



Communication

The performance and degradation mechanism of sulfamethazine from wastewater using IFAS-MBR



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ABSTRACT

Sulfamethazine (SMZ) is an important sulfonamide antibiotic. Although the concentration in the environment is small, it is harmful. The drug residues can be transferred, transformed or accumulated, affecting the growth of animals and plants. In this study, the integrated fixed-film activated sludge membrane bioreactor (IFAS-MBR) were constructed to investigate the performance and degradation mechanism of SMZ. The addition of SMZ had a significant impact on the removal of the chemical oxygen demand (COD) and ammonia nitrogen ($\text{NH}_4^+\text{-N}$). The optimal operating conditions were hydraulic retention time (HRT) at 10 h and solid retention time (SRT) at 80 d, respectively. On this basis, the effects of different SMZ concentrations on nutrient removal, degradation, and sludge characteristics were compared. The removal efficiency of SMZ increased with the increase of SMZ concentration. The maximum removal rate was as high as 87%. The SMZ dosage also had an obvious effect on sludge characteristics. As the SMZ concentration increased, the extracellular polymer substances (EPS) concentration and the membrane resistance both decreased, which were beneficial for the reduction of membrane fouling. Finally, seven kinds of SMZ biodegradation intermediates were identified, and the possible degradation pathways were speculated. The microbial community results showed that the microbial diversity and richness in the reactor decreased after adding SMZ to the influent. The relative abundance of Bacteroidetes, Actinobacteria, Saccharibacteria and Nitrospirae increased at the phylum level. Sphingobacteria and Betaproteobacteria became dominant species at the class level. The relative abundance of norank-p-Saccharibacteria and Nitrospirae increased significantly, and norank-p-Saccharibacteria may be the dominant bacteria for SMZ degradation.

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The active sludge return system was developed in moving bed biofilm reactor-membrane bioreactor (MBBR-MBR) to form an integrated fixed-film activated sludge bioreactor (IFAS-MBR) technology [1]. This can not only reduce the content of metabolites, but also improve the sludge properties to simultaneously slow down and reduce sludge production in membrane fouling. IFAS-MBR also displayed a higher endurance for shock loading than the MBR [2]. As a novel wastewater treatment process, there has limited report about antibiotics removal by IFAS-MBR.

As an important component of antibiotics, sulfonamides have been widely detected in urban rivers, effluent from sewage treatment plants, groundwater and even drinking water [3]. These are most common in municipal sewage treatment plants and pose a serious threat to water quality and human health [4]. In recent years, the removal methods of trace sulfonamide antibiotics in urban sewage include activated carbon adsorption [5], resin adsorption [6], ozone catalytic oxidation [7], biological aerated filter [8], constructed wetland, MBR and so on. But the most effective and economical way is MBR. Some researchers have used MBR to treat sulfamethazine (SMZ) wastewater to study the long-term effects of different concentrations of SMZ on its degradation efficiency and sludge characteristics [9]. A lab-scale anaerobic/hypoxia/oxygen membrane bioreactor ($\text{A}_1/\text{A}_2/\text{O}$ -MBR) was used to study the removal performance of nine sulfonamides (SAs) at

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ambient concentrations [10]. However, the complete pathway and degradation mechanism of SMZ in IFAS-MBR require further study.

Therefore, the objective of this study is to investigate the performance and degradation mechanism of SMZ from wastewater using IFAS-MBR. Membrane fouling in the reactor with different SMZ concentration was studied by extracellular polymeric substances (EPS) and soluble microbial products (SMP). Moreover, the intermediate products of SMZ degradation were analyzed by LC-MS and the microbial community characteristics were determined by high-throughput sequencing.

The schematic structure of the laboratory scale reactor is shown in Fig. S1 (Supporting information). The IFAS-MBR consisted of an MBBR unit (R_1) and an MBR unit (R_2). Detailed introduction of reactor and SMZ is in Supporting information (Fig. S2). In this study, three different hydraulic retention times (HRTs) (6, 8 and 10 h), three different solid retention time (SRTs) (20, 40 and 80 d) and three different SMZ concentrations (50 $\mu\text{g/L}$, 100 $\mu\text{g/L}$, 150 $\mu\text{g/L}$) were used to compare the performance of IFAS-MBR.

