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Influence of biodegradable polybutylene succinate and non-biodegradable polyvinyl chloride microplastics on anammox sludge: Performance evaluation, suppression effect and metagenomic analysis

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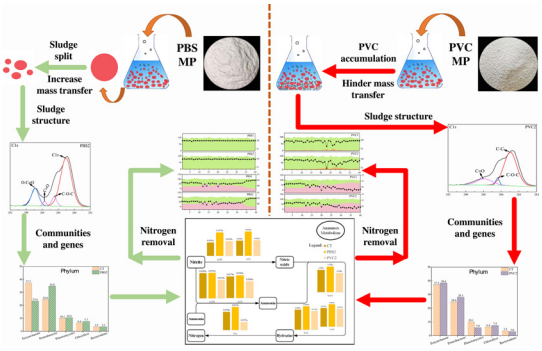
GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics (MPs) has been widely detected in wastewater treatment plants. However, there is a lack of research on its influence on anaerobic ammonia oxidation (anammox) process. Therefore, the effects of polybutylene succinate (PBS) and polyvinyl chloride (PVC) MPs on the nitrogen removal performance, microbial community and metabolites of anammox sludge were investigated. Results showed that PBS and PVC MPs reduced the nitrite removal efficiency of the anammox sludge, and PVC1 (0.1 g/L PVC) group was the most significant at 19.2 %. Fourier transform infrared (FTIR) and X-ray photoelectron spectroscopy (XPS) spectra showed that PBS2 (0.5 g/L PBS) group increased the polysaccharide content in the anammox sludge. This may be because of the byproduct, which was produce during the biodegradation of PBS MPs, and decrease the agglomeration capacity of sludge, so as to increase the mass transfer. PBS2 group reduced the relative abundance of *Methanosaeta* (10.18 %) and the methane modules, and stimulated the anammox bacteria *Ca. Brocadia* (1.17 %) and the relative nitrogen metabolism modules. PVC2 group reduced the relative abundance of *Ca. Brocadia* (3.02 %), while was enriched *Methanosaeta* (2.1 %). Non-biodegradable PVC MPs was more harmful to anammox sludge, which would draw attention to the entry of PVC MPs into the anammox system.

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1. Introduction

Excessive discharge of nitrogen into water environments will cause eutrophication, resulting in ecological and economic losses, so the development of a cost-effective nitrogen removal process has been focused on wastewater treatment (Zhou et al., 2020). Traditional nitrification and denitrification process for nitrogen removal requires oxygenation and the addition of organic carbon source in the operation process, which will result in a high cost of wastewater treatment (He et al., 2020). The emergence of the anaerobic ammonium oxidation (anammox) process has improved upon this problem. It uses nitrite as an electron donor to convert ammonium to N_2, and generates a certain amount of nitrate nitrogen in the anaerobic environment. Anammox process does not require organic matter as an electron donor; therefore, it is more energy-efficient and is considered a promising denitrification technology (Chen et al., 2019; Kartal et al., 2011). Due to its superior performance, the anammox process was widely used in wastewater treatment plant (WWTP), with approximately 100 systems using anammox technology worldwide as of 2014 (Lackner et al., 2014). Additionally, there have been many adaptations to the anammox process, including partial nitrification-anaerobic ammonium oxidation (PN/A) and partial denitrification-anaerobic ammonium oxidation (PDA) (Chen et al., 2020; Xu et al., 2020).

Although anammox process in wastewater nitrogen removal has many advantages, application and industrialization of the anammox process, the stable operation of anammox process is often inhibited by such factors as dissolved oxygen, pH and inhibitory substrate concentration (Ji et al., 2019; Tomaszewski et al., 2017; Zhang et al., 2019c). With increasing industrial development, more and more novel pollutants, such as nano-metals, organic matter, and antibiotics, *etc.* have brought new challenges to the performance of anammox process, which is sensitive to various inhibitors. (Li et al., 2020b; Yang et al., 2020a; Zhang et al., 2019c, 2017). Xu et al. (2019) had evaluated the performance, sludge characteristics and microbial community of anammox biogranules under long-term nickel oxide nanoparticles (NiO NPs) exposure, which the anammox system performance differed under the stresses of different NiO NPs concentrations. Zhang et al. (2019b) had researched the fate of antibiotic resistance genes in anammox process under oxytetracycline and sulfamethoxazole stresses, and the results showed that the anammox performance was inhibited when the oxytetracycline or sulfamethoxazole concentration increased from 0.5 to 1.0 mg/L. Therefore, it is of great significance to investigate the inhibitory effect of emerging pollutants on the anaerobic anammox sludge and provide a reference for the stable operation of anammox bioreactor for the treatment wastewater. When anaerobic granular sludge is used as inoculation sludge to start the anammox reactor, there will be a symbiosis between methanogens and anammox bacteria (Chen et al., 2016). Ammonia acts as a co-metabolic substrate along with methane, which is partially produced by the methane monooxygenase (MMO) of methanotrophs (Kim et al., 2020). Methane is often produce in anaerobic environments by acetoclastic methanogenesis and hydrogenotrophic methanogenesis. Acetoclastic methanogens use acetic acid and hydrogenotrophic methanogens use CO_2 and hydrogen as substrates to produce methane (Zhang et al., 2018a). However, when the concentration of volatile fatty acids (VFA) is too high, the pH value will be reduce, which will inhibit the methane generation in the anaerobic digester. Therefore, bicarbonate is often added in anaerobic digester as a buffer to regulate the pH value (Lin et al., 2013). Zhang et al. (2018c) found that carbonate increased the abundance of *Methanosaeta* and reduced the Gibbs free energy of methane butyrate. In addition, researchers had found that high concentrations of sodium bicarbonate can affect the concentration of CO_2 enough to promote hydrogenotrophic methanogenesis formation (Lin et al., 2013).

Microplastics (MPs) originate from the degradation of larger plastic items or are directly produced as < 5 mm particles (Mason et al., 2016). Due to their tiny nature and wide existence in water, atmosphere and soil, MPs can be taken up by a variety of organisms (Liu et al., 2019b). The biological uptake of MPs will seriously affect the key physiological functions of these organisms (Gardon et al., 2018), such as digestion, reproduction and respiratory capacity (Sussarellu et al., 2016). Currently, MPs have been widely detected in WWTPs, and the MPs concentrations in the effluent of WWTPs were in the range of 0–447 particle/L (Sun et al., 2019). In addition, some researchers found that the concentration of MPs was positively correlate with population size (Li et al., 2018). Therefore, it is particularly important to remove microplastics before they enter the environment. MPs can be divided into non-biodegradable and biodegradable MPs. Non-biodegradable MPs, including polyvinyl chloride (PVC), polyethylene (PE), *etc.* will persist in the environment for several decades (Gong et al., 2019). Biodegradable MPs are considered as substitutes for difficult-to-degrade MPs. They are usually polymerized from natural polymers (starch and cellulose) and plasticizers, including polylactic acid (PLA), polybutylene succinate (PBS), and polyhydroxy butyrate (PHB) (Chinaglia et al., 2018; Tosin et al., 2019). Biodegradable plastics have been widely used as a substitute for traditional non-degradable plastics. Whether MPs influence wastewater treatment processes or not, which are base on biological treatment technologies is still controversial. Some researchers studied the effects of three types of MPs on wastewater treatment processes and concluded that they have little effect on nitrification and denitrification processes (Liu et al., 2019a). However, other researchers found that MPs slightly inhibit the nitrification of activated sludge and reduce the conversion rate of ammonia nitrogen (Sun et al., 2018). In addition, researchers have found that microplastics inhibit the production of methane during anaerobic digestion (Li et al., 2020a). These studies focused on the impact of non-biodegradable MPs on traditional sludge. It is necessary to study the toxicity of MPs on anammox sludge to ensure the efficient operation of anammox system. However, our knowledge of the effect of biodegradable and non-biodegradable MPs on the nitrogen removal capacity and methanogens of anammox sludge and its mechanisms are still lacking. Based on next-generation sequencing technologies, metagenomic analysis, including amplicon and shotgun sequencing, has sufficient sequencing depth and high accuracy to cover complex microbial nitrogen transformation in micro-ecosystems (Yang et al., 2020a,b). This method can be used to evaluate the response of various steps of nitrogen and methanogenesis conversion to MPs stress.

Consequently, this paper used the PBS, PVC and anammox sludge to investigate the effects of MPs on (1) the removal of ammonia nitrogen and nitrite nitrogen; (2) the functional groups and binding energy of anammox sludge using XPS and FTIR; and (3) nitrogen and methane metabolism of the microbial community using whole-genome metagenomics sequencing. This work aims to elucidate the effects of different types of MPs inhibition on the anammox sludge and provide a reference for the efficient operation of anammox process.

2. Materials and methods

2.1. Wastewater composition and sludge inoculum

The inoculated sludge used in this experiment was mature anaerobic granular sludge collected from an anaerobic tank at the Liquan Brewery in Guilin, China. The sludge acclimation reactor was an anaerobic baffled reactor (ABR) with synthetic wastewater using the method of Zhang et al. (2018b) (Table S1). Approximately 100 mg/L of NH_4-N and NO_2-N were added to the wastewater in the form of $(NH_4)_2SO_4$ and $NaNO_2$, respectively (Zhang et al., 2018b). Jiang et al. (2020b) had explored advanced nitrogen removal *via* partial nitrification-anammox biofilm reactor driven by high dissolved oxygen, anammox bacteria accounted for 2.39 % of total bacteria in the whole biofilm, contributing 90.0 % to nitrogen removal. Therefore, considering the cost and operation management of actual engineering applications, nitrogen gas was not blown into the influent during the acclimation, and

deoxidizer was not added. In addition, the reactor was not shaded and the domestication temperature was room temperature. After the sludge acclimation for 97 days, the NH_4-N and NO_2-N removal rates were steady at 98 % and 70 %, respectively, thus, these results suggested that there is a certain amount of anammox bacteria in the ABR reactor (Zhang et al., 2019a, b).

