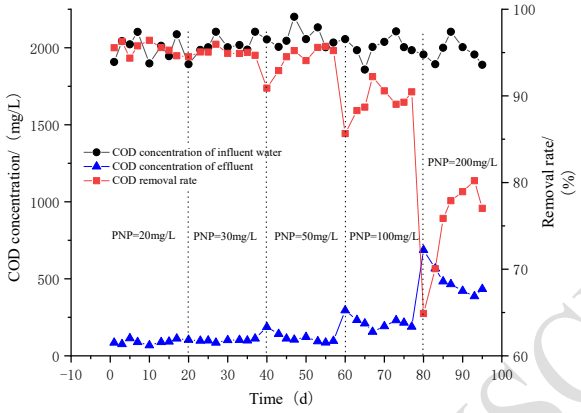


Figure 2. Effect of initial nitrophenol concentration on organic matter removal.

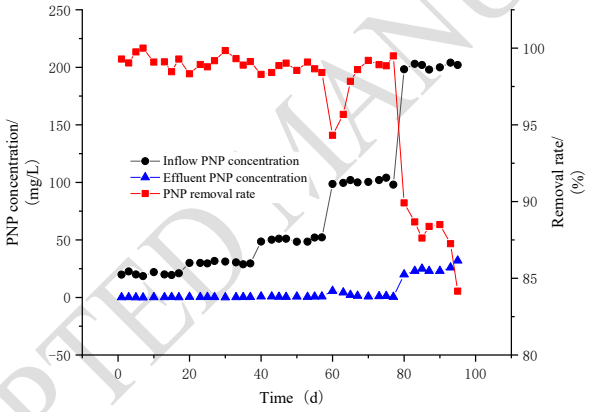


Figure 3. Effect of Influent PNP Concentration on PNP Removal.

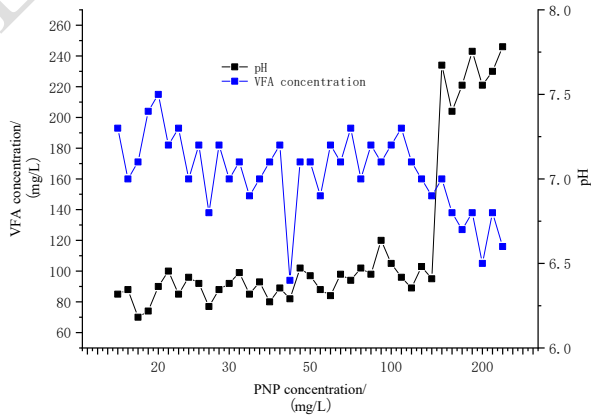


Figure 4. Effect of Nitrophenol Concentration on VFA and pH.

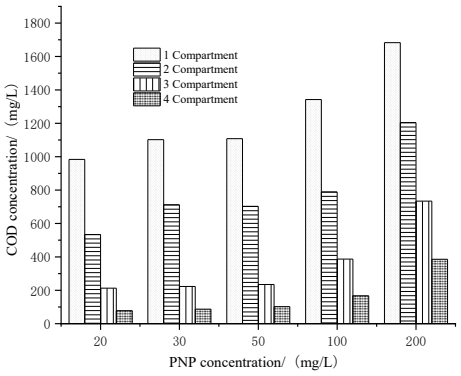


Figure 5. Changes of COD concentration in different compartments under different PNP concentrations.

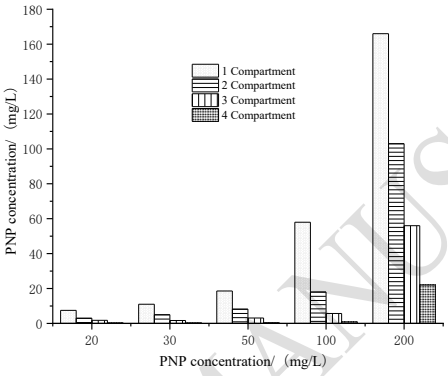


Figure 6. Change of PNP in each compartment of reactor under different PNP concentration.