The specific measurement method is shown in the Supporting information. Chemical oxygen demand (COD) and ammonia nitrogen ($\text{NH}_4^+\text{-N}$) were detected using Hach methods 8000 and 10031, respectively [11]. Solid phase extraction was required before HPLC can be used to determine the SMZ content [12,13]. Quantitative analysis was performed by the external standard method [9]. LC-MS was used to identify the biological metabolic intermediates of SMZ [14,15]. The total extracellular polymeric substances (T-EPS, include EPS and SMP) were extracted by the method of cation exchange resin (CER, Sigma-Aldrich, USA) [16]. The contents of the EPS and SMP were characterized using total organic carbon (TOC), DNA, protein, and polysaccharide. The resistance in series model has been adapted to consider membrane fouling [17]. The total filtration resistance was defined as the following (Eq. (1)):

$$R_t = R_c + R_f + R_m \quad (1)$$

where R_t is the total filtration resistance; R_m is the intrinsic membrane resistance; R_f is the internal fouling resistance due to fouling mechanisms, and R_c is the cake layer resistance that can be removed by physical cleaning.

Sludges were taken from MBBR and MBR reactors with SMZ influent concentrations of 0, 50, 100, 150 $\mu\text{g/L}$, numbered SMZ_0_MBBR, SMZ_0_MBR, SMZ_50_MBBR, SMZ_50_MBR, SMZ_100_MBBR, SMZ_100_MBR, SMZ_150_MBBR, SMZ_150_MBR, respectively. High-throughput sequencing was performed according to the Illumina platform sequencing process.

The performance of IFAS-MBR at different HRTs is illustrated in Fig. 1a. When SMZ was not added to the influent, the removal efficiencies of COD and $\text{NH}_4^+\text{-N}$ were 96.5% and 99.6%, respectively, when the HRT was 8 h. When SMZ was added to the influent, the removal efficiencies of COD and $\text{NH}_4^+\text{-N}$ were approximately 89%–92% and 95.5%–97.34%, respectively. It can be observed that the removal rates of COD and $\text{NH}_4^+\text{-N}$ were decreased when SMZ

was added into the bioreactors. It may relate to the toxicity of SMZ to the microorganisms that degrade COD. The amino groups in the structure of SMZ degradation were separated and existed in the solution as NH_4^+ , which increased the concentration of ammonia nitrogen. The removal efficiencies of SMZ at different HRT were approximately 63.5%–73.5%, which increased as HRT increased. This suggested that at the condition of long HRT, the SMZ removal efficiency was significantly positive. Previous results have shown that the prolongation of HRT reduced the tendency for membrane pollution and promoted the diversity, symbiosis, and biodegradation efficiency of some micro-pollutants [18]. This study draws the same conclusion, which may be due to the higher adsorption and biodegradation of SMZ at longer HRT. Since the major removal mechanism of the system was biodegradation, the attachment growth mode was ideal for enriching slow-growing bacteria and culturing specific degrading bacteria. Thereby an effective removal of micro-contaminants was obtained.

The performance of IFAS-MBR at different SRTs is shown in Fig. 1b. When SMZ was not added to the influent, the removal efficiencies of COD and $\text{NH}_4^+\text{-N}$ were approximately 93.5%–97.2% and 99.1%–99.7%, respectively. The removal efficiencies of COD and $\text{NH}_4^+\text{-N}$ were very higher at the SRT 80 days. When SMZ was added to the influent, the removal efficiencies of COD and $\text{NH}_4^+\text{-N}$ were approximately 89%–93% and 95%–97%, respectively. The removal efficiencies of COD and $\text{NH}_4^+\text{-N}$ were reduced, compared with the SMZ was not added. The possible reasons are the same as discussed above. The removal efficiency of SMZ increased from 69.5% to 78.5% as SRT increased. This also means that longer SRT is more conducive to the survival of slow-growing bacteria and the adaptation of some SMZ-degraded microorganisms to degrade certain persistent pollutants [19].

The performance of IFAS-MBR at different SMZ influent concentrations is presented in Fig. 1c. The removal efficiencies of COD and $\text{NH}_4^+\text{-N}$ were approximately 89%–94% and 95%–99%, respectively. Previous studies suggested that the addition of SMZ had no significant influence on the removal of COD and $\text{NH}_4^+\text{-N}$ at high SMZ concentration (1–5 mg/L) [9]. However, in this study, the removal of COD and $\text{NH}_4^+\text{-N}$ was reduced. This can due to the simulated domestic wastewater with a low concentration of SMZ at 50–150 $\mu\text{g/L}$ was used in this research. Therefore, the results were different with high SMZ concentration condition in previous research. This indicated that the SMZ concentration had a certain inhibitory effect on the biodegradation and nitrification of COD and $\text{NH}_4^+\text{-N}$. In addition, the removal efficiency of SMZ increased with the increased of influent concentration.