2.2. Experimental device and operation

In this study, PVC MPs and PBS MPs were purchased from Dongguan Xunjie Plastic Scientific Technology Co., Ltd. (Dongguan, China). The average diameter of the purchased PVC MPs and PBS MPs were 0.5 mm, both in white powder form, according to the common dimensions of MPs entering the WWTP (Li et al., 2020a). The experiment was performed using five 500 m L-conical flasks containing 150 mL of anammox sludge that had been uniformly mixed and cultured in the ABR reactor for 146 days. Considering that the increasing trend of MPs content in the WWTP (Li et al., 2018), the concentration selected in the experiment is higher than the actual concentration in the WWTP: one low concentration (0.1 g/L) and one high concentration (0.5 g/L). The five treatment groups were as follows: group control (CT) had no MPs; groups PBS1 and PBS2 contained 0.1 and 0.5 g/L PBS, respectively; and groups PVC1 and PVC2 contained 0.1 and 0.5 g/L PVC (Qin et al., 2020). The experiment used synthetic wastewater without deoxidizing (Table S1). The daily wastewater intake was 350 mL, the hydraulic retention time (HRT) was 24 h and the pH was controlled at 7.5 − 8.0. All conical flasks were sealed without shading, and placed in a constant temperature incubator at 32 ˚C for 40 days. During the experiment, 10 mL of influent and effluent samples were taken daily to measure the concentration of nitrite nitrogen, ammonia nitrogen, and nitrate nitrogen. In addition, on the final day of the experiment (day 40), 40 mL of anammox sludge was taken for FTIR, XPS and metagenomic analyses.

2.3. Analytical methods

Nitrite nitrogen, ammonia nitrogen and nitrate nitrogen concentrations in the water samples were determined using standard methods after filtering with a 0.45 μm filter (APHA, 2005). FTIR spectrometer (Perkin Elmer, Spectrum Two) was used to determine functional groups in the anammox sludge from day 40. Briefly, the anammox sludge was dried at 55 ˚C for 24 h, mixed with potassium bromide at a ratio of 1:100, ground with an agate mortar, and then pressed for FTIR spectra analysis. Using pure potassium bromide as the reference, the scanning wavelength was set to 400-4000 cm^{-1} (Yang et al., 2020a). XPS spectra analysis of the 5 mL anammox sludge samples was performed on an X-ray photoelectron spectrometer (Thermo scientific, 250 Xi) after 24 h drying at 55 ˚C. The beam spot was 500 μm, with a full spectral pass energy of 100 eV (1.0 eV step size) and a narrow spectral pass energy of 30 eV (0.06 eV step length). All binding energies were referenced to the C1s (285.0 eV) peak to compensate for surface charge effects.

2.4. Metagenomic analysis

Anammox sludge was collected at the end of the 40-day experiment for metagenomic analysis of groups CT, PBS2 and PVC2. The anammox sludge samples were centrifuged at 10,000 rpm for 3 min and then the supernatant was discarded to obtain the biomass. DNA was extracted using an OMEGA kit as per the manufacturer's instructions. DNA integrity was verified by 1% agarose gel electrophoresis (Zhao et al., 2018). DNA samples were sequenced on the Illumina HiSeq platform. Fast quality control (QC) was used to visually evaluate the quality of the original and QC filtered data. Processed sequences were an average length of 150 base pairs (bp) (Lei et al., 2019). The original reads were filtered using Trimmomatic (2.0 − 1.0), which removed N-base sequences and low-quality data to obtain relatively accurate valid sequences (Bolger et al., 2014). The software IDBAUD was used to splice and assemble high-quality sequences, obtaining contigs that based on the overlapping relationship between sequences, and selecting the best Kmer assembly results (Oh et al., 2018). Prodigal was used to perform open reading frame (ORF) prediction on the splicing results, select genes of ≥100 bp in length, and then translate them into amino acid sequences (Lei et al., 2019). DIAMOND with BLASTP homology alignment of genomic protein sequences and the NCBI non-redundant protein sequence database were used to obtain functional annotation and homologous species information. Then, according to the NCBI microbial taxonomy information database, the gene taxonomy annotation information was obtained and the relative microbial abundances were determined for each taxonomic level (Bhattacharyya et al., 2016). The gene automatic annotation server GhostKOALA was used to compare the protein sequences of the gene set with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to obtain the corresponding KO numbers, thereby obtaining the model annotation information of the sequences (Fontana et al., 2018).

3. Results and discussion

3.1. Effect on the nitrogen removal rate

The ammonia nitrogen concentration in the influent was maintained at 90 − 103 mg/L throughout the 40-day experimental period (Fig. 1). The average ammonia nitrogen removal rates were 99.5 % (CT, control group), 99.4 % (PBS1, 0.1 g/L PBS), 99.0 % (PBS2, 0.5 g/L PBS), 95.7 % (PVC1, 0.1 g/L PVC) and 93.3 % (PVC2, 0.5 g/L PVC), respectively. It was demonstrate that the non-biodegradable PVC MPs had an evident impact on the average ammonia nitrogen removal rate than the biodegradable PBS MPs. As far as the average ammonia nitrogen removal efficiency was concerned, there was little difference between PBS1 and PBS2 and CT group. However, compared with the control group, the average ammonia nitrogen removal rates of 0.1 g/L and 0.5 g/L PVC decreased by 3.8 % and 6.2 %, respectively. The nitrite nitrogen removal in the conical flasks after adding PVC and PBS MPs were presented in Fig. 2. The average removal rates of nitrite nitrogen in CT, PBS1, PBS2, PVC1 and PVC2 were 72.3 %, 73.9 %, 67.7 %, 53.2 % and 60.7 %, respectively. The trend of CT group, PBS1 and PBS2 group was basically the same, which nitrite concentration in the effluent increased slightly from days 0 to 25, and then gradually decreased from days 25 to 40. However, in the PVC1 and PVC2 groups, the effluent nitrite concentration gradually increased. At day 40, the effluent nitrite concentrations of PVC1 and PVC2 were 68.7 and 57.6 mg/L, respectively. PBS had a smaller effect on nitrite nitrogen in the effluent and even exhibited a promoting effect with 0.1 g/L PBS. PVC MPs had a greater effect on nitrite nitrogen in the effluent, which was consistent with the results of ammonia nitrogen. PBS MPs is a type of polymer, with its main chain containing ester groups that can be attacked by various microbial enzymes. Its ester groups can be hydrolyzed by enzymes to water-soluble oligomers and monomers, eventually generating carbon dioxide and water that can be metabolized by microbial (Gan et al., 2001; Pan et al., 2018). 0.1 g/L PBS could be more quickly decomposed and used by organisms, thereby promoting the nitrogen removal efficiency. The non-biodegradability of PVC MPs made it accumulate in the anammox sludge, which impeded the mass transfer between organisms, thereby affecting the nitrogen removal efficiency.

The theoretical molar ratio of NO_3-N/NH_4-N proposed by Strous et al. (1998) is 0.26 for the anammox equation. From days 0 to 25, the average NO_3-N/NH_4-N molar ratios in the CT, PBS1, PBS2, PVC1 and PVC2 groups were 0.217, 0.2, 0.186, 0.171 and 0.168, respectively. The molar ratio of the CT group was closer to the theoretical value, while that of PBS was larger than that of PVC, and the decrease in the molar ratio was positively relate to the concentration. From days 25 to 40, the average NO_3-N/NH_4-N molar ratios in the CT, PBS1, PBS2, PVC1 and

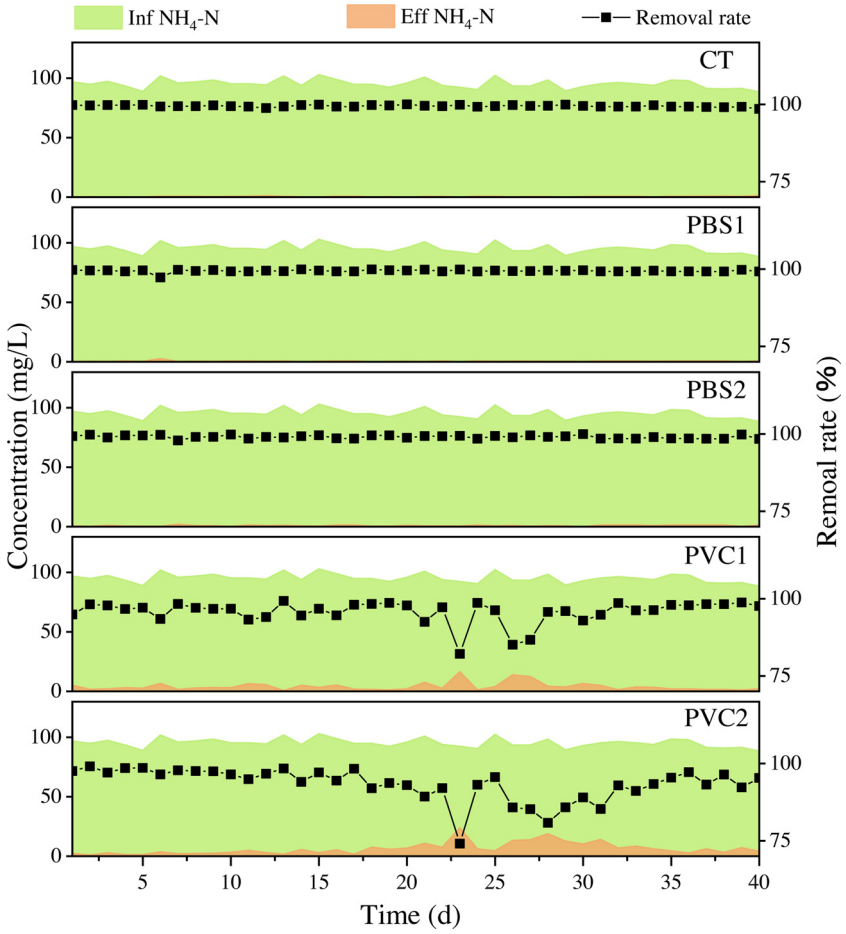

Fig. 1. Ammonia nitrogen removal effect: CT-the control group, PBS1-0.1 g/L PBS, PBS2-0.5 g/L PBS, PVC1-0.1 g/L PVC, PVC2-0.5 g/L PVC.

