

Effect of Polypropylene Microplastics Concentration on Wastewater Denitrification

Xiongshuang Su^{1,2,3}, Cheng Chen^{2,3}, Jia Li², Shun Lu², Guihua Xu^{2,*}

¹College of River and Ocean Engineering, Chongqing Jiaotong University, Chongqing, China

²Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing, China

³Chongqing School, University of Chinese Academy of Sciences, Chongqing, China

Email address:

xuguihua@cigit.ac.cn (Guihua Xu)

*Corresponding author

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Abstract: Microplastics have been ubiquitously in the environment, and a large number of microplastics have entered wastewater treatment plants, and their effects on activated sludge denitrification are rarely reported. Polypropylene (PP) microplastics were selected as the research object for denitrification research. Herein, we set different concentrations of PP microplastics to study their effect on sewage denitrification. Several indicators including NO_3^- -N, NO_2^- -N, Total-N (TN), extracellular polymer substances, Zeta potential, and N_2O production, etc., were employed to evaluate the denitrification effect. Results demonstrated that the denitrification process was affected by the concentration of PP microplastics. Its concentration may damage the denitrification of sludge, especially in low concentrations, due to its slowing down the NO_2^- -N reduction process. Meanwhile, it will release an amount of greenhouse gas N_2O as the concentration is positively correlated. However, the removal of NO_3^- -N is almost unaffected in different concentrations of PP microplastics during the denitrification process. When the concentration of PP microplastics reached 60 mg L^{-1} , the accumulation of NO_2^- -N in the effluent increased significantly. Accordingly, the extracellular polymer and potential indicators also showed that PP microplastics will reduce the flocculation capacity of the sludge, resulting in a decrease in the denitrification efficiency of the sludge overall. When the concentration of PP microplastics exceeded 100 mg L^{-1} , the impact of microplastics on denitrification water quality indicators was relatively small, but it will increase the N_2O emission with a negative impact. In addition, the activated sludge denitrification pathway can be affected by the addition of different microplastics and requires further investigations in the future.

Keywords: Microplastics, Polypropylene, Denitrification, Activated Sludge

1. Introduction

Microplastics ($d \leq 5 \text{ mm}$) first entered the public's field of vision as Thompson [1] put forward this concept for the first time since 2004. Microplastic pollution has attracted worldwide attention in recent years, however, the effects of microplastics still keep difficult to understand comprehensively, especially the existence of microplastics in the ocean [2], soil [3, 4], and atmosphere [5]. Moreover, plastic products often use plasticizers, colorants, and other chemicals to enhance their physical behaviors. But it became harmful as it turned into smaller plastics, furthermore, the

size (low than 5 mm) of smaller plastics brought a worse impact towards the environment. Because of the properties of small particle size and large specific surface area, microplastics can absorb polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls, heavy metals, persistent organic compounds, and other hazardous substances during environmental migration [6-8], increasing in toxicity of microplastics and threatening the safety of the environment. Consequently, microplastics has suddenly become a new kind of environmental pollutant with high risk [9] in the environment. Scholars noticed microplastics' potential threats and studied their possible detection, occurrence, and

removal mechanism. Sun *et al.* [10] investigated the interaction of microplastics and PAHs resulted in the decrease in total counts of blood cells, the change of blood components, and the inhibition of blood cell phagocytosis on marine bivalves. Further analysis showed that the mixture of microplastics and PAHs may increase the content of reactive oxygen species (ROS) in cells, leading to lipid peroxidation and DNA damage, and reducing the vitality of blood cells, thus having toxic effects on blood parameters and destroying important molecular signaling pathways. Banaee *et al.* [11] observed that polyethylene (PE) and Cd exposure ($200 \mu\text{g L}^{-1}$) had a synergistic effect on carp, which enhanced the sublethal effect. And the blood biochemical and immune parameters were also changed after 30 days of exposure. That is, the existence of microplastics will threaten the normal life activities of living organisms.

The sewage treatment plant is an important part of urban wastewater remediation, and biological treatment is a cost-effective and environment-friendly method for wastewater denitrification [9]. According to the previous research, sewage treatment plants will intercept more than 90% of microplastics, and the removal efficiency will reach 90.52% [12]. Even so, sewage treatment plants still become one of the main sources of environmental microplastics [12, 13]. Biological treatment in sewage treatment plants is the core of water treatment, in which microorganisms play an absolutely important role [14]. Relevant studies indicate that anaerobic digestion of sludge [15] and other biological processes will be negatively affected to varying degrees in the presence of microplastics. Meanwhile, the microbial community structure and function related to nitrogen metabolism will change correspondingly, and nitrogen metabolism will become slow [16, 17]. Therefore, microplastics will also affect the biochemical cycle of carbon [18].

Yang *et al.* [16] studied microplastics-polystyrene in constructed wetlands and found that microplastics harmed the nitrification process of wetlands. With the accumulation of time, the removal efficiency of total nitrogen in microplastics decreased by 29.5–40.6%. Simultaneously, microorganisms absorbed microplastics into the body through endocytosis, which destroyed the cell membrane integrity and the balance of reactive oxygen species in cells and inhibited the activities of enzymes and electron transport systems. Seeley *et al.* [17] conducted a microplastic experiment on salt marsh sediments in coastal areas and found that the presence of microplastics would change the microbial community composition and nitrogen cycle process of sediments, especially nitrification and denitrification.