The characteristics of EPS and SMP are shown in Fig. 2. The concentrations of the four components (TOC, DNA, protein and polysaccharide) in the EPS and SMP decreased with increasing SMZ concentration. Previous researchers believed that EPS and SMP were the significant causes of membrane fouling [20]. It has been reported that EPS and SMP have an important impact on the characteristics and function of the sludge. Among them, EPS has a

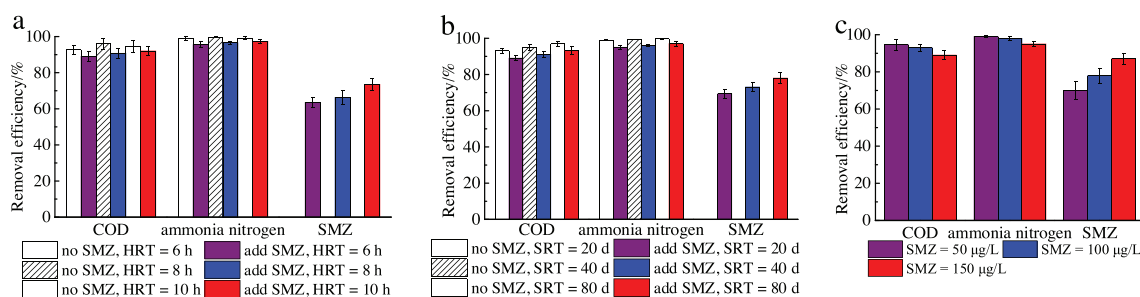


Fig. 1. The performance of IFAS-MBR under different HRTs (a), different SRTs (b), different SMZ influent concentrations (c). The values are average of 30 sample values.

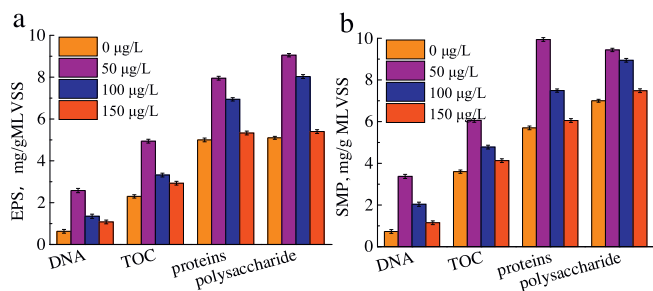


Fig. 2. EPS (a) and SMP (b) characterized by DNA, TOC, protein and polysaccharide in the MBR at different SMZ influent concentrations. The values are average of 30 sample values.

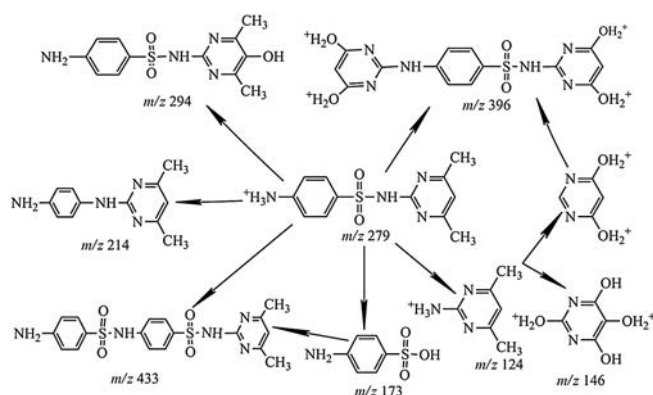


Fig. 3. Degradation pathway of sulfamethazine in the IFAS-MBR.

major impact on the removal of some micro-pollutants. The causes of membrane fouling may be related to functional bacteria in sludge, which will be discussed below. The membrane resistances results were shown in Table S1 (Supporting information). The results showed that all the resistance (R , R_f , R_c) also decreased as SMZ concentration increased. Most of the filtration resistance was attributed to R_f (70%–82%). The proportion of R_f relative to R clearly increased as the SMZ concentration at high SMZ concentration condition. R_c accounted for 17%–24% of R in the reactors, and the ratio increased with increasing SMZ concentration.

The LC–MS analysis of the main reaction intermediates is shown in Fig. S3 (Supporting information). Analysis of each corresponding

substance revealed that the substance with an m/z of 217 was produced by removing sulfur dioxide from SMZ. The substance with an m/z of 124 was a product of a bond between sulfate and nitrogen. The hydroxylated product of the pyrimidine ring had an m/z of 146. The material with an m/z of 295 was derived from the hydroxylation of SMZ. Because these types of intermediate products were not stable, the measurements of these products were difficult, and research is currently conducted only by inference. Based on the detected intermediates, the speculated degradation pathway of SMZ in the IFAS-MBR reactor is shown in Fig. 3.