2.2. Impact of external carbon Source concentration

Maintain the influent PNP concentration at around 100 mg/L, HRT at 24 hours, and dissolved oxygen concentration at about 0.3 mg/L. Using glucose as the primary carbon source, gradually reduce the influent COD at different concentrations such as 2000, 1500, 1000, and 500 mg/L. Analyze the impact of influent organic matter concentration on the treatment effect. The changes in COD in the effluent are shown in Figure 7, the effect of organic matter concentration in the influent on PNP is shown in Figure 8, and the impact on VFA and pH is shown in Figure 9.

The removal rate of COD decreased with the decrease of organic matter concentration in the influent throughout the experimental process. Still, the concentration of COD in the effluent changed relatively little, ranging from 230-310mg/L. This is because PNP contributes much of COD after being degraded into intermediate products. When the COD concentration is above 1000 mg/L, the removal rate of PNP remains above 88%. When the COD concentration is around 500 mg/L, the degradation rate of PNP changes to 45% -60%, indicating that the content of organic matter in the influent significantly impacts the degradation of PNP. The COD concentration and PNP concentration in the effluent of each compartment of the reactor under different influent COD concentrations are shown in Figures 10 and 11. As the COD concentration in the influent continues to decrease, the first and second COD concentration of the effluent from the three compartments gradually decreases.

In contrast, the COD concentration of the effluent from the fourth compartment does not differ significantly. When the COD concentration in the influent is 500mg/L, the PNP concentration in the

effluent of each compartment increases. PNP is both a toxic substance to microorganisms and a carbon source matrix. The presence of PNP has an inhibitory effect on the biodegradation of glucose, and the degradation of glucose can promote the conversion of PNP. It can provide a readily available carbon source to promote the rapid proliferation of microorganisms in the reactor. When its concentration is low, it is insufficient to support further growth of microorganisms. In order to maintain a high PNP removal effect, the influent should contain sufficient amounts of easily degradable matrix [Toja et al.2021]. The concentration of VFA in the effluent slightly increases with the decrease of COD but is less than 200mg/L, which is within the normal range. The pH value slightly decreased.