PVC2 groups were 0.97, 0.55, 0.53, 0.176 and 0.177, respectively. The average NO_3-N/NH_4-N ratio in the control and PBS groups increased notably, indicating that the content of NO_3-N in the effluent increased. In the anaerobic and constant temperature environment, the decrease of temperature leads to the increase of dissolved oxygen in the synthetic wastewater, which leads to the conversion of NO_2-N into NO_3-N (nitrification reaction), which was the main reason for the rapid increase of NO_3-N content in the effluent. However, due to the non-biodegradability of PVC MPs, it accumulated in the conical flasks, which some extent obstructed the mass transfer between the wastewater and anammox sludge, causing a little change in the molar ratio. Conversely, PBS MPs was easily biodegraded, so there were no obstacles to mass transfer.

3.2. Effect of microplastics on surface characterization of anammox sludge

In order to better grasp the elemental composition and functional group changes of the anammox sludge in the presence of PBS and PVC MPs, FTIR and XPS spectra were used to analyze the difference between the CT group and PBS2 and PVC2 groups on day 40. The FTIR spectra (Fig. S1) of the three anammox sludge samples showed that there were significant peaks at 3698 − 3705 cm^{-1}, which belong to OH− stretching vibration, and the indicator was a hydrocarbon (Yang et al., 2020a). In the PBS2 and PVC2 groups, there were peaks attributed to C−OC− ring vibration at 1099 and 1097 cm^{-1}, respectively, indicating the presence of polysaccharide (Yang et al., 2020a). Similar to previous studies on PBS, the peaks appeared in the PBS group at 2922 and 1712 cm^{-1}, of which the C–H and methyl groups at 2922 cm^{-1}, and the carbonyl (C═O) group at 1712 cm^{-1} in the PBS group (Zhu et al., 2015). The peaks of C–H, COC−− were more difficult to find in the PVC2 group than in the PBS2 group, indicating that the addition of 0.5 g/LPBS significantly increases the content of methyl, polysaccharide. In 25–40 days, the anammox sludge appeared flocculent in the PBS2 group, which indicated that the structure of the anammox sludge had been damaged. This may cause an increase in methyl, polysaccharide in the PBS2 group.

XPS was used to investigate the evolution of carbon and nitrogen functional groups in different groups. XPS spectra of the three groups showed that all anammox sludge samples contained C1s, O1s and N1s peaks (Fig. 3). The main elements of anammox sludge include C, O and N. In addition, the Cl2p peak (200 eV) also appeared in the PVC2 group, confirming the presence of PVC MPs material in the sludge (containing chlorine element). In the high-resolution C1s spectrum (Fig. 4), two fitting peaks appeared in the CT, PBS2 and PVC2 groups: C1s (284.96–285.04 eV) and COC (286.4–286.8 eV), and COC−−−− fitting peaks indicated the presence of polysaccharide in the three groups. Compared with the C1s and C−OC− peak areas of the CT group, the addition of PBS significantly increased the area of the two peaks, while the addition of PVC slightly increased the C1s peak area and decreased the C−OC− peak area (Table S2), indicating that the addition of PBS2 MPs would increase the content of polysaccharide. In addition, there were N−C = O peaks (288.1 eV) in the control group; O−C = O (288.9 eV) and C═O bonds (287.7 eV) in the PBS2 group; and C═O bonds (287.9 eV) in the PVC2 group (Liu et al., 2010). The N1s high-resolution spectrum showed two fitting peaks in the CT, PBS2 and PVC2 groups: N1s (399.63–399.88 eV) and -NH_4^+ (401.3–401.7 eV) (Yang et al., 2020a). The -NH_4^+ bond basically came from lysine and arginine in the sludge (Liu et al., 2010). PBS2 MPs reduced the relative atomic weight of the -NH_4^+ bond by 7.63 %, while PVC2 MPs decreased it by 2.12 % (Table S3), indicating that the addition of PBS2 and PVC2 MPs

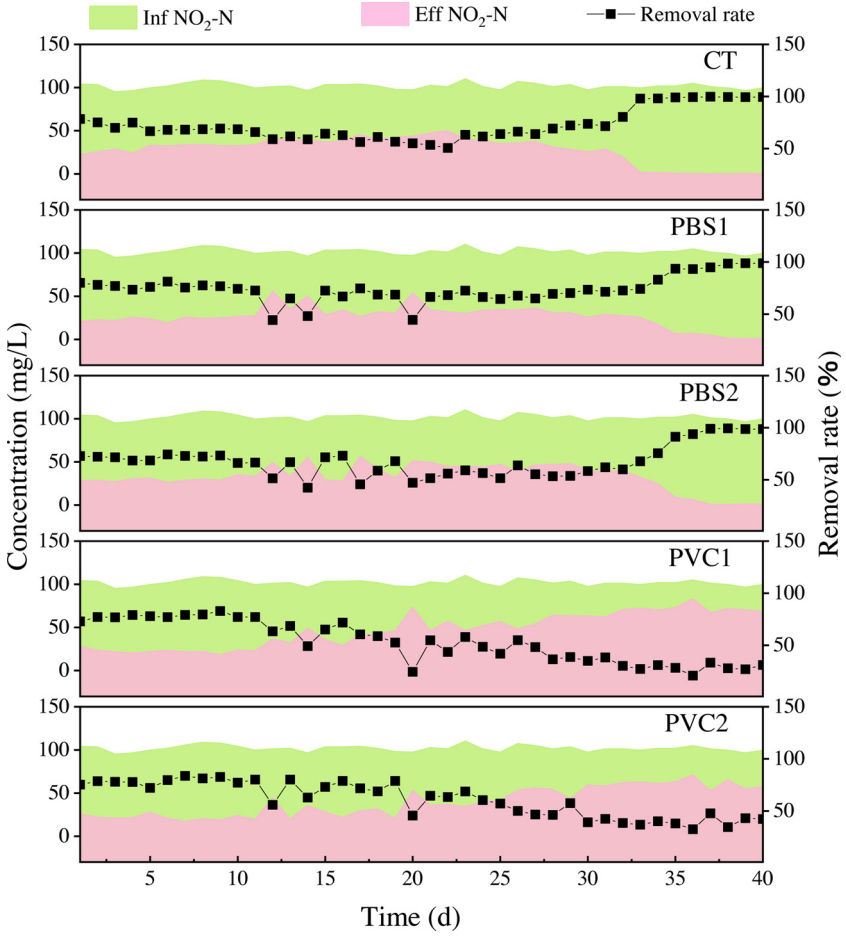

Fig. 2. Nitrite nitrogen removal effect: CT-the control group, PBS1-0.1 g/L PBS, PBS2-0.5 g/L PBS, PVC1-0.1 g/L PVC, PVC2-0.5 g/L PVC.

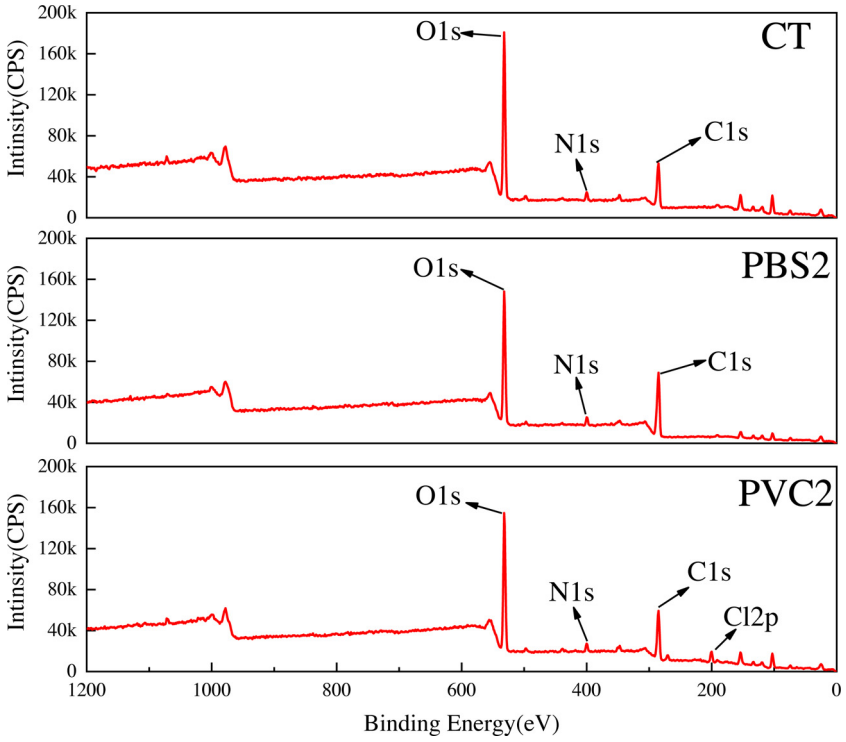

Fig. 3. XPS survey of sludge: CT-the control group, PBS2-0.5 g/L PBS, PVC2-0.5 g/L PVC.