The microbial community results are shown in Table S2 (Supporting information). The diversity and richness of the microorganisms decreased after SMZ was added in the reactor, and the addition of SMZ inhibited some microorganisms. As shown in Fig. 4a, Proteobacteria and Bacteroidetes were the dominant phyla in the reactors with different SMZ influent concentration, which were similar to other microbial communities, such as soil and sewage [21]. Proteobacteria decreased from 63.27% to 41.27% while Bacteroidetes increased from 9.50% to 43.51%, when 50 µg/L SMZ was added in the influent. It has been reported that Proteobacteria included a variety of functionally important bacteria for organic and nitrogen removal [22]. Thus, the higher abundance of Proteobacteria could account for the slightly better removal of pollutants in the SMZ-0 than in the SMZ-50. It is reported that Bacteroidetes could potentially release more membrane foulants such as proteinaceous EPS [23]. Also, Bacteroidetes possess fimbriae, which could help them attach to the supporting material surfaces [24]. After the addition of SMZ, faster membrane fouling occurred in the SMZ-50 due to the relatively high abundance of Bacteroidetes in the sludge. However, the content of Bacteroidetes in SMZ-100 and SMZ-150 did not change much, which also indicated that the membrane fouling not only be affected by Bacteroidetes.

After the addition of SMZ, Sphingobacteria and Betaproteobacteria became the most abundant dominant species (Fig. 4b). It has been reported that these specific bacteria were the pioneers of surface colonization on membranes [25]. It can be stated that these shared dominant bacteria may be responsible for the initial membrane fouling. The relative abundance of Saccharibacteria in SMZ-150 was significantly increased at the level of the phylum, while the relative abundance of norank-p-Saccharibacteria also increased significantly at the level of the class. The norank-p-Saccharibacteria has been reported to play an important role in promoting the biosynthesis of antibiotics, the biosynthesis of secondary metabolites and the degradation of pyrimidines [26]. The norank-p-Saccharibacteria may be the dominant bacteria for SMZ degradation.

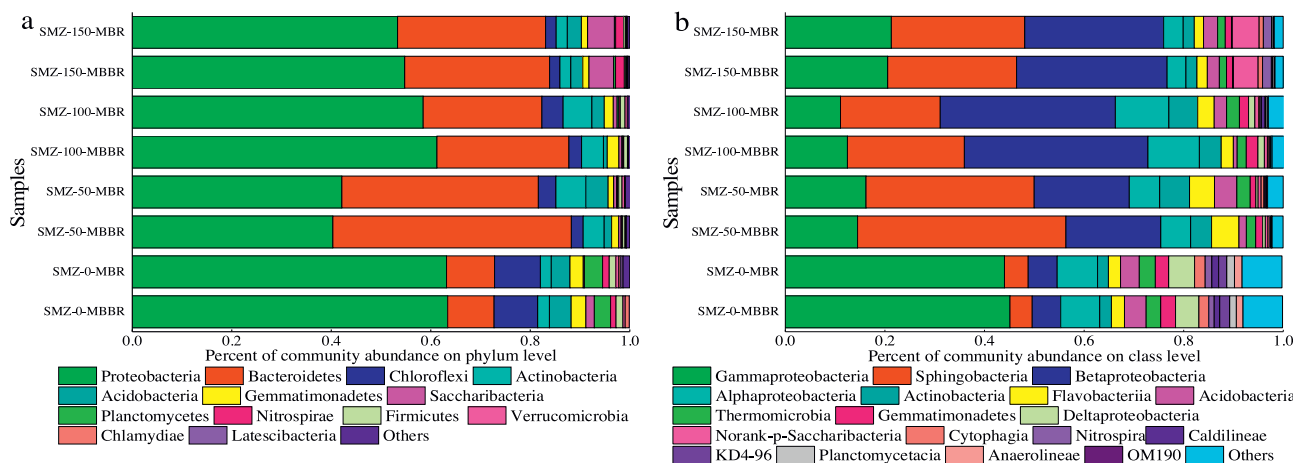


Fig. 4. Analysis of microbial diversity at a different taxonomic level for different SMZ influent concentrations samples: phylum (a); class (b).

In summary, when treating synthetic wastewater in the IFAS-MBR reactor at different HRT and SRT operating conditions, the removal rates of COD and $\text{NH}_4^+\text{-N}$ decreased after the addition of SMZ. For the SMZ removal effect, the optimal HRT and SRT were 10 h and 80 d, respectively. The biodegradation rate of SMZ increased as the influent SMZ concentration increased. The concentration of EPS and SMP decreased with the increase of SMZ concentration. The results showed that increasing the SMZ concentration can slow down membrane fouling. Seven kinds of SMZ biodegradation intermediates were identified by the analysis, and the possible degradation pathways were speculated. After the addition of SMZ, the relative abundance of Bacteroidetes, Actinobacteria, Saccharibacteria and Nitrospirae increased significantly at the level of the phylum. Faster membrane fouling occurred in the SMZ-50 due to the relatively high abundance of Bacteroidetes in the sludge. Sphingobacteria and Betaproteobacteria became the most abundant dominant species. The norank-p-Saccharibacteria may be the dominant bacteria for SMZ degradation.

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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.ccl.2019.08.031>.

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