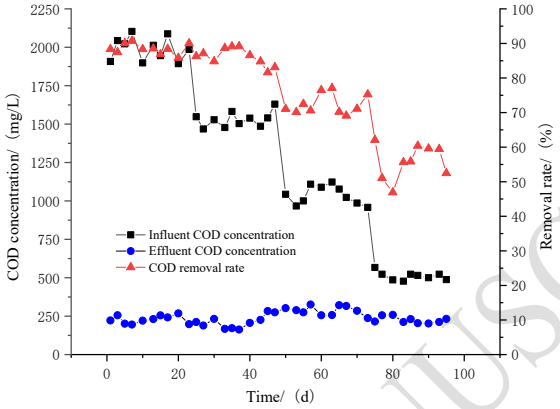


Figure 7. The impact of influent COD changes on COD removal

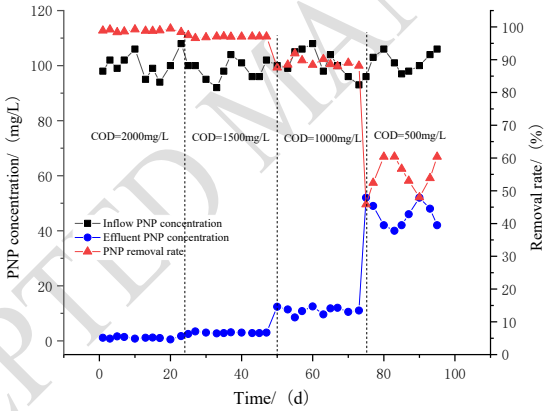


Figure 8. The effect of influent COD variation on PNP removal

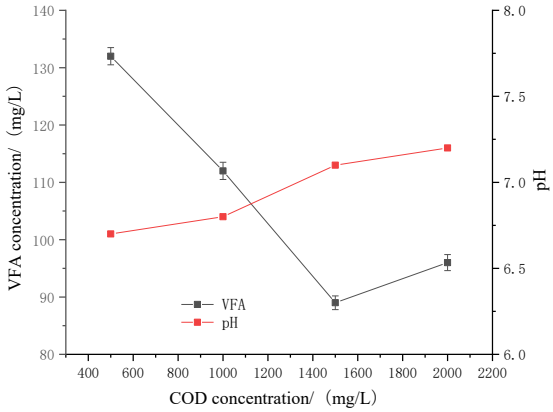


Figure 9. The influence of influent COD changes on VFA and pH

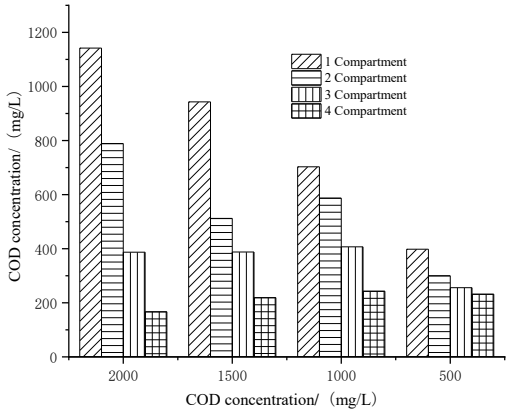


Figure 10. Changes of COD in each compartment of the reactor at different COD concentrations

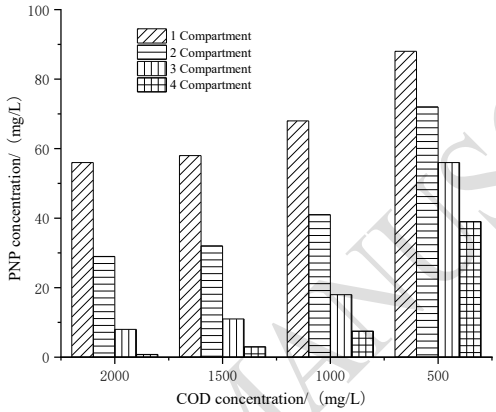


Figure 11. Changes of PNP in each compartment of the reactor under different COD concentrations

2.3. Impact of HRT

Maintain the COD concentration in the reactor's influent at around 2000 mg/L and the PNP concentration at about 100 mg/L, gradually reduce the HRT and operate for 24, 18, and 12 hours to investigate the impact of HRT changes on the removal efficiency.

Figures 12 and 13 show that with the decrease of HRT, the COD and PNP removal rate does not change much and can be maintained above 85%. After shortening the HRT, it did not affect the degradation of organic matter and PNP, mainly because the microorganisms in the reactor have adapted to PNP wastewater, and the granular sludge can withstand a specific load impact load. However, the concentration of VFA in the effluent has increased from 87 mg/L to 190mg/L. The pH change is relatively stable.