In addition, some researchers reported that microplastics could reduce the abundance of anaerobic digestion bacteria, nitrifying bacteria, denitrifying bacteria, heterotrophic nitrification-aerobic denitrifying bacteria, and bacteria capable of degrading phenolic compounds, and inhibit the biological transformation of ammonia nitrogen ($\text{NH}_4^+\text{-N}$) [19, 20]. However, different results will be shown according to different environmental conditions and different types of microplastics in the study of the influence of microplastics on

the nitrogen cycle. It is mostly negative for refractory microplastics. The degradable microplastics could be regarded as a carbon source, providing energy for microorganisms, and promoting nitrification and denitrification. Tang *et al.* [21] explored degradable and refractory microplastics polybutylene succinic acid (PBSA) and PE and concluded that both microplastics and refractory microplastics had a greater impact on nitrate removal. Seeley *et al.* [17] and Sun *et al.* [22] also proved that degradable microplastics can promote the nitrogen cycle process of organisms, while refractory microplastics can inhibit the nitrogen removal effect. However, degradable microplastics will increase the secretion of extracellular polymer in the process of degradation, which will affect the cohesion of sludge.

Microplastic affects the microbial process, either promoting or inhibiting it. Therefore, the research on the influence of microplastics on the denitrification process in sewage treatment plants can provide an important theoretical basis for microplastic's influence on the microbial process, thus providing theoretical guidance for the optimal design of sewage treatment plants. In this paper, the effect of the addition of different concentrations of PP microplastics on denitrification was studied, and the effect of microplastics on the denitrification process was reflected by the corresponding water quality index, biological index, and gas production, to reveal the effect of microplastics on biological denitrification in sewage treatment plants.

2. Materials and Methods

2.1. Establishment and Operation of Denitrification Reactor

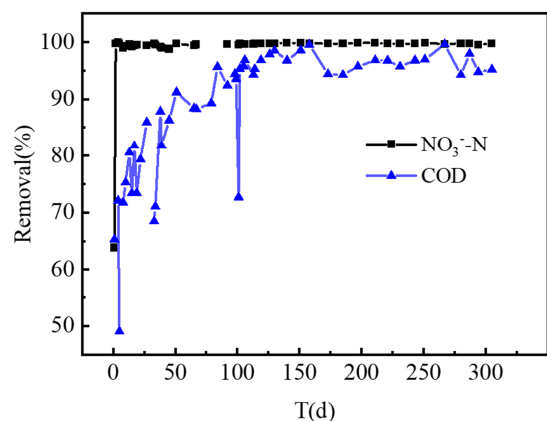


Figure 1. Reactor operation data.

The highly activated sludge was taken back from the end of the aeration tank of Xiaojiahe Sewage Treatment Plant in Chongqing and settled by standing still. After standing, the sludge was domesticated and cultured in a laboratory denitrification reactor. The reactor has a volume of 6 L and an effective volume of 4.55 L and adopts a sequencing batch reactor (SBR) for culture, with a period of 8 h, and the water is automatically fed in and out by using a time delay. The

influent water is artificially distributed by NO_3^- -N $100 \text{ mg}\cdot\text{L}^{-1}$ (supplied by sodium nitrate), chemical oxygen demand (COD) $600 \text{ mg}\cdot\text{L}^{-1}$ (supplied by sodium acetate trihydrate, and glucose), $0.2 \text{ g}\cdot\text{L}^{-1}$ dipotassium hydrogen phosphate, $0.1 \text{ mL}\cdot\text{L}^{-1}$ trace elements and constants. The denitrification reactor runs until the index is stable, that is, the removal of NO_3^- -N and COD in the effluent reaches over 99% and 80% (Figure 1), and the effluent does not contain nitrite nitrogen. At this time, the sludge concentration in the reactor is $4000 \pm 200 \text{ mg}\cdot\text{L}^{-1}$.

It is estimated that the maximum concentration of microplastics can reach $0.5 \text{ g}\cdot\text{L}^{-1}$ [23] in the sewage treatment plant. Therefore, in this experiment, the concentration of microplastics was set at 0, 10, 30, 60, 100, 200, and $500 \text{ mg}\cdot\text{L}^{-1}$ PP microplastics, and it was divided into low concentration group and high concentration group with $100 \text{ mg}\cdot\text{L}^{-1}$ as the boundary. Take out the sludge from the stable denitrification reactor, transfer it to a 250 mL conical flask, and add microplastics with corresponding concentrations, with two parallel units in each group. After adding nutrients and microplastics to the conical flask, the purity was rinsed with 99.999% nitrogen to remove the air in the conical flask, seal it, and then put it in a constant-temperature shaking table for oscillating reaction. The experimental temperature is controlled at $(27.5 \pm 0.5)^\circ\text{C}$ and the rotating speed is $160 \text{ r}\cdot\text{min}^{-1}$. The denitrification reaction cycle is 5 h, and samples are taken every 0.5 h.