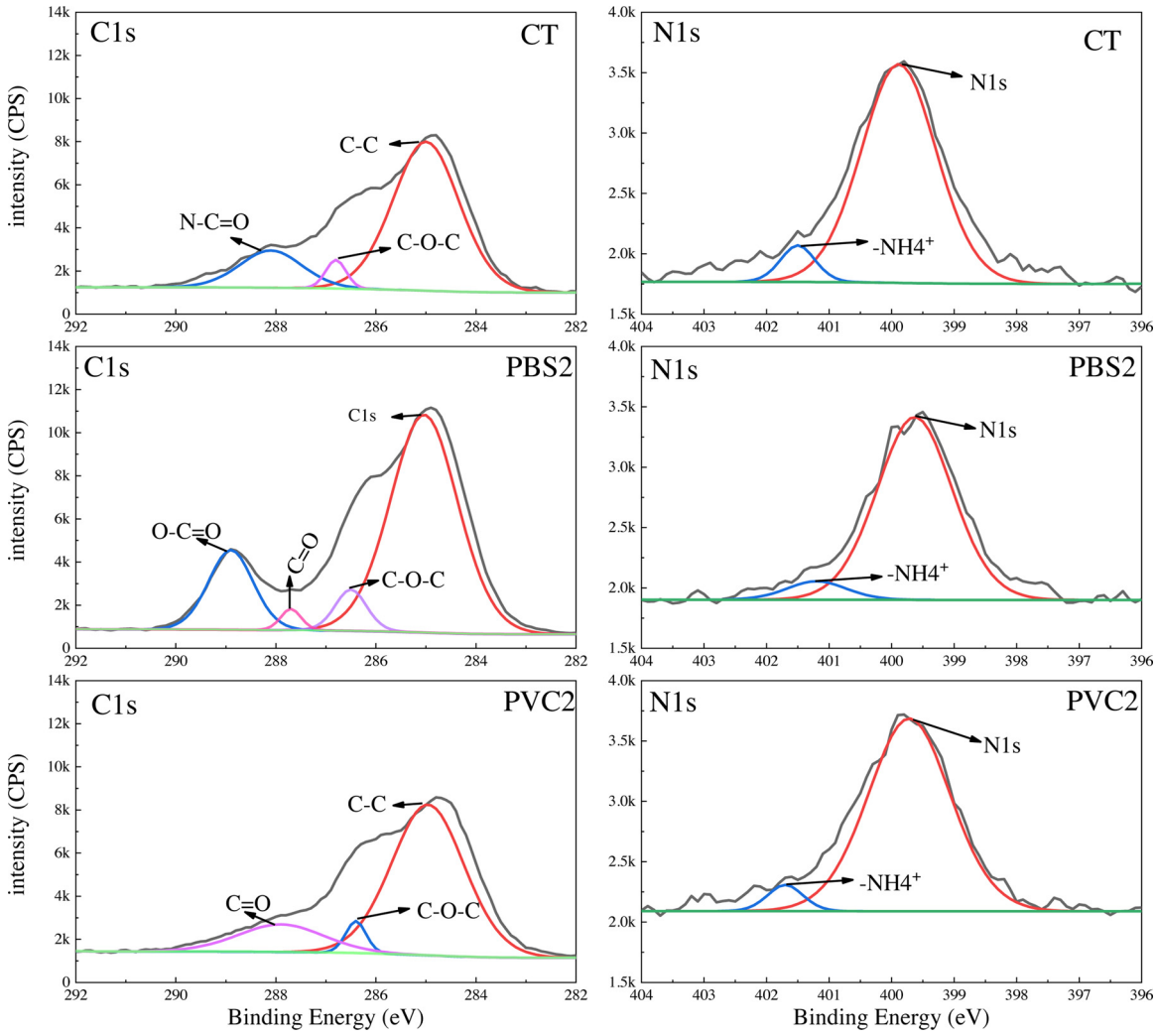

Fig. 4. C1s, N1s high-resolution XPS spectra: CT-the control group, PBS2-0.5 g/L PBS, PVC2-0.5 g/L PVC.

reduced the amount of amino acids in the sludge.

Extracellular polymeric substance (EPS) is a mixture of macromolecules produced by sludge, which is mainly composed of polysaccharides, proteins and humic acids. The hydrophilic part is mainly composed of polysaccharides, while the hydrophobic part is mainly composed of proteins and amino acids (Huangfu et al., 2019b). In addition, EPS has a strong adsorption ability and can protect bacteria from toxic substances. FTIR and XPS spectra analysis showed that, compared with the CT group, the addition of PBS2 MPs could increase the polysaccharide in the sludge and reduce the content of amino acids, so as to increase the hydrophilicity and decrease the hydrophobicity of the sludge. The decrease of sludge surface hydrophobicity will increase the remainder Gibbs free energy on the surface of microbial cells, thus reducing the adsorption capacity of cells and reducing the viscosity of sludge (Sanin et al., 2003). The decrease of sludge viscosity will weaken the agglomeration between sludge particles and loosen and break the structure of sludge (Jiang et al., 2020a).

3.3. Effect on microbial structure in anammox sludge

To better understand the effects of MPs with different properties on related functional bacteria in the anammox sludge, the relative abundance of microorganisms at the phylum and genus levels was analyzed. The distribution abundance of microbial communities of groups CT (control), PBS2 (0.5 g/L PBS) and PVC2 (0.5 g/L PVC) were first analyzed at the phylum level (Fig. 5a). The anammox sludge had a rich microbial diversity, among which Euryarchaeota, Proteobacteria and Planctomycetes were dominant. The relative abundance of Euryarchaeota in the CT, PBS2, and PVC2 groups was 37.21 %, 23.37 % and 38.45 %, respectively. Euryarchaeota is an archaea that can participate in methane production in an anaerobic environment (Guo et al., 2019). Some researchers have found that when denitrifying sludge was used as the inoculation sludge and only HCO_3^- was used as the carbon source to cultivate an anammox sludge, Euryarchaeota with high abundance still existed after 40 days of cultivation (Chen et al., 2016). In this experiment, the inoculation sludge was anaerobic granular sludge, it could be illustrated that the selection of inoculation sludge would have an impact on the microbial community. The proportions of Proteobacteria in CT, PBS2 and PBS2 were 24.60 %, 34.83 % and 28.07 %, respectively. It is well known that Proteobacteria contain important anaerobic denitrifying bacteria (Huang et al., 2019a). The addition of 0.5 g/L PBS significantly stimulated the enrichment of the Proteobacteria (10.23 %). Anammox bacteria are subject to biological classification as Planctomycetes, which are Gram-negative bacteria that are suitable for growth in limited dissolved oxygen condition (Yangin-Gomec et al., 2017). The proportions of Planctomycetes in CT, PBS2 and PVC2 were 10.07 %, 10.55 % and 5.91 %, respectively. The abundance of Planctomycetes did not reach the highest in the three groups of conical flasks, which was closely related to the absence of deoxygenation in the influent. PBS2 group increased the abundance of Planctomycetes by 0.48 %. The non-biodegradable PVC MPs inhibited the growth of anaerobic ammonia-oxidizing bacteria, which reduced

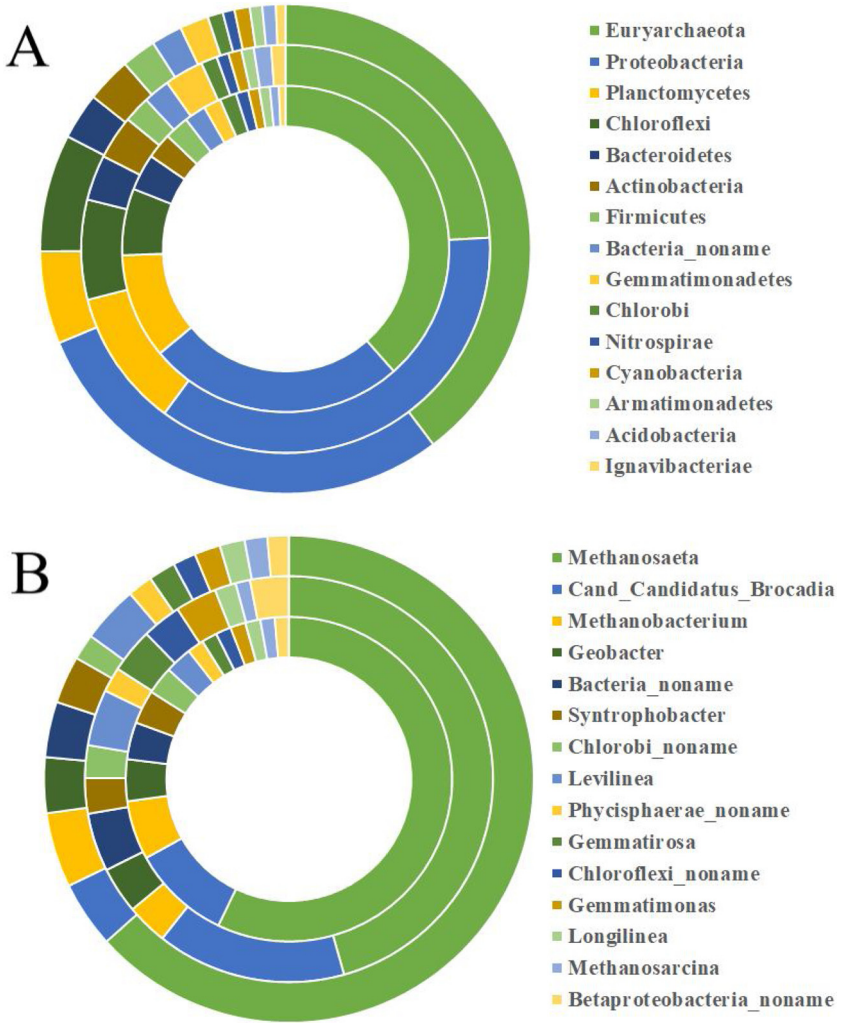

Fig. 5. Relative abundance of bacterial at phylum level (A), genus level (B): the inner circle was the control group (CT), the middle circle was 0.5 g/L PBS (PBS2), and the outer circle was 0.5 g/L PVC (PVC2).