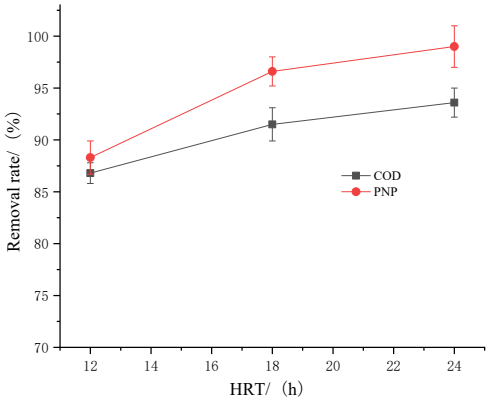


Figure 12. The impact of HRT changes on COD and PNP

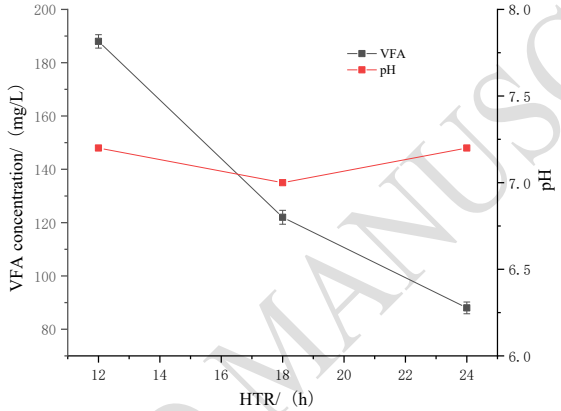


Figure 13. The impact of HRT changes on VFA and pH

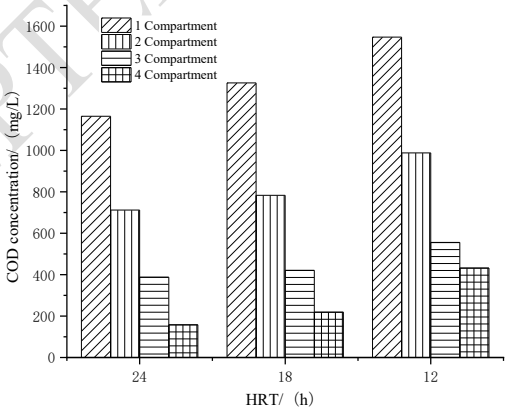


Figure 14. Changes of COD in each compartment of the reactor under different HRT conditions

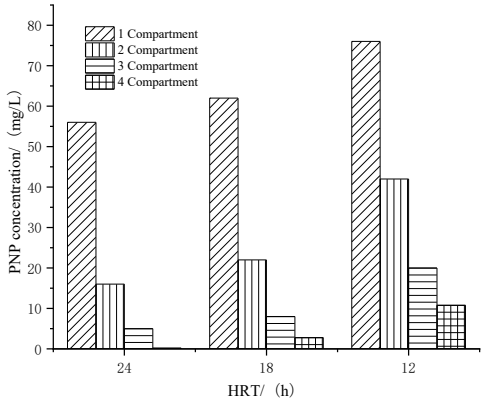


Figure 15. Changes of PNP in each compartment of the reactor under different HRT conditions

Figures 14 and 15 show that the COD concentration in the first chamber gradually increases as HRT decreases. As HRT decreases, on the one hand, the contact time between microorganisms and pollutants decreases, and on the other hand, the organic load borne by the first chamber increases. leading to an increase in the COD concentration in its effluent. When HRT=24 hours and HRT=18 hours, the COD concentration in the effluent did not change much but shortened to 12 hours, and the COD concentration in each compartment showed a significant increase; The variation of PNP concentration in each compartment with HRT is the same as in COD.

2.4. Impact of DO

Maintain the COD concentration in the influent of the reactor at around 2000 mg/L and PNP concentration at about 100 mg/L, and control the reactor at different DO concentrations of 0.1, 0.3, 0.5, and 0.7 mg/L with HRT for 24 hours. Observe the removal rate of COD and PNP by the reactor, as shown in Figure 16, And the changes in VFA and pH are shown in Figure 17.

It can be seen that with the increase of DO concentration, although the change in COD removal rate is not significant, its removal first increases and then decreases. Anaerobic conditions are conducive to the degradation of high-concentration organic matter, while aerobic conditions further reduce the concentration of organic matter. Figures 18 and 19 show that COD and PNP concentrations in each compartment are lower at DO concentrations of 0.3-0.5mg/L than at DO concentrations of 0.1mg/L and 0.7mg/L. Under experimental conditions, when the DO concentration is between 0.3 and 0.5mg/L, the removal rate of COD is the highest, as there are anaerobic and aerobic zones in the granular sludge. Anaerobic and aerobic microorganisms cooperate, resulting in the highest removal rate of COD [Song et al.2021,Qin et al.2022]. When the DO value is less than 0.3mg/L, the anaerobic zone in the granular sludge increases, and the aerobic area decreases, resulting in the inability of effective aerobic treatment of organic matter after anaerobic degradation and, ultimately, a decrease in COD removal efficiency. When the DO concentration reaches 0.7mg/L, the anaerobic zone becomes smaller, leading to weaker anaerobic degradation ability and decreased COD removal rate.