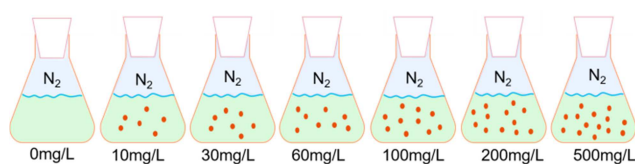


Figure 2. Batch experimental design.

2.2. Determination of Water Quality Index and Zeta Potential

The water quality index can directly indicate the influence of microplastics on denitrification from a macro level. During the experiment, the water samples collected every 0.5 h were filtered by a $0.45 \mu\text{m}$ filter membrane, and the indexes of nitrate-nitrogen (NO_3^- -N), nitrite-nitrogen (NO_2^- -N), and total nitrogen (TN) in water quality were determined by ultraviolet spectrophotometry and rapid digestion-ultraviolet spectrophotometry, respectively, and the data were recorded and analyzed. In addition, at the end of the experiment, after the sludge settles stably, the supernatant of the experiment is collected, and the Zeta potential of the sludge is measured and analyzed by Malvern potential analyzer. Zeta potential is mainly used to evaluate the dispersion degree of particles in water, and it can be used to evaluate the influence of microplastics on biological flocs in experiments.

2.3. Extraction of Extracellular Polymer Substances (EPS)

The modified thermal extraction method was used for

extraction [24]. Take 15 mL of the sludge-water mixture from the denitrification experiment, centrifuge it for 10 min at 4°C for $4000 \text{ r}\cdot\text{min}^{-1}$, discard the supernatant, restore the volume with 0.9%NaCl solution, and wash it three times repeatedly. Then, 0.9%NaCl solution was used to restore the volume, heated at 80°C for 30 min, centrifuged at $8000 \text{ r}\cdot\text{min}^{-1}$ for 3 min, and the supernatant was collected as the sludge EPS extract. Anthrone-concentrated sulfuric acid method and BCA kit method were used to determine polysaccharides and protein in EPS.

2.4. N_2O Gas Collection

N_2O , the greenhouse gas produced in the process of sludge denitrification, is collected by a disposable calibrated syringe, and the needle is controlled by a three-way valve during collection to prevent the outside air from entering. The collected N_2O is immediately injected into a gas chromatographic sampling bottle and determined by a gas chromatograph (GC-2014).

2.5. Data Analysis

The data analyzed in the experiment are mainly processed by Microsoft EXCEL 2019 and Origin 2021b.

3. Results and Discussion

3.1. The Influence of Different Concentrations of PP Microplastics on the Water Quality of the Denitrification Process

As shown in Figure 3a, with the increase of PP microplastic concentration, the overall change of NO_3^- -N content in the experimental group with low microplastic concentration showed a relatively consistent trend with that in the control group, and the effluent concentration did not change much. Specifically, the concentrations of NO_3^- -N were 2.36, 1.61, $3.57 \text{ mg}\cdot\text{L}^{-1}$, and the control was $0.95 \text{ mg}\cdot\text{L}^{-1}$. Almost all NO_3^- -N contained in the water body was removed, and the removal rate reached 98%, that is, the low concentration of PP microplastics would not affect the removal of NO_3^- -N in the biological denitrification process. However, the presence of PP microplastics will affect the removal of NO_2^- -N (Figure 3c). In the first 60 minutes, the content of NO_2^- -N in the water of the experimental group added with microplastics was higher than that of the control group, and the accumulated amount reached the maximum $8.24 \text{ mg}\cdot\text{L}^{-1}$ when the PP microplastics concentration was $30 \text{ mg}\cdot\text{L}^{-1}$. Within 60~150 minutes, the content of NO_2^- -N in the experimental group was similar to that in the control group. After 150 minutes, the reduction rate of NO_2^- -N in the experimental group slowed down and the content gradually accumulated. By the end of the reaction, the content of NO_2^- -N in the experimental group was higher than that in the control group at $0.34 \text{ mg}\cdot\text{L}^{-1}$, the accumulated amounts are respectively 0.85, 0.56, $1.31 \text{ mg}\cdot\text{L}^{-1}$, in which the inhibition of NO_2^- -N reached the maximum when the concentration of PP microplastics was $60 \text{ mg}\cdot\text{L}^{-1}$, and the removal effect

decreased by 10.4% compared with that of the control group. That is, with the increase of microplastic concentration, the

inhibition of NO_2^- -N reduction became more obvious [25] in the low microplastic group.