the abundance of Planctomycetes by 4.16 %, thus explaining why PVC reduced the average removal rate of ammonia nitrogen and nitrite nitrogen. In addition, Chloroflexi (6.39–7.67 %), Bacteroidetes (2.96–3.51 %), Actinobacteria (2.46–3.35 %), Nitrospirae (0.75–1.1 %) and Cyanobacteria (0.97–1.06 %) were identified. Chloroflexi can degrade large molecules, such as carbohydrates and cellular substances, under anaerobic conditions (Guo et al., 2019). Nitrospirae can oxidize nitrite to nitrate and compete with anammox bacteria for nitrite (Wang et al., 2018). Cyanobacteria is a photoautotrophic microorganism, which can synthesize organic compounds and produce H_2 using CO_2 and H_2O under the action of light energy (Srirangan et al., 2011). Since the reactors were not shaded during the stages of domesticating anammox sludge and adding microplastics, a certain amount of Cyanobacteria was present in the sludge. *Methanosaeta* and *Candidatus Brocadia* (*Ca. Brocadia*) were the main genera in the sludge at genus level (Fig. 5b). Other identified genera included *Methanobacterium* (1.41–3.48 %), *Geobacter* (1.6–2.25 %) and *Syntrophobacter* (1.22–1.82 %). *Methanosaeta* can use acetate as the substrate to produce methane, which is dominant at a low acetic acid concentration (Kurade et al., 2019; Zhang et al., 2018a). *Methanobacterium* is a hydrogenotrophic methanogens which could produce methane by reducing CO_2 with H_2 (Tejerizo et al., 2017). In addition, some researchers have found that the addition of carbonate can increase the abundance of *Methanobacterium* (Zhang et al., 2018c). *Methanosaeta* decreased by 10.18 % in the PBS2 group, but increased by 2.1 % in the PVC2 group. In addition, *Methanobacterium* had the lowest abundance in the PBS2 group (1.41 %). Thus, the methane production efficiency in the PBS2 group should be lower than that in the CT and the PVC2 groups. *Ca. Brocadia*, which can be well enriched in the context of poor nutrition, was one of the main genera of the Planctomycetes (Pradhan et al., 2019). The relative abundance of *Ca. Brocadia* in the CT, PBS2, and PVC2 groups was 5.31 %, 6.48 % and 2.29 %, respectively. The addition of PBS increased *Ca. Brocadia* by 1.17 %, while the addition of PVC MPs decreased its abundance by 3.02 %.

Analysis of the phylum and genus in the PBS-amended anammox sludge showed that the denitrifying (Proteobacteria) and anammox bacteria (Planctomycetes and *Ca. Brocadia*) can be significantly increased, indicating that PBS MPs can increase the abundance of functional bacteria to a certain extent. Biodegradation of PBS MPs produces short-chain polymers and monomers such as butanediol-succinate-butanediol, butanediol and succinic acid, which decreases the crystallinity (Shi et al., 2019). Studies have shown that sludge contains polymer-degrading lipase, which can specifically act on ester bonds (Gessesse et al., 2003). During PBS MPs biodegradation, the intermediate product succinic acid may create an acidic environment in the conical flask. Subsequently, the sludge structure breaks under the acidic environment, increasing the mass transfer rate of the wastewater. Meanwhile, butanediol and succinic acid can also provide a carbon source to promote growth of the microorganism. In addition, *Methanosaeta* and *Methanobacterium* with a high abundance were found in the three

groups of conical bottles without adding organic carbon source. Its possible reasons are as follows: (1) Acetic acid was synthesized by CO_2 from the dissolution of HCO_3^- in the synthetic water and H_2 produced by cyanobacteria photosynthesis under the action of homoacetogenic bacteria, and then used by *Methanosaeta* (Kurade et al., 2019); (2) CO_2 and H_2 are directly utilized by the hydrogenotrophic methanogens *Methanobacterium* (Zhang et al., 2018c). During the experiment, a large amount of PVC accumulation was observed in the PVC group, which hindered mass transfer between the wastewater and sludge and increased the anaerobic reaction, which may lead to the enrichment of methanogens (*Methanosaeta* and *Methanobacterium*) in the PVC group. In addition, dissolved oxygen in the influent affects the abundance of Planctomycetes in the sludge and the process of nitration. In order to improve the abundance of anammox bacteria, deoxidizer can be added or nitrogen gas can be blown off the influent.

3.4. Comparative metagenomic analysis

3.4.1. Nitrogen module and metabolic analysis

Mapping the abundance of nitrogen metabolism modules based on module annotations in KEGG in metagenomics. Microbial nitrogen metabolism is of great significance to wastewater treatment and microbial growth. Nitrogen transformation mainly includes nitrogen fixation, assimilatory nitrate reduction, dissimilatory nitrate reduction, denitrification, nitrification and anammox (Yang et al., 2020b). PBS2 group promoted conversion of the nitrate/nitrite transport system (M00438), nitrification (M00528), denitrification (M00529), dissimilatory nitrate reduction (M00530), assimilation nitrate reduction (M00531) and nitrate assimilation (M00615), with denitrification (0.1 %) and dissimilation nitrate reduction (0.069 %) being the most significant (Fig. 6). Some researchers had used PBS as a biofilm material, which could provide a carbon source for sludge during biodegradation in a fixed-bed reactor, thereby achieving a higher denitrification rate (Zhu et al., 2015). PBS group reduced nitrogen fixation in the anammox sludge (M00175) by about 0.0755 %, which may be attributed to the damaged anammox sludge in the PBS2 group, which weakened the nitrogen fixation ability. The PVC2 only slightly increased nitrogen fixation (0.01 %), while the abundance of other nitrogen metabolism modules was significantly reduce. The addition of 0.5 g/L PBS increased the relative abundance of nitrogen metabolism modules, while the addition of 0.5 g/L PVC reduced their relative abundance, which helped to explain why the addition of PBS MPs increased the abundance of the phyla Proteobacteria and Planctomycetes and the genus *Ca. Brocadia*. In contrast, the opposite effect was presente with PVC addition.

It is well known that NH_4-N and NO_2-N are substrates for the anammox reaction. NO_2-N can be reduced to nitrite (NO), an intermediate in the anammox reaction produced by nitrite reductase, which mainly

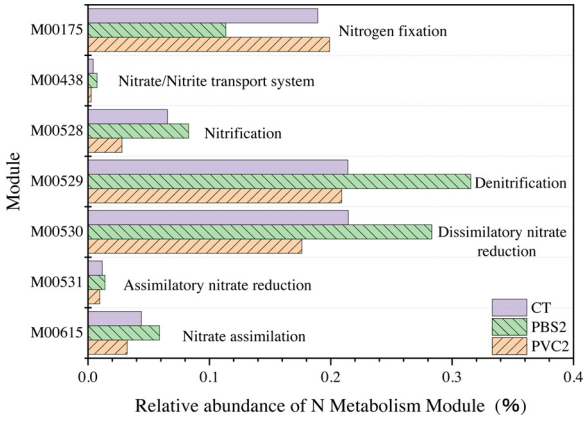

Fig. 6. Abundance of nitrogen metabolism module: CT-the control group, PBS2-0.5 g/L PBS, PVC2-0.5 g/L PVC.

involves the genes nirK and nirS (EC: 1.7.2.1) (Kartal et al., 2011). The intermediate products NO or hydroxylamine and NH_4-N generate hydrazine under the action of hydrazine synthase, which genes involved in this reaction mainly include hzsA, hzsB and hzsC (EC: 1.7.2.7), which are considered to be key discriminate anammox genes (Oshiki et al., 2016; Strous et al., 1998). Shimamura et al. (2007) found that the hydrazine dehydrogenase (HZO) of the anammox bacterial strain KSU-1 could dehydrogenate hydrazine to generate N_2. However, researchers, such as Strous et al. (1998) and Schalk et al. (2000), thought that the hydroxylamine oxidoreductase (hao) could oxidize hydrazine to generate nitrogen. The main anammox genes found in this study were nirK, nirS, hzsA, hzsB, hzsC and hao (Fig. 7), while the HZO gene was not found. In addition, the nrfH and nrfA genes (EC: 1.7.2.2), which could reduce nitrate, were found. The gene abundance graph showed that the addition of 0.5 g/L PBS increased the abundance of hzsA, hzsB and hzsC by 0.2 %, 0.063 % and 0.049 %, respectively, compared with the control group, while it decreased by 0.18 %, 0.014 % and 0.021 % respectively in the PVC2 group (Fig. 7). The PBS2 group increased the abundance of all the nitrogen metabolism genes, while the PVC2 group reduced the abundance of all nitrogen metabolism genes except nirK and nirS, which coincided with the microbial community analysis and nitrogen metabolism module. This may be because the addition of PBS made anammox sludge break up, increasing the mass transfer between sludge and wastewater, making more bottom flow to anammox bacteria, providing more energy for the synthesis of enzymes and the growth of anammox bacteria. In addition, the increase of the abundance of key functional genes (HZS, nirK, nirS) led to the corresponding enhancement of the activity of key enzymes, which enhanced the denitrification ability of the PBS group. However, the accumulation of a large amount of PVC in the PVC group hindered the mass transfer between wastewater and sludge, which reduced the flow to anammox bacterial substrates, thereby reducing the bacterial abundance of anammox.