The changes in DO have little impact on the degradation of PNP, as well as VFA and pH, mainly because the microorganisms in the reactor can adapt to PNP degradation after domestication. In contrast, PNP degradation can be reduced or oxidized under anaerobic and aerobic conditions[Luo et al.2021].

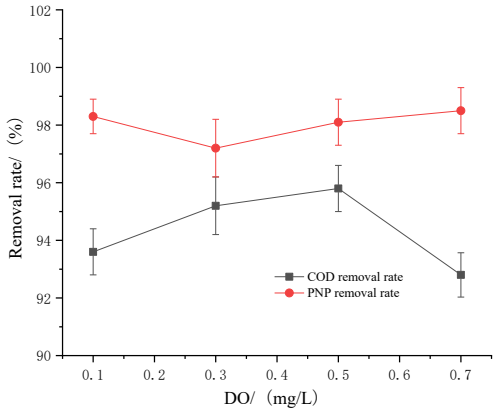


Figure 16. The impact of DO changes on COD and PNP

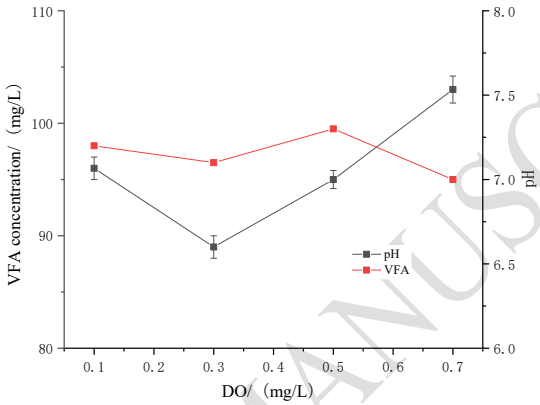


Figure 17. The impact of DO changes on VFA and pH

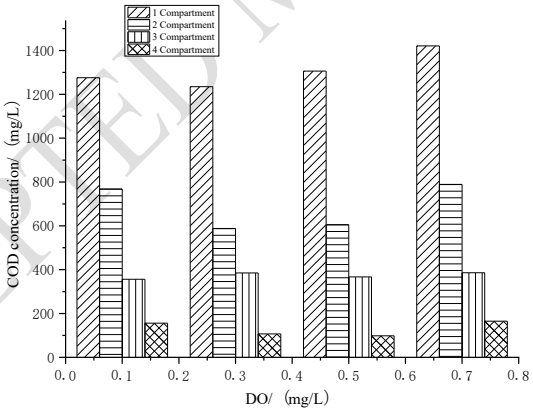


Figure 18. Changes of COD in each compartment of the reactor under different DO concentrations.

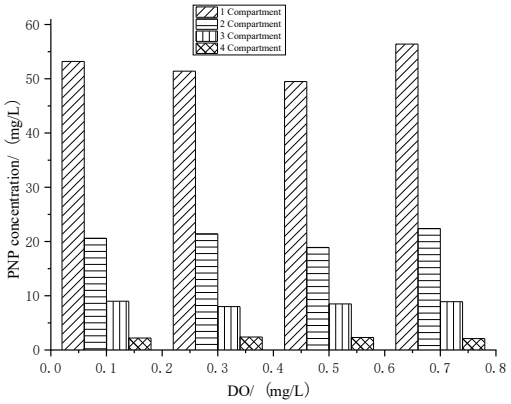


Figure 19. Changes of PNP in each compartment of the reactor under different DO concentration.

2.5. High throughput sequencing analysis of granular sludge

(1) Microbial diversity analysis

The abundance and diversity of microbial communities can be reflected through the diversity analysis of samples. The analysis index of microbial diversity within mOBR is shown in Table 1. It can be seen that there are specific differences in the richness index and Diversity index of the microbial community in each compartment. The species richness in the first compartment and the third compartment is high, the species complexity in the first compartment is low, and the microorganisms in the fourth compartment are mainly Methanogen, with low community diversity but a large proportion. Generally speaking, the richer the number of bacterial species and community diversity, the better the removal effect of pollutants.

Table 1. Indicators of Microbial Abundance and Diversity.