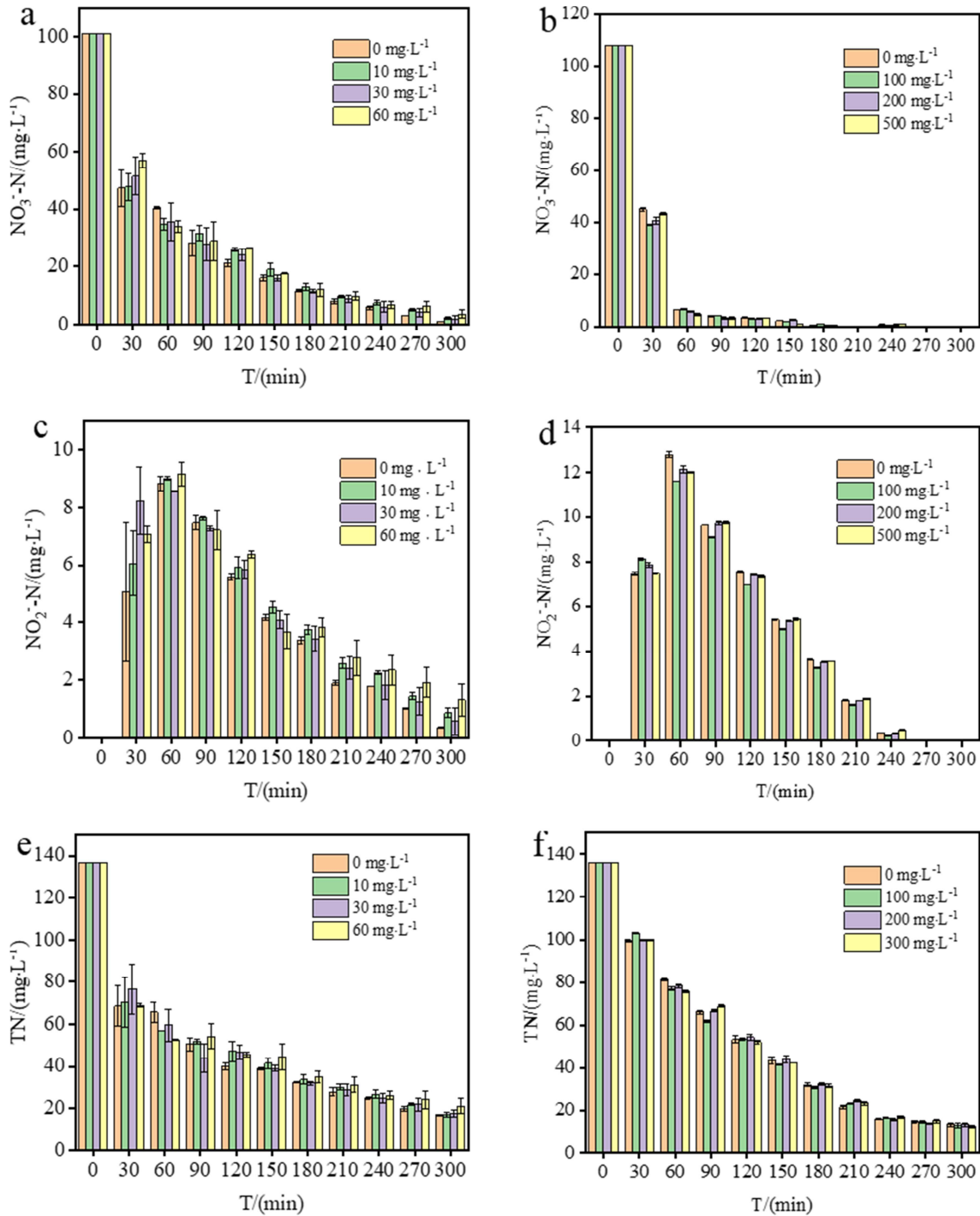


Figure 3. The effect of microplastics concentration on denitrification water quality index (a, b: NO_3^- -N; c, d: NO_2^- -N; e, f: TN).

When the concentration of PP microplastics exceeds $100 \text{ mg}\cdot\text{L}^{-1}$, compared with the control group, the removal of NO_3^- -N is still not affected by microplastics (Figure 3b), which proves that the reduction of NO_3^- -N is not affected by microplastics and has nothing to do with the concentration of microplastics [26]; Compared with the control group (Figure 3d), the removal trend of NO_2^- -N is not obvious, but within 60~240 min, the content of NO_2^- -N in the experimental group

with the concentration of $100 \text{ mg}\cdot\text{L}^{-1}$ is lower than that in the control group. At the end of the final reaction, the content of NO_2^- -N is consistent with that of the control group, and the removal effect is not affected by microplastics. At the end of the reaction, the measured results of TN (Figure 3e, Figure 3f) were high in both low and high concentrations, which may be due to the high content of TN caused by the presence of ammonia nitrogen and some nitrogen-containing

intermediate metabolites in water.

