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Formation, characteristics and microbial community of aerobic granular sludge in the presence of sulfadiazine at environmentally relevant concentrations

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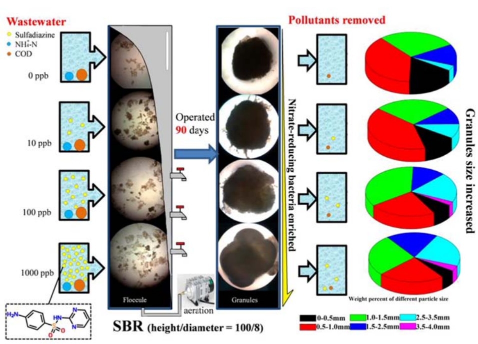
GRAPHICAL ABSTRACT



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ABSTRACT

The growing occurrence of antibiotics in water environment is causing increasing concern. To investigate the impact of frequently detected sulfadiazine on the formation of aerobic granular sludge, four sequencing batch reactors (SBRs) were set up with different environmentally relevant concentrations of sulfadiazine. Results showed that sulfadiazine pressure could lead to larger and more compact sludge particles and cause slight effect on reactor performance. Presence of sulfadiazine apparently increased the extracellular polymeric substances (EPS) secretion of microorganisms. Quantitative polymerase chain reaction (qPCR) showed that the abundances of sulfanilamide resistance genes in sludge increased with addition of sulfadiazine significantly. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was used to predict functional genes, results showed that sulfadiazine led to an increase of specific functional genes. Thereby, it concluded that microorganisms could change the community structure by acclimating of functional bacteria and antibiotic resistance species to adapt to the antibiotic stress.

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1. Introduction

As human and veterinary pharmaceuticals are detected frequently in the environment, antibiotics have caused more and more concern due to their potential biological damage on human and other organism. Sulfa antibiotics are universal broad-spectrum synthetic antibiotics that can effectively control bacterial infections in the field of medicine. Because of the wide use of the low-cost medicine, these kinds of antibiotics could be detected in sewage effluents, municipal wastewater, surface water and even ground water (Yang et al., 2013). Antibiotic concentration in domestic sewage and hospital sewage are of ppt to ppb level, while wastewater from the pharmaceutical factory could reach ppm levels (Larsson et al., 2007). All of the concentrations of antibiotics which were detected in the water environment could be defined as environmentally relevant concentrations.

Aerobic granular sludge with a unique spherical structure could be a promising system to treat antibiotics containing wastewater (Shi et al., 2011). Compared with conventional biological wastewater treatment system, aerobic granular sludge has excellent settling properties and anti-shock loading capability (Sarma et al., 2017). In recent years, some researchers began to focus on the effect of antibiotics at environmentally relevant concentrations on mature aerobic granular sludge. Research indicated that 32 μM of fluoroquinolones did not affect the activities of the ammonium oxidation bacteria (AOB) and nitrite oxidation bacteria (NOB) in aerobic granular sludge but inhibited the activity of polyphosphate-accumulating organisms (PAOs) and also changed microbial community (Amorim et al., 2014). It was found that 100 ppb of sulfadimidine, oxytetracycline and ciprofloxacin had no significant effect on physical characteristics of mature aerobic granular sludge, but reduced pollutants removal rate and enhanced resistance of bacteria (Liu et al., 2016). In activated sludge systems, studies found that even low-level concentrations of antibiotics will have a certain impact on treatment performance and microbial communities (Novo et al., 2013; Zhang et al., 2016; Wang et al., 2017). Since antibiotic can cause influence on activated sludge and mature granular sludge, it may be influential to the formation process of aerobic granular sludge, while there is a lack of information about it.

Low-level concentrations of antibiotics could facilitate the formation of biofilms, which was considered as the defense response mechanism of bacteria under antibiotic pressure (Kaplan et al., 2012; Hoffman et al., 2005; Tan et al., 2016). Previous study also found that antibiotics induced the aggregation of activated sludge (Song et al., 2015). Aerobic granular sludge was a kind of special biofilm aggregated by activated sludge (Yuan et al., 2017). However, research about the formation process of aerobic granular sludge under antibiotic pressure was rarely carried out.

The purpose of this paper is to assess the possible effect on the formation of aerobic granular sludge under antibiotic pressure at environmentally relevant concentrations. Sulfadiazine was used as the antibiotic selection pressure because of its frequent detection in water environment. This paper mainly focuses on the following aspects: I. the influence of different antibiotic concentrations on the process of granulation, II. the change of EPS concentration in the reactors, III. the sulfadiazine removal rate in the formation process of aerobic granular sludge and after formation, IV. the abundance change of sulfanilamide resistance genes, V. the change of microbial community in aerobic granular sludge. This study can promote the understanding of the impact of antibiotics on aerobic granular sludge system and provide some help to degrade antibiotics in wastewater treatment.