Sample	Seq Num	OTU Num	Shannon index	ACE index	Chao1 index	Coverage	Simpson
1 Compartment	70496	5116	5.18	63984.08	32171.75	0.92	0.04
2 Compartment	53146	4966	5.57	54223.98	26006.98	0.93	0.02
3 Compartment	63697	5738	5.35	67459.15	33689.13	0.93	0.04
4 Compartment	33756	3674	5.23	31205.02	16709.52	0.94	0.05

(2) Analysis of microbial community structure

Figure 20 shows the microbial Wayne diagram, from which it can be seen that the diversity of microbial community structure in the samples of each compartment is relatively affluent. Among them, 800

bacterial OTUs and specific bacteria are in each compartment. Figure 21 shows the level of microorganisms in each compartment, including Pseudomonadota, Chloroflexota, and Bacteroidota. Pseudomonadota can effectively degrade organic matter in wastewater, and Chloroflexota can effectively degrade carbohydrates in water [Rout et al.2022;Tournier et al.2023]; Bacteroidota can effectively degrade complex organics and macromolecular substances [Gao et al.2019;Yang et al.2022]. Actinomycetota strongly tolerates phenols, Polycyclic aromatic hydrocarbons, and other pollutants [Semenova et al.2022].

From Figure 22, it can be seen that the species with higher abundance have been detected at the genus level, including Chlorobium, Chlorobaculum, longline, Levilinea, Treponema, etc., all of which mainly function in breaking down proteins, metabolizing carbohydrates, generating organic acids, and oxidizing acetic acid; Metanobacterium, Syntrophobacter, and Syntrophomonas are genera of bacteria that can participate in methane production. Desulfovibrio, Thiobacillus, sulfurisoma, and others all have the function of organic acid oxidation and can reduce S element; Saccharibacteria, Pseudomonas, Petrimonas, Smitherlla, Candidatu, and other bacterial genera can degrade substances such as phenols and hydrocarbons.

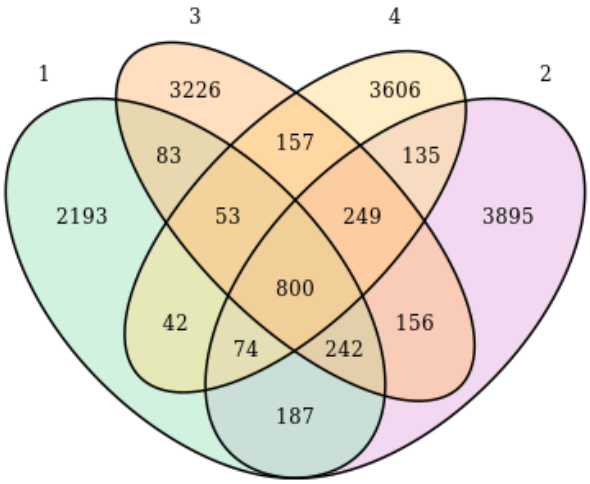


Figure 20. The OTU venn diagram of activated sludge group.

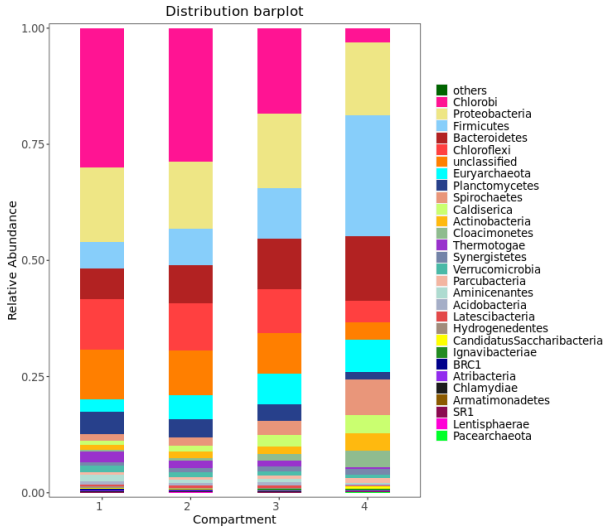


Figure 21. The taxonomic composition distribution in samples at the phylum level.