Through analysis, the reduction of NO_2^- -N is related to the external environment of microorganisms. In the presence of PP microplastics in the solution, the probability of collision between oscillating microorganisms and microplastics increases, the sludge flocs become unstable under the shear of microplastics, and the mass transfer capacity among microorganisms decreases [21], which leads to a decrease of NO_2^- -N uptake by microorganisms and the increase of NO_2^- -N accumulation in water; however, when the concentration of microplastics exceeds $100 \text{ mg}\cdot\text{L}^{-1}$, although the shear force of PP microplastics still exists in the solution, the high concentration of microplastics will provide a new anaerobic environment for microorganisms [27], thus providing an attached surface for denitrifying microorganisms. Therefore, in the high concentration microplastics group, the reduction rate of NO_2^- -N is almost the same as that of the control group, and the final nitrogen removal effect in water is not affected by microplastics. He et al. [25] studied the results of sludge nutrient removal by microplastic concentration. When the microplastic concentration was lower than $100 \text{ mg}\cdot\text{L}^{-1}$, the reduction of NO_2^- -N was also inhibited by microplastics and had nothing to do with the particle size of microplastics. Shi et al. [28] also got the conclusion that when the concentration of microplastics was low, the accumulation of NO_2^- -N increased through the experiment on the microplastics of aerobic denitrification [28]. Li et al. [27] experimental results show that when the concentration of microplastics is high, it will promote denitrification, which is also consistent with the results of this experiment. That is, when a high concentration of microplastics exists,

microplastics provide a new place for microbial reaction, which accelerates the denitrification rate, thus offsetting the decline of the denitrification effect caused by shearing in microplastics. However, in other kinds of literature, high concentration microplastics will also inhibit the reduction of NO_2^- -N because of their different types. Therefore, attention should be paid to the influence of different types of microplastics on the denitrification process in the future.

3.2. Effects of Different Concentrations of PP Microplastics on Zeta Potential and Extracellular Polymer Substances of Denitrifying Sludge

As shown in Figure 4a, in the low concentration experimental group, when the concentration is 10 and $30 \text{ mg}\cdot\text{L}^{-1}$, the absolute value of Zeta potential is slightly larger than that of the control group, indicating that the dispersion ability of particles in the solution is stronger than that of the control group in these two concentration gradients. According to theoretical analysis, the higher the potential value, the higher the possibility of sludge flocs being acted by microplastics shear force, which makes sludge flocs unstable and reduces the denitrification capacity. The increase in the number of free particles in the solution is more obvious in the high concentration microplastics group (Figure 4b). In the high concentration group, compared with the control group, the absolute value of potential increased, which was related to the instability of sludge flocs and the increase of free microorganisms, and also related to the potential of microplastics itself, because microplastics themselves also showed negative electricity [29].

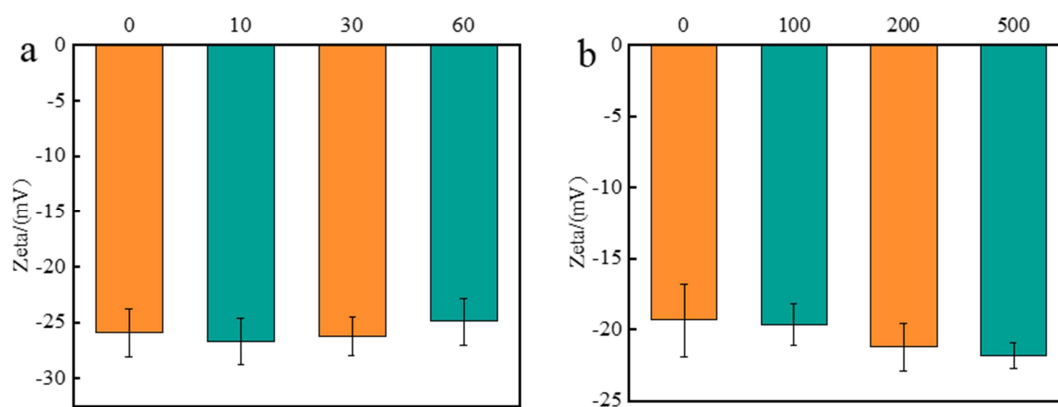


Figure 4. Effect of microplastics concentration on potential value (a: low concentration; b: high concentration).

At the same time, through the measurement of extracellular polymer substances (EPS), it can be seen that in the low concentration experiment (Figure 5a), the overall concentration of polysaccharides decreased at 10 and $30 \text{ mg}\cdot\text{L}^{-1}$, which is consistent with the result of the increase of the absolute value of the above potential, because the EPS content has a positive correlation with the stability of sludge flocs [30]. In the high concentration experimental group (Figure 5b), the total content of EPS was higher than that of the control group, which indicated that under this condition, although microbial flocs were subjected to the shear force of

microplastics, the anaerobic environment was increased due to the high concentration of microplastics. In addition, because microplastics had a certain adsorption performance [31], microorganisms could colonize with microplastics as the carrier, thus secreting extracellular polymers to adapt to the new external environment and forming a new microbial microenvironment in microplastics with microplastics. The increase of EPS content helps to promote the connection between microorganisms [32], and microorganisms can quickly take in nutrients contained in water and consume inorganic nitrogen in the water.