3.4.2. Methane module and metabolic changes

To better understand the reasons for the high abundance of methanogens in the absence of organic carbon source, the corresponding module graph of methane metabolism was analyzed (Fig. 8). Methane production mainly involved four modules: methanol methane production (M00356); acetate methane production (M00357); methylamine, dimethylamine, trimethylamine methane production (M00563); and carbon dioxide methane production (M00567) (Bhattacharyya et al., 2016). Fig. 8 showed that the methane production in this experiment was dominated by the acetate and CO_2 (hydrogen reduction of carbon dioxide) pathways. This demonstrates the rationality of the hypothesis in part 3.3. Microbial community analysis shows that the PBS2 group would increase mass transfer and the abundance of the *Ca. Brocadia* genus. Compared with the CT group, the M00356, M00357, M00563 and M00567 modules in the PBS2 group were reduce by 0.153 %, 0.434 %, 0.017 % and 0.636 %, respectively. It may be due to the damaged sludge structure and the corresponding reduction in its methane production capacity. In addition, PVC2 group had almost no effect on the relative abundance of the methanogenesis modules, and the relative abundance of the carbon dioxide methanogenesis pathway was slightly greater in the PVC2 group (1.759 %) than in the CT group (1.757 %). The addition of 0.5 g/L PBS would significantly affect the methane production capacity of the sludge.

The methane-metabolic pathway map (Fig. 9) showed that specific enzymes involved in carbon dioxide methanogenesis mainly include methoxymethane dehydrogenase (EC: 1.2.7.12), formylmethylfuran tetrahydrometopterin-*N*-formyltransferase (EC: 2.3.1.101), methenyltetrahydromethanopterin cyclohydrolase (EC: 3.5.4.27), methylenetetrahydrometopterin dehydrogenase (EC: 1.5.98.1) and 5,10-methylenetetramethyl methotrexate reductase (EC: 1.5.98.2) (Bhattacharyya et al., 2016). In addition, the unique enzymes found in the acetic acid methanogenesis pathway include acetate kinase (EC:

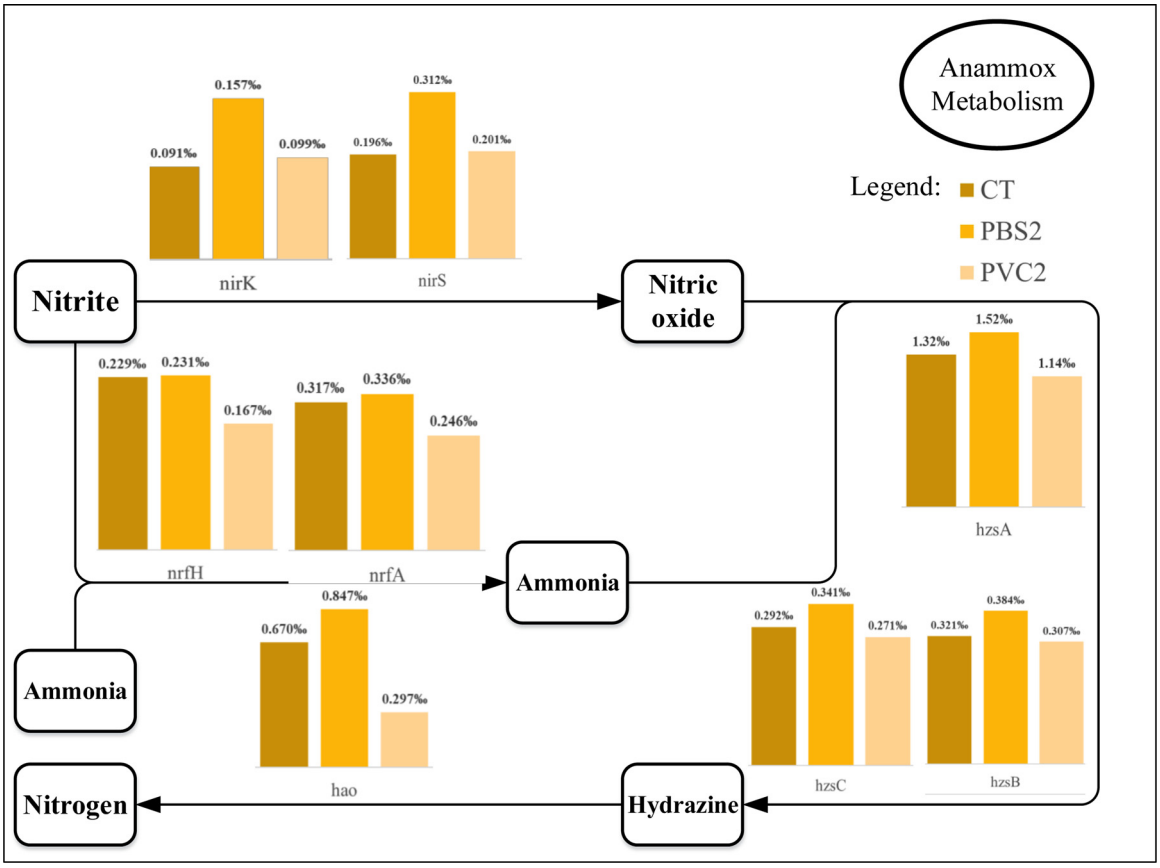

Fig. 7. Anammox metabolism schematic: CT-the control group, PBS2-0.5 g/L PBS, PVC2-0.5 g/L PVC.

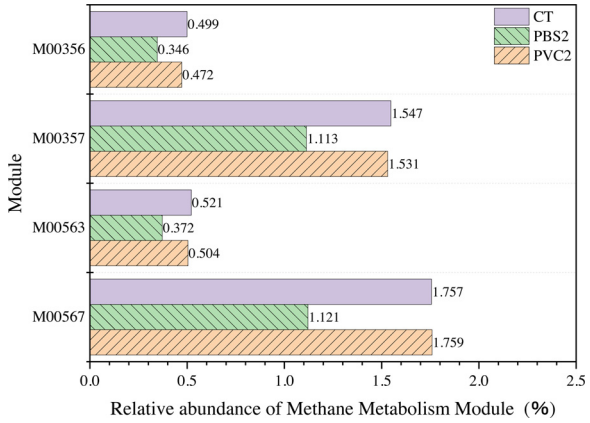

Fig. 8. Abundance of methane generation module: CT-the control group, PBS2-0.5 g/L PBS, PVC2-0.5 g/L PVC.

2.7.2.1), phosphate acetyltransferase (EC: 2.3.1.8), acetyl-CoA synthetase (EC: 6.2.1.1) and acetyl-CoA decarbonylation/synthetase (EC: 2.1.1.245 and EC: 2.3.1.169), with the first three being key to acetyl-CoA synthesis(Zhao et al., 2020). Finally, enzymes shared in carbon dioxide and acetate methanogenesis were tetrahydromethoxin-S-methyltransferase (EC: 2.1.1.86), methyl coenzyme M reductase (EC: 2.8.4.1) and heterodisulfide reductase (EC: 1.8.98.1 and EC: 1.8.98.4). The above genes were found in the three groups of sludge (Table S4). In addition, there was a way for CO_2 to produce acetic acid by carbon monoxide dehydrogenase (EC: 1.2.7.4) and acetyl-coenzyme A synthase, which provides a reaction substrate for *Methanosaeta*. Consistent with the trend of the microbial community, 0.5 g/L PBS addition distinctly reduced the abundance of functional genes, which involved in the production of methane and acetic acid, while 0.5 g/L PVC was only slightly reduced (Table S4).

4. Conclusions

In the present study, MPs had a certain negative impact on the nitrogen removal performance and functional groups of the anammox sludge, especially PVC MPs. The average ammonia nitrogen and nitrite nitrogen removal rates of 0.5 g/L PVC MPs decreased by 6.2 % and 11.6 %, respectively. PVC MPs caused accumulation and Cl2p peak appeared in the XPS spectra of the anammox sludge. PVC MPs had an obviously inhibitory effect on anammox bacteria Planctomycetes. However, PBS MPs reduced the relative abundance of the methanogen *Methanosaeta* and methane modules, while stimulated the anammox bacteria *Ca. Brocadia*.

CRediT authorship contribution statement

Linqin Tang: Writing - original draft, Writing - review & editing. **Chengyuan Su:** Writing - original draft, Writing - review & editing. **Yu Chen:** Data curation. **Yunchuan Xian:** Data curation. **Xinyue Hui:** Data curation. **Ziyu Ye:** Data curation. **Menglin Chen:** Supervision. **Fenghua Zhu:** Data curation. **He Zhong:** Data curation.