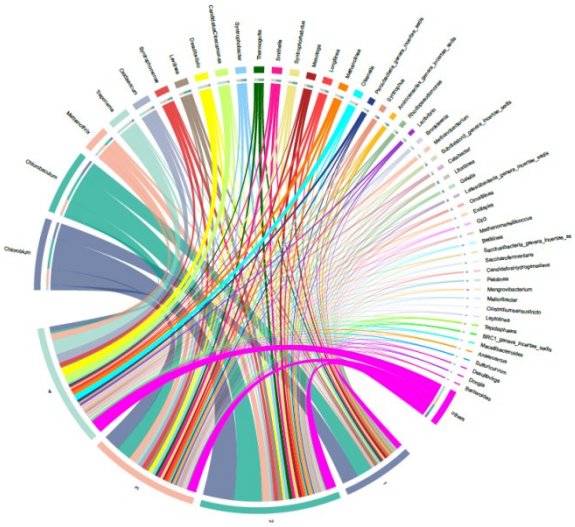


Figure 22. Taxonomic composition distribution in samples at the genus level

(3) Differences in microbial communities among different compartments

Using PCA analysis and sample similarity tree diagrams, the differences in microbial community structure among different compartments can be analyzed. The specific results are shown in Figure 23. In the PCA diagram, the smaller the distance between two points, the higher the degree of community aggregation and the greater the similarity of the samples. There are varying degrees of differences among various products without high similarity. The highest point is at 4, followed by 3, and the lowest is at 2 and 1; 1. The relative distance between points 2 and 3 is close, indicating that the difference in microbial community structure between them is negligible compared to point 4. This is because wastewater containing nitrophenols first enters the first compartment (point 4), where nitrophenols undergo primary degradation and then join other compartments in sequence. Therefore, there

is a significant difference in microbial communities between the first compartment and the three different compartments.

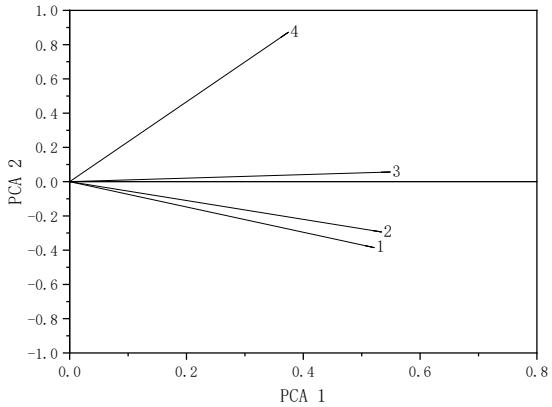


Figure 23. PCA analysis of microbial community structure in each compartment.

3. Conclusion

(1) The micro oxygen baffle reactor has good degradation performance for nitrophenol wastewater. When the influent's organic matter (glucose) content is around 2000 mg/L, HRT is maintained for 24 hours, and the PNP concentration is below 100 mg/L, the COD removal rate stabilizes at 93.68%. When the PNP concentration increases to 200 mg/L, the COD removal rate decreases to about 77%. The removal rate of COD decreases with the decrease of organic matter concentration in the influent. Still, the change in COD concentration in the effluent is relatively small, ranging from 230-310mg/L. With the decrease of HRT, the removal rate of COD and PNP in the reactor does not change much and can be maintained above 85%. After shortening HRT, organic matter and PNP degradation are not significantly affected. When the DO concentration is between 0.3-0.5mg/L, the removal rate of COD is the highest, indicating that micro aeration is beneficial for removing pollutants.

(2) The degradation of nitrophenols is mainly concentrated in the first two compartments of the micro oxygen baffled reactor, with the first compartment playing a leading role and the subsequent compartments becoming beneficial supplements for the system to cope with load shocks.

(3) The microbial community composition in each reactor compartment was analyzed by high-throughput sequencing. The species abundance in the first and third compartments was high. The microbial groups in the reactor mainly included Bacteroidetes, Firmicutes, Proteobacteria, Spirochaetes, and Actinobacteria.

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