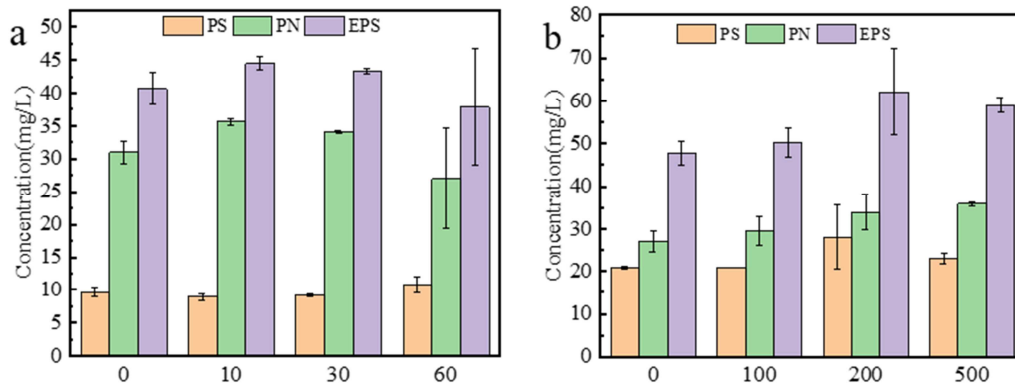


Figure 5. Effect of microplastics concentration on potential value (a: low concentration; b: high concentration).

3.3. Effect of Different Concentrations of PP Microplastics on Nitrous Oxide (N_2O) Emission from Denitrifying Sludge

As one of the greenhouse gases, N_2O causes about 265 times the greenhouse effect of carbon dioxide [33], and its ability to destroy the ozone layer exceeds that of carbon dioxide. Denitrification is the main denitrification reaction, and N_2O is inevitable, but it should be reduced as much as possible. As shown in Figure 6a, the N_2O emission in the denitrification experiment group with a low concentration of microplastics was higher than that in the control group, with an average increase of about 0.3 ppm in the experiment groups of 30 and 60 $mg \cdot L^{-1}$, which was twice that of the control group. Similarly, in the high concentration experimental group, the N_2O release of microplastics added with PP also increased a lot compared with the control group (Figure 6b). Generally speaking, the addition of PP

microplastics will lead to an increase in N_2O release [27, 34], and the amount of N_2O release has nothing to do with the concentration, but only with the addition of microplastics. N_2O is a more intense greenhouse gas than CO_2 , and the addition of microplastics increases its emission, which is unfavorable to sewage treatment plants and the environment. Therefore, it is necessary to control microplastics in the process of sewage treatment.

In Figure 6c, the result of denitrification potential is consistent with the water quality index. When the concentration of microplastics was 10, 30, and 60 $mg \cdot L^{-1}$, the total N_2O production was lower than that of the control group, indicating that the total nitrogen content of microorganisms was lower than that of the control group. N_2O released by denitrification potential in high concentration is roughly equal to that of the control group (Figure 6d), and the nitrogen intake by microorganisms is consistent with the water quality index.

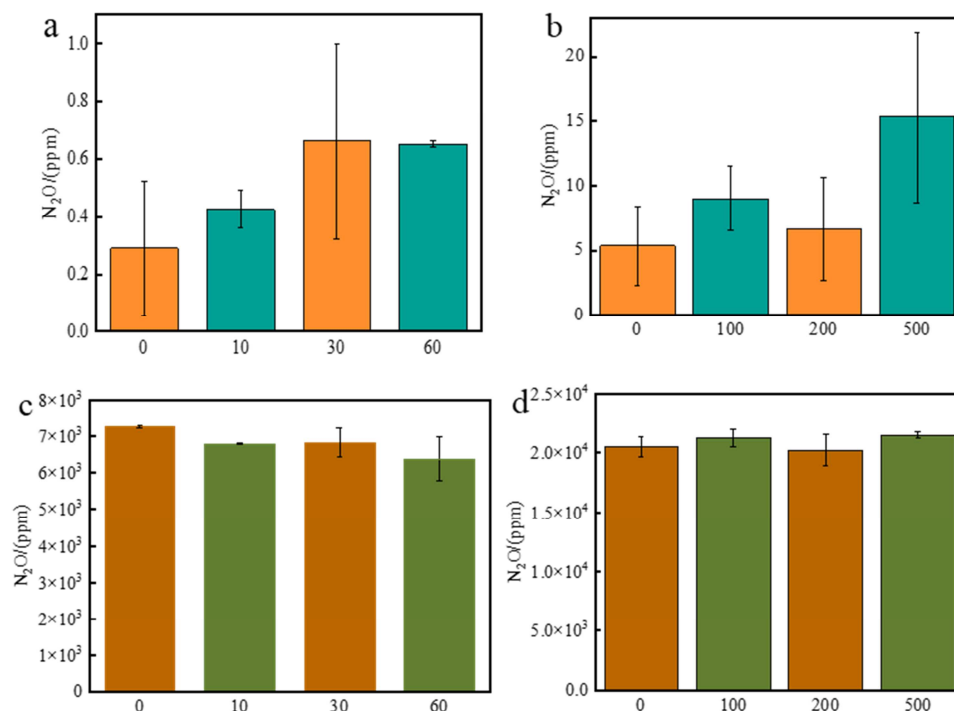


Figure 6. N_2O emission (a, b: natural emission; c, d: denitrification potential).