Declaration of Competing Interest

The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

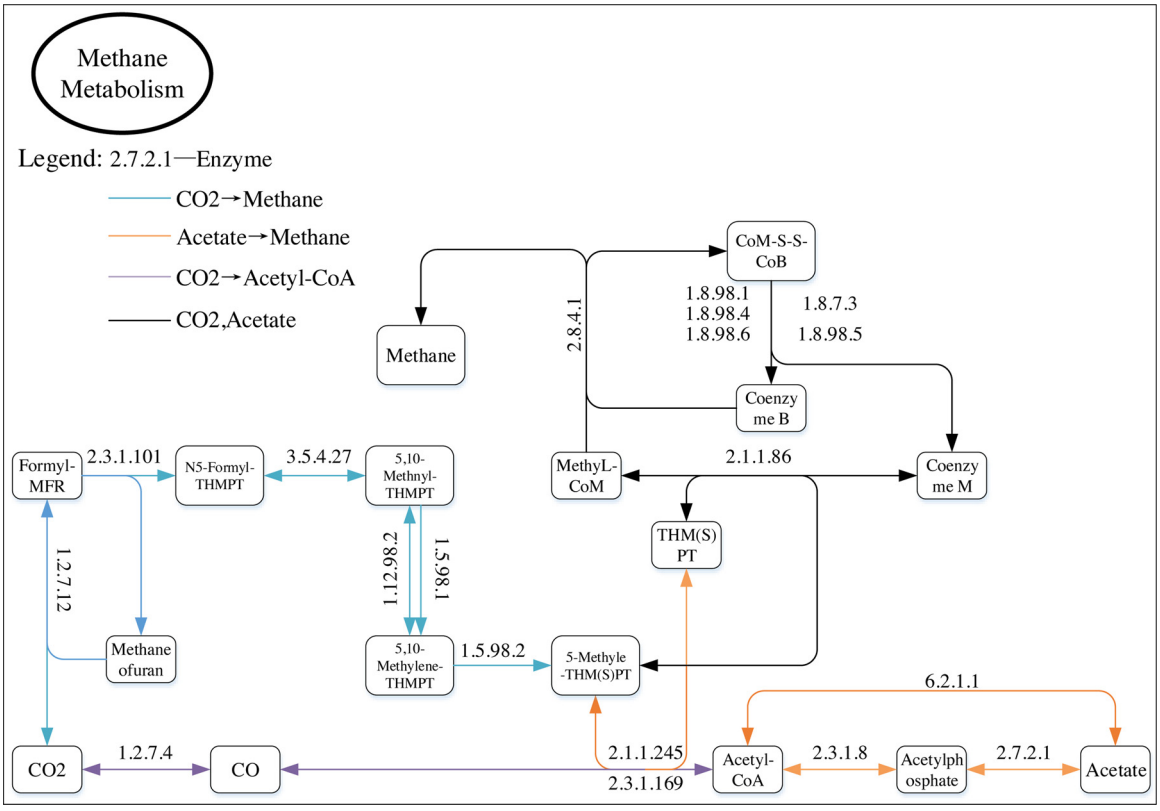

Fig. 9. Methane metabolism pathway: the blue line was a unique line for carbon dioxide methanogenesis; the orange line was for acetate methane production and there are lines; and the black line was a specific route for CO_2 to produce acetyl-CoA; the black line was CO_2, and acetate was a common line for methane production.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2020.123337.

References

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, 21st edition. American Public Health Association (APHA), Washington, DC, United States of America.
Bhattacharyya, P., Roy, K.S., Das, M., Ray, S., Balachandar, D., Karthikeyan, S., Nayak, A.K., Mohapatra, T., 2016. Elucidation of rice rhizosphere metagenome in relation to methane and nitrogen metabolism under elevated carbon dioxide and temperature using whole genome metagenomic approach. Sci. Total Environ. 542, 886–898.
Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120.
Chen, H., Hu, H.Y., Chen, Q.Q., Shi, M.L., Jin, R.C., 2016. Successful start-up of the anammox process: influence of the seeding strategy on performance and granule properties. Bioresour. Technol. 211, 594–602.
Chen, R., Takemura, Y., Liu, Y., Ji, J.Y., Sakuma, S., Kubota, K., Ma, H.Y., Li, Y.Y., 2019. Using partial nitrification and anammox to remove nitrogen from low-strength wastewater by co-immobilizing biofilm inside a moving bed bioreactor. ACS Sustain. Chem. Eng. 7, 1353–1361.
Chen, Y.Z., Zhao, Z.C., Liu, H., Ma, Y.H., An, F.J., Huang, J.M., Shao, Z.W., 2020. Achieving stable two-stage mainstream partial-nitrification/anammox (PN/A) operation via intermittent aeration. Chemosphere 245, 125650.
Chinaglia, S., Tosin, M., Degli-Innocenti, F., 2018. Biodegradation rate of biodegradable plastics at molecular level. Polym. Degrad. Stab. 147, 237–244.
Fontana, A., Campanaro, S., Treu, L., Kougias, P.G., Cappa, F., Morelli, L., Angelidaki, I., 2018. Performance and genome-centric metagenomics of thermophilic single and two-stage anaerobic digesters treating cheese wastes. Water Res. 134, 181–191.
Gan, Z.H., Abe, H., Doi, Y., 2001. Crystallization, melting, and enzymatic degradation of diodegradable poly(butylene succinate-co-14 mol ethylene succinate) copolyester. Biomacromolecules 2, 313–321.
Gardon, T., Reisser, C., Soyez, C., Quillien, V., Le Moullac, G., 2018. Microplastics affect energy balance and gametogenesis in the pearl oyster pinctada margaritifera. Environ. Sci. Technol. 52, 5277–5286.
Gessesse, A., Dueholm, T., Petersen, S.B., Nielsen, P.H., 2003. Lipase and protease extraction from activated sludge. Water Res. 37, 3652–3657.
Gong, W.W., Jiang, M.Y., Han, P., Liang, G., Zhang, T.T., Liu, G.N., 2019. Comparative analysis on the sorption kinetics and isotherms of fipronil on nondegradable and biodegradable microplastics. Environ. Pollut. 254, 112927.
Guo, Q.W., Li, N.N., Chen, S.L., Chen, Y., Xie, S.G., 2019. Response of freshwater sediment archaeal community to metal spill. Chemosphere 217, 584–590.
He, T.X., Xie, D.T., Ni, J.P., Li, Z., Li, Z.L., 2020. Nitrous oxide produced directly from ammonium, nitrate and nitrite during nitrification and denitrification. J. Hazard. Mater. 388, 122114.
Huang, W.Y., She, Z.L., Gao, M.M., Wang, Q., Jin, C.J., Zhao, Y.G., Guo, L., 2019a. Effect of anaerobic/aerobic duration on nitrogen removal and microbial community in a simultaneous partial nitrification and denitrification system under low salinity. Sci. Total Environ. 651, 859–870.
Huangfu, X.L., Xu, Y.H., Liu, C.H., He, Q., Ma, J., Ma, C.X., Huang, R.X., 2019b. A review on the interactions between engineered nanoparticles with extracellular and intracellular polymeric substances from wastewater treatment aggregates. Chemosphere 219, 766–783.
Ji, X.M., Wu, Z.Y., Sung, S.H., Lee, P.H., 2019. Metagenomics and metatranscriptomics analyses reveal oxygen detoxification and mixotrophic potentials of an enriched anammox culture in a continuous stirred-tank reactor. Water Res. 166, 115039.
Jiang, B., Zeng, Q.Z., Liu, J.X., Hou, Y., Xu, J., Li, H.X., Shi, S.N., Ma, F., 2020a. Enhanced treatment performance of phenol wastewater and membrane antifouling by biochar-assisted EMBR. Bioresour. Technol. 306, 123147.
Jiang, H., Peng, Y.Z., Lia, X.Y., Zhang, F.Z., Wang, Z., Ren, S., 2020b. Advanced nitrogen removal from mature landfill leachate via partial nitrification-anammox biofilm reactor (PNABR) driven by high dissolved oxygen (DO): protection mechanism of aerobic biofilm. Bioresour. Technol. 306, 123119.
Kartal, B., Maalcke, W.J., de Almeida, N.M., Cirpus, I., Gloerich, J., Geerts, W., Op den Camp, H.J.M., Harhangi, H.R., Janssen-Megens, E.M., Francoijs, K.J., Stunnenberg, H.G., Keltjens, J.T., Jetten, M.S.M., Strous, M., 2011. Molecular mechanism of anaerobic ammonium oxidation. Nature 479, 127–130.
Kim, I.T., Lee, Y.E., Jeong, Y., Yoo, Y.S., 2020. A novel method to remove nitrogen from reject water in wastewater treatment plants using a methane- and methanol-dependent bacterial consortium. Water Res. 172, 115512.
Kurade, M.B., Saha, S., Salama, E.S., Patil, S.M., Govindwar, S.P., Jeon, B.H., 2019. Acetoclastic methanogenesis led by Methanosarcina in anaerobic co-digestion of fats,