2. Materials and methods

2.1. Chemicals

Sulfadiazine of 99.0% purity was purchased from Aladdin Industrial Corporation. Sulfadiazine was dissolved in 0.007 mol/L ammonium hydroxide to obtain stock solution of 240 mg/L for feeding reactors. In order to guarantee the experimental accuracy, all of the other chemicals are analytical reagent (AR) and obtained from Sinopharm Chemical Reagent Company (China).

2.2. Reactors set up and operation

Four sequencing batch reactors with a working volume of 4 L were set up and seeded with activated sludge collected from the secondary sedimentation tank of Everbright Water wastewater treatment plant in Jinan, China. Before experiments, activated sludge was acclimated in the aforementioned four sequencing batch reactors for about two weeks. The initial mixed liquid suspended solids concentrations (MLSS) in the four reactors were measured by Standard Methods for the Examination of Water and Wastewater (APHA, 1995), and were approximately 1800 mg/L. All of the reactors had a height-diameter ratio of 100/8 and were run at room temperature (20 ± 2 °C). The reactors were operated continuously in a 4-h cycle, consisting of 12 min feeding, 210 min aeration (aeration rate of 0.3 m^3/h), 1 min settling and 17 min effluent. In all the reactors the exchange volume was half of the total volume of 4 L with a hydraulic retention time of 8 h.

To observe the effects of sulfadiazine on the formation of aerobic granular sludge and microbial community in reactors, R_0 was set as the control experiment without sulfadiazine addition and since 20th day R_1–R_3 were added in different concentrations of sulfadiazine (10 ppb, 100 ppb, 1000 ppb respectively). The whole operation of reactors was divided into two stages: stage 1 (without sulfadiazine addition in the 20 days at the beginning) and stage 2 (with sulfadiazine addition in treatment groups from 20 to 90th days). During the reactors operation, the operating parameters were controlled properly: pH at 7.5–8.0, temperature at 20 ± 2 °C, solids retention time (SRT) at 20 day and dissolved oxygen at 5–6 mg/L.

2.3. Synthetic wastewater composition

The reactors were fed with synthetic wastewater in this study. Sodium acetate was used as the organic carbon source and the nitrogen source was ammonium chloride in the synthetic wastewater with 500 mg/L COD, 100 mg/L NH_4^+-N (COD/N = 5). Sodium bicarbonate was the inorganic carbon source for autotrophic bacteria and also maintained pH at 7.0–7.5. The rest parts of the synthetic wastewater were micronutrients, consisting of $NaH_2PO_4{\cdot}2H_2O$ 40 mg/L, $CaCl_2{\cdot}2H_2O$ 80 mg/L, $MgSO_4{\cdot}7H_2O$ 90 mg/L, KCl 36 mg/L, $FeSO_4{\cdot}7H_2O$ 25 mg/L, Yeast extract 1 mg/L, EDTA 3 mg/L, $FeCl_3{\cdot}6H_2O$ 0.45 mg/L, H_3BO_3 0.045 mg/L, $CuSO_4{\cdot}5H_2O$ 0.009 mg/L, KI 0.054 mg/L, $MnCl_2{\cdot}4H_2O$ 0.036 mg/L, $Na_2MoO_4{\cdot}2H_2O$ 0.018 mg/L, $ZnSO_4{\cdot}7H_2O$ 0.036 mg/L, $CoCl_2{\cdot}6H_2O$ 0.045 mg/L (Loosdrecht et al., 2016).

2.4. Analytical methods

Water samples of 100 mL were collected from reactors in effluent stage every week to determinate COD, NH_4^+-N, NO_2^--N, NO_3^--N by standard methods (APHA, 1995). Potassium dichromate method with distilled water as the blank was used to determine the concentration of COD. Nessler's reagent colorimetric method, hydrochloric acid–alpha naphthylamine method and UV spectrophotometry method were used to determine the concentration of NH_4^+-N, NO_2^--N, and NO_3^--N, respectively. Sludge samples were sampled from reactors when sludge and water were completely mixed in aeration stage. The sample volumes for mixed liquor volatile suspended solids (MLVSS) determination and sludge volume index (SVI) determination were both 100 mL. Dry weight method was used for the determination of MLVSS and SVI. Particle size distribution was determined by the sieving method (Liu et al., 2015). Measuring cylinder was used to measure settling velocity by recording the falling time that a single granule free dropped down from a certain height in water (Zheng et al., 2006). Integrity coefficient