4. Conclusion

In this study, different concentrations of PP microplastics were used to study the biological denitrification process in sewage treatment plants. The results showed that the denitrification effect was affected by the concentration of microplastics at different concentrations. When the concentration of PP microplastics is lower than $100 \text{ mg} \cdot \text{L}^{-1}$, the reduction process of $\text{NO}_2^- \text{-N}$ will be slowed down, and the sludge flocs will become unstable due to the shear force. And the concentration of PP microplastics is $60 \text{ mg} \cdot \text{L}^{-1}$, the inhibition of denitrification is the most obvious, whether it is water quality index or biochemical index; however, when the concentration exceeds $100 \text{ mg} \cdot \text{L}^{-1}$, it has little influence on the water quality index, and the denitrification process is almost unaffected.

On the whole, in the presence of microplastics, N_2O production is always higher than that of the control group, but there is no positive correlation with the concentration of microplastics. Therefore, it is necessary to control microplastics in sewage treatment plants. And in the future, we also should further explore the influence pathway of the microbial community, to provide strong data support for optimizing the influence of microplastics in sewage treatment plants.

Acknowledgements

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References

- [1] R. C. Thompson, Y. Olsen, R. P. Mitchell, A. Davis, S. J. Rowland, A. W. John, D. McGonigle, A. E. Russell, Lost at sea: where is all the plastic?, *science* 304 (2004) 838-838.
- [2] A. Khoironi, H. Hadiyanto, S. Anggoro, S. Sudarno, Evaluation of polypropylene plastic degradation and microplastic identification in sediments at Tambak Lorok coastal area, Semarang, Indonesia, *Mar Pollut Bull*, 151 (2020) 110868.
- [3] D. Huang, X. Wang, L. Yin, S. Chen, J. Tao, W. Zhou, H. Chen, G. Zhang, R. Xiao, Research progress of microplastics in soil-plant system: Ecological effects and potential risks, *Sci Total Environ*, (2021) 151487.
- [4] H. Ya, B. Jiang, Y. Xing, T. Zhang, M. Lv, X. Wang, Recent advances on ecological effects of microplastics on soil environment, *Sci Total Environ*, 798 (2021) 149338.
- [5] I. Batool, A. Qadir, J. M. Levermore, F. J. Kelly, Dynamics of airborne microplastics, appraisal and distributional behaviour in atmosphere; a review, *Sci Total Environ*, (2021) 150745.
- [6] Z. Zhang, Y. Chen, Effects of microplastics on wastewater and sewage sludge treatment and their removal: A review, *Chemical Engineering Journal*, 382 (2020).
- [7] S. Lu, M. Hummel, Z. Gu, Y. Wang, K. Wang, R. Pathak, Y. Zhou, H. Jia, X. Qi, X. Zhao, B. Xu, X. Liu, Highly efficient urea oxidation via nesting nano-nickel oxide in eggshell membrane-derived carbon. *ACS Sustainable Chemistry & Engineering*, 9.4 (2021) 1703-1713.
- [8] X. Zhao, S. Lu, W. Li, S. Zhang, K. Li, K. Nawaz, P. Wang, G. Yang, A. Ragauskas, S. Ozcan, E. Webb, Epoxy as Filler or Matrix for Polymer Composites, *IntechOpen*, (2022) DOI: 10.5772/intechopen.102448.
- [9] J. Tang, Z. Wu, L. Wan, W. Cai, S. Chen, X. Wang, J. Luo, Z. Zhou, J. Zhao, S. Lin, Differential enrichment and physiological impacts of ingested microplastics in scleractinian corals in situ, *J Hazard Mater*, 404 (2020) 124205.
- [10] S. Sun, W. Shi, Y. Tang, Y. Han, X. Du, W. Zhou, W. Zhang, C. Sun, G. Liu, The toxic impacts of microplastics (MPs) and polycyclic aromatic hydrocarbons (PAHs) on haematic parameters in a marine bivalve species and their potential mechanisms of action, *Sci Total Environ*, 783 (2021) 147003.
- [11] M. Banaee, S. Soltanian, A. Sureda, A. Gholamhosseini, B. N. Haghi, M. Akhlaghi, A. Derikvandy, Evaluation of single and combined effects of cadmium and micro-plastic particles on biochemical and immunological parameters of common carp (*Cyprinus carpio*), *Chemosphere*, 236 (2019) 124335.
- [12] Z. Long, Z. Pan, W. Wang, J. Ren, X. Yu, L. Lin, H. Lin, H. Chen, X. Jin, Microplastic abundance, characteristics, and removal in wastewater treatment plants in a coastal city of China, *Water Res*, 155 (2019) 255-265.
- [13] S. Lu, M. Hummel, Z. Gu, Y. Gu, Z. Cen, L. Wei, Y. Zhou, C. Zhang, C. Yang, Trash to treasure: A novel chemical route to synthesis of NiO/C for hydrogen production, *International Journal of Hydrogen Energy*, 44 (2019) 16144-16153.
- [14] A. Castellano-Hinojosa, P. Maza-Márquez, J. González-López, B. Rodelas, Influence of operation parameters on the shaping of the denitrification communities in full-scale municipal sewage treatment plants, *Journal of Water Process Engineering*, 37 (2020) 101464-101472.
- [15] W. Wei, Q. S. Huang, J. Sun, X. Dai, B. J. Ni, Revealing the Mechanisms of Polyethylene Microplastics Affecting Anaerobic Digestion of Waste Activated Sludge, *Environ Sci Technol*, 53 (2019) 9604-9613.
- [16] X. Yang, Q. He, F. Guo, X. Sun, J. Zhang, M. Chen, J. Vymazal, Y. Chen, Nanoplastics Disturb Nitrogen Removal in Constructed Wetlands: Responses of Microbes and Macrophytes, *Environ Sci Technol*, 54 (2020) 14007-14016.
- [17] M. E. Seeley, B. Song, R. Passie, R. C. Hale, Microplastics affect sedimentary microbial communities and nitrogen cycling, *Nat Commun*, 11 (2020) 2372.
- [18] H. Liu, X. Yang, G. Liu, C. Liang, S. Xue, H. Chen, C. J. Ritsema, V. Geissen, Response of soil dissolved organic matter to microplastic addition in Chinese loess soil, *Chemosphere*, 185 (2017) 907-917.