oil and grease for enhanced production of methane. Bioresour. Technol. 272, 351–359.
Lackner, S., Gilbert, E.M., Vlaeminck, S.E., Joss, A., Horn, H., van Loosdrecht, M.C.M., 2014. Full-scale partial nitritation/anammox experiences-An application survey. Water Res. 55, 292–303.
Lei, Y.Q., Sun, D.Z., Dang, Y., Feng, X.L., Huo, D., Liu, C.Q., Zheng, K., Holmes, D.E., 2019. Metagenomic analysis reveals that activated carbon aids anaerobic digestion of raw incineration leachate by promoting direct interspecies electron transfer. Water Res. 161, 570–580.
Li, X.W., Chen, L.B., Mei, Q.Q., Dong, B., Dai, X.H., Ding, G.J., Zeng, E.Y., 2018. Microplastics in sewage sludge from the wastewater treatment plants in China. Water Res. 142, 75–85.
Li, L., Geng, S.X., Li, Z.Y., Song, K., 2020a. Effect of microplastic on anaerobic digestion of wasted activated sludge. Chemosphere 247, 125874.
Li, L., Song, K., Yeerken, S., Geng, S., Liu, D., Dai, Z.L., Xie, F.Z., Zhou, X.H., Wang, Q.L., 2020b. Effect evaluation of microplastics on activated sludge nitrification and denitrification. Sci. Total Environ. 707, 135953.
Lin, Y.C., Lü, F., Shao, L.M., He, P.J., 2013. Influence of bicarbonate buffer on the methanogenetic pathway during thermophilic anaerobic digestion. Bioresour. Technol. 137, 245–253.
Liu, X.M., Sheng, G.P., Luo, H.W., Zhang, F., Yuan, S.J., Xu, J., Zeng, R.J., Wu, J.G., Yu, H.Q., 2010. Contribution of extracellular polymeric substances (EPS) to the sludge aggregation. Environ. Sci. Technol. 44, 4355–4360.
Liu, H., Zhou, X., Ding, W.Q., Zhang, Z.H., Nghiem, L.D., Sun, J., Wang, Q.L., 2019a. Do microplastics affect biological wastewater treatment performance? Implications from bacterial activity experiments. ACS Sustain. Chem. Eng. 7, 20097–20101.
Liu, K., Wang, X.H., Fang, T., Xu, P., Zhu, L.X., Li, D.J., 2019b. Source and potential risk assessment of suspended atmospheric microplastics in Shanghai. Sci. Total Environ. 675, 462–471.
Mason, S.A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., Fink, P., Papazissimos, D., Rogers, D.L., 2016. Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. Environ. Pollut. 218, 1045–1054.
Oh, S., Hammes, F., Liu, W.T., 2018. Metagenomic characterization of biofilter microbial communities in a full-scale drinking water treatment plant. Water Res. 128, 278–285.
Oshiki, M., Ali, M., Shinyako-Hata, K., Satoh, H., Okabe, S., 2016. Hydroxylamine-dependent anaerobic ammonium oxidation (anammox) by “Candidatus Brocadia sinica”. Environ. Microbiol. 18, 3133–3143.
Pan, W.J., Bai, Z.H., Su, T.T., Wang, Z.Y., 2018. Enzymatic degradation of poly(butylene succinate) with different molecular weights by cutinase. Int. J. Biol. Macromol. 111, 1040–1046.
Pradhan, N., Swa Thi, S., Wuertz, S., 2019. Inhibition factors and kinetic model for anaerobic ammonia oxidation in a granular sludge bioreactor with Candidatus Brocadia. Chem. Eng. J., 123618.
Qin, R.H., Su, C.Y., Liu, W.H., Tang, L.Q., Li, X.J., Deng, X., Wang, A.L., Chen, Z.P., 2020. Effects of exposure to polyether sulfone microplastic on the nitrifying process and microbial community structure in aerobic granular sludge. Bioresour. Technol. 302, 122827.
Sanin, S.L., Sanin, F.D., Bryers, J.D., 2003. Effect of starvation on the adhesive properties of xenobiotic degrading bacteria. Process Biochem. 38, 909–914.
Schalk, J., de Vries, S., Kuenen, J.G., Jetten, M.S.M., 2000. Involvement of a novel hydroxylamine oxidoreductase in anaerobic ammonium oxidation. Biochemistry 39, 5405–5412.
Shi, K., Su, T.T., Wang, Z.Y., 2019. Comparison of poly (butylene succinate) biodegradation by fusarium solani cutinase and candida antarctica lipase. Polym. Degrad. Stab. 164, 55–60.
Srirangan, K., Pyne, M.E., Perry Chou, C., 2011. Biochemical and genetic engineering strategies to enhance hydrogen production in photosynthetic algae and cyanobacteria. Bioresour. Technol. 102, 8589–8604.
Strous, M., Heijnen, S., Kuenen, J.G., Jetten, M., 1998. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. Appl. Microbiol. Biotechnol. 50, 589–596.
Sun, X.M., Chen, B.J., Li, Q.F., Liu, N., Xia, B., Zhu, L., Qu, K.M., 2018. Toxicities of polystyrene nano- and microplastics toward marine bacterium Halomonas alkaliphila. Sci. Total Environ. 642, 1378–1385.
Sun, J., Dai, X.H., Wang, Q.L., van Loosdrecht, M.C.M., Ni, B.J., 2019. Microplastics in wastewater treatment plants: Detection, occurrence and removal. Water Res. 152, 21–37.
Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le, G.N., Quillien, V., Mingant, C., Epelboin, Y., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. 113, 2430–2435.
Tejerizo, G.T., Kim, Y.S., Maus, I., Wibberg, D., Winkler, A., Off, S., Pühler, A., Scherer, P., Schlüter, A., 2017. Genome sequence of Methanobacterium congolense strain Buetzberg, a hydrogenotrophic, methanogenic archaeon, isolated from a mesophilic industrial-scale biogas plant utilizing bio-waste. J. Biotechnol. 247, 1–5.
Tomaszewski, M., Cema, G., Ziembińska-Buczyńska, A., 2017. Significance of pH control in anammox process performance at low temperature. Chemosphere 185, 439–444.
Tosin, M., Pischedda, A., Degli-Innocenti, F., 2019. Biodegradation kinetics in soil of a multi-constituent biodegradable plastic. Polym. Degrad. Stab. 166, 213–218.
Wang, C., Liu, S.T., Xu, X.C., Zhang, C.L., Wang, D., Yang, F.L., 2018. Achieving mainstream nitrogen removal through simultaneous partial nitrification, anammox and denitrification process in an integrated fixed film activated sludge reactor. Chemosphere 203, 457–466.
Xu, J.J., Cheng, Y.F., Xu, L.Z.J., Liu, Y.Y., Zhu, B.Q., Fan, N.S., Huang, B.C., Jin, R.C., 2019. The revolution of performance, sludge characteristics and microbial community of anammox biogranules under long-term NiO NPs exposure. Sci. Total Environ. 649, 440–447.
Xu, X.X., Ma, B., Lu, W.K., Feng, D.B., Wei, Y., Ge, C.J., Peng, Y.Z., 2020. Effective nitrogen removal in a granule-based partial-denitrification/anammox reactor treating low C/N sewage. Bioresour. Technol. 297, 122467.
Yang, G.J., Zhang, N., Yang, J.N., Fu, Q., Wang, Y., Wang, D.B., Tang, L., Xia, J.F., Liu, X.R., Li, X.M., Yang, Q., Liu, Y.W., Wang, Q.L., Ni, B.J., 2020a. Interaction between perfluorooctanoic acid and aerobic granular sludge. Water Res. 169, 115249.
Yang, X.Y., Chen, Y., Guo, F.C., Liu, X.B., Su, X.X., He, Q., 2020b. Metagenomic analysis of the biotoxicity of titanium dioxide nanoparticles to microbial nitrogen transformation in constructed wetlands. J. Hazard. Mater. 384, 121376.
Yangin-Gomec, C., Pekyavas, G., Sapmaz, T., Aydin, S., Ince, B., Akyol, Ç., Ince, O., 2017. Microbial monitoring of ammonia removal in a UASB reactor treating pre-digested chicken manure with anaerobic granular inoculum. Bioresour. Technol. 241, 332–339.
Zhang, Z.Z., Xu, J.J., Shi, Z.J., Cheng, Y.F., Ji, Z.Q., Deng, R., Jin, R.C., 2017. Short-term impacts of Cu, CuO, ZnO and Ag nanoparticles (NPs) on anammox sludge: CuNPs make a difference. Bioresour. Technol. 235, 281–291.
Zhang, W., Dai, K., Xia, X.Y., Wang, H.J., Chen, Y., Lu, Y.Z., Zhang, F., Zeng, R.J., 2018a. Free acetic acid as the key factor for the inhibition of hydrogenotrophic methanogenesis in mesophilic mixed culture fermentation. Bioresour. Technol. 264, 17–23.
Zhang, X.J., Chen, Z., Ma, Y.P., Zhao, J., Chen, T., Fu, H.Q., Zhai, H.F., 2018b. Acute and persistent toxicity of Cd(II) to the microbial community of Anammox process. Bioresour. Technol. 261, 453–457.
Zhang, Y.P., Li, J.Z., Liu, F.Q., Yan, H., Li, J.L., Zhang, X., 2018c. Reduction of Gibbs free energy and enhancement of Methanosaeta by bicarbonate to promote anaerobic syntrophic butyrate oxidation. Bioresour. Technol. 267, 209–217.
Zhang, K., Lyu, L., Kang, T., Yao, S., Ma, Y.G., Pan, Y., Wang, Y.Z., Furukawa, K., Hao, L.Y., Zhu, T., 2019a. A rapid and effective way to cultivate anammox granular sludge through vibration. Int. Biodeter. Biodegr. 143, 104704.
Zhang, Q.Q., Bai, Y.H., Wu, J., Xu, L.Z.J., Zhu, W.Q., Tian, G.M., Zheng, P., Xu, X.Y., Jin, R.C., 2019b. Microbial community evolution and fate of antibiotic resistance genes in anammox process under oxytetracycline and sulfamethoxazole stresses. Bioresour. Technol. 293, 122096.
Zhang, X.J., Chen, Z.M., Y.P, Chen, T., Zhang, J., Zhang, H., Zheng, S.H., Jia, J.P., 2019c. Impacts of erythromycin antibiotic on Anammox process: performance and microbial community structure. Biochem. Eng. J. 143, 1–8.
Zhao, Y.P., Liu, S.F., Jiang, B., Feng, Y., Zhu, T.T., Tao, H.C., Tang, X., Liu, S.T., 2018. Genome-centered metagenomics analysis reveals the symbiotic organisms possessing ability to cross-feed with Anammox bacteria in Anammox consortia. Environ. Sci. Technol. 52, 11285–11296.
Zhao, Z.Q., Wang, J.F., Li, Y., Zhu, T.T., Yu, Q.L., Wang, T.T., Liang, S., Zhang, Y.B., 2020. Why do DIETers like drinking: metagenomic analysis for methane and energy metabolism during anaerobic digestion with ethanol. Water Res. 171, 115425.
Zhou, Y., Wang, L.L., Zhou, Y.Y., Mao, X.Z., 2020. Eutrophication control strategies for highly anthropogenic influenced coastal waters. Sci. Total Environ. 705, 135760.
Zhu, S.M., Deng, Y.L., Ruan, Y.J., Guo, X.S., Shi, M.M., Shen, J.Z., 2015. Biological denitrification using poly(butylene succinate) as carbon source and biofilm carrier for recirculating aquaculture system effluent treatment. Bioresour. Technol. 192, 603–610.