- [19] A. McCormick, T. J. Hoellein, S. A. Mason, J. Schluep, J. J. Kelly, Microplastic is an Abundant and Distinct Microbial Habitat in an Urban River, *Environmental Science & Technology*, 48 (2014) 11863.
- [20] Sun, Xuemei, Chen, Bijuan, Li, Qiufen, Liu, Nan, Xia, Bin, Toxicities of polystyrene nano- and microplastics toward marine bacterium *Halomonas alkaliphila*, *Science of the Total Environment*, (2018).
- [21] L. Tang, C. Su, Y. Chen, Y. Xian, X. Hui, Z. Ye, M. Chen, F. Zhu, H. Zhong, Influence of biodegradable polybutylene succinate and non-biodegradable polyvinyl chloride microplastics on anammox sludge: Performance evaluation, suppression effect and metagenomic analysis, *J Hazard Mater*, 401 (2020) 123337.
- [22] Y. Sun, X. Ren, J. Pan, Z. Zhang, T. H. Tsui, L. Luo, Q. Wang, Effect of microplastics on greenhouse gas and ammonia emissions during aerobic composting, *Sci Total Environ*, 737 (2020) 139856.
- [23] A. Hl, A. Sx, W. A. Shuai, B. Jya, Y. A. Peng, A. Yc, A. Jg, F. A. Fang, New insight into the effect of short-term exposure to polystyrene nanoparticles on activated sludge performance, *Journal of Water Process Engineering*, 38.
- [24] X. Y. Li, S. F. Yang, Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge, *Water Res*, 41 (2007) 1022-1030.
- [25] Y. He, L. Li, K. Song, Q. Liu, Z. Li, F. Xie, X. Zhao, Effect of microplastic particle size to the nutrients removal in activated sludge system, *Mar Pollut Bull*, 163 (2021) 111972.
- [26] Y. Cui, J. Gao, D. Zhang, D. Li, H. Dai, Z. Wang, Y. Zhao, Responses of performance, antibiotic resistance genes and bacterial communities of partial nitrification system to polyamide microplastics, *Bioresour Technol*, 341 (2021) 125767.
- [27] L. Li, K. Song, S. Yeerken, S. Geng, D. Liu, Z. Dai, F. Xie, X. Zhou, Q. Wang, Effect evaluation of microplastics on activated sludge nitrification and denitrification, *Sci Total Environ*, 707 (2020) 135953.
- [28] W. C. Shi, M. Y. Gui, J. Y. Du, Z. F. Ma, D. S. Wu, Effects of typical microplastics on the denitrification characteristics and denitrification related genes of aerobic denitrifying bacteria, *Chinese Journal of Environmental Engineering*, 15 (2021) 1333-1343.
- [29] K. Xie, Q. W. Guo, W. Zhang, S. L. Chen, Z. W. Xiong, Study on Removal of Microplastic Particles from Waters by PFS, *Guangdong chem*, 48 (2021) 92-95.
- [30] X. H. Zhou, Y. Pan, Q. Q. Chen, M. Deng, Y. Zhen, Dynamic changes of EPS during sludge bulking and recovery in sludge transfer SBR process, *Chinese Journal of Environmental Engineering*, 10 (2016) 5643-5647.
- [31] O. S. Alimi, J. Farner Budarz, L. M. Hernandez, N. Tufenkji, Microplastics and Nanoplastics in Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport, *Environ Sci Technol*, 52 (2018) 1704-1724.
- [32] H. Wang, Y. Zheng, B. Zhu, F. Zhao, In situ role of extracellular polymeric substances in microbial electron transfer by *Methylobacter* sp. LW13, *Fundamental Research*, (2021).
- [33] A. Mancina, D. R. Chadwick, S. M. Waters, D. J. Krol, Uncertainties in direct N₂O emissions from grazing ruminant excreta (EF3PRP) in national greenhouse gas inventories, *Sci Total Environ*, 803 (2022) 149935.
- [34] X. Ren, J. Tang, X. Liu, Q. Liu, Effects of microplastics on greenhouse gas emissions and the microbial community in fertilized soil, *Environ Pollut*, 256 (2020) 113347.