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Response of aerobic granules and flocs on thiamethoxam inhibition. Part 1: aerobic granule cultivation and experimental study

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ABSTRACT

Aerobic granular sludge (AGS) technology is an effective tool for treating toxic wastewater because of its structural composition. Thiamethoxam (TMX), which is widely used as an insecticide on plants and animals all over the world, significantly inhibits bacterial activity during wastewater treatment. In this study, the seed sludge collected from an aeration tank was cultivated to an AGS. The performance of the AGS vs. an aerobic flocculent sludge (AFS) was compared during treatment of synthetic wastewater in the presence of TMX. Compared with AFS, AGS removed chemical oxygen demand (COD) more efficiently. The protein (PN) content was higher than the polysaccharide (PS) content for both AGS and AFS. When exposed to high concentrations of TMX, both the extracellular polymeric substances (EPS) content and the COD removal efficiency decreased more for the AFS than the AGS. Thus, the AGS has greater detoxification potential than AFS when exposed to TMX.

Keywords: Toxic inhibition; Aerobic flocculent sludge; Aerobic granular sludge; Extracellular polymeric substances; Thiamethoxam

1. Introduction

Aerobic granular sludge (AGS) technology is considered to be a promising biotechnology for wastewater treatment and has drawn increasing attention in recent years [1–4]. AGS consists of self-immobilized aggregates of microorganisms; it has a high sedimentation capacity and is resistant to impact loads and complex environmental conditions [5–7].

Thiamethoxam (3,5-disubstituted-4-nitroimino-1,3,5-oxadiazinanes) (TMX), a major class of neonicotinoid insecticide, is effective for crop protection and flea control on animals [8]. The vast majority of TMX eventually contaminates nectar and pollen by diffusing throughout plant tissues [9]. Due to its strong neurotoxicity and wide usage, TMX causes various risks and can easily enter the environment [10].

It has been shown that AGS has the ability to degrade phenol wastewater over activated sludge [5]. Rafiee et al. [11] investigated the biodegradation of 4-Chorophenol and found that AGS was resistant to the toxic inhibition of xenobiotic than suspended flocs. It has also been reported that AGS more effectively removes chemical oxygen demand (COD) than aerobic flocculent sludge (AFS) when exposed to dye-laden textile wastewater [12].

However, when differentiating between AGS and AFS, few investigations have examined the toxic inhibitory impact on extracellular polymeric substances (EPS). In addition, few studies have compared the effects of TMX inhibition in AGS vs. AFS. Therefore, the purpose of this investigation was to cultivate AGS and estimate TMX inhibition for AGS and AFS. Our results can provide useful information on the behavior of AGS and AFS when exposed to toxic substances.

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2. Materials and methods

2.1. Reactor operation

The seed sludge used in the sequencing batch reactor (SBR) was collected from an aeration tank at the Zhuzhuanjing Wastewater Treatment Plant in Hefei, China. The activated sludge was pre-cultured in the SBR at room temperature (approximately 20°C). The SBR had a working volume of 6.0 L with an internal diameter of 12.0 cm and a height of 77 cm. The mixed liquid suspended solid (MLSS) in the SBR was maintained at approximately 3.0 g L^{-1} at a pH of 7.5 ± 0.5, and the dissolved oxygen (DO) was kept at approximately 8.0 mg L^{-1}. The SBR was operated in 4 h cycles, which included 10 min of influent feeding, 225 min of aeration, 3 min of settling and 2 min of effluent withdrawal.

2.2. Synthetic wastewater

The synthetic wastewater used in the experiments included sucrose, NH_4Cl and Na_2HPO_4 as carbon, nitrogen and phosphorus sources for microbial growth, respectively. The composition of the microelement solution was based on the results of previous reports [13].

2.3. Batch experiments

The experiments were divided into two groups for AGS and AFS, respectively. To obtain a representative sample, AGS was taken from the SBR during the aeration phase and crushed using a pestle, after which it was identified as AFS. In this way, we could investigate the effect of diffusion of TMX on AGS and AFS on the same condition.

In each group, seven 250-mL Erlenmeyer flasks were used in parallel at temperatures of approximately 20°C for the batch experiments in SBR mode including four phases of influent, aeration, sedimentation, and effluent. One flask was used as a control without TMX and the other six were dosed with 50, 100, 200, 300, 400, and 500 mg L^{-1} TMX. In each experiment, the MLSS was kept at approximately 3.0 g L^{-1} and the pH was kept at approximately 7.5. The flask experiment was run for 3 h and the DO in the solutions was maintained at approximately 8.0 mg L^{-1}, using air supplied with an air compressor to each flask.

2.4. Analytical methods

2.4.1. Scanning electron microscopy (SEM)

The washed granules were placed in 10-mL bottles that contained 2.5% glutaraldehyde and fixation was executed overnight at 4°C. Subsequently, the granules were sequentially dehydrated with ethanol solutions of increasing concentration: 10%, 30%, 50%, 70%, 80%, and 90%. Finally, they were washed three times in a 100% ethanol wash. Next, the samples were dried in a freeze dryer for approximately 6 h. The samples were placed in the SEM holder and covered with gold in a sputter coating unit. They were imaged on a Scanning Electron Microscope (JEOL Ltd., Japan).

2.4.2. EPS extraction and excitation-emission matrix (EEM) fluorescence spectroscopy

The EPS from both AGS and AFS were extracted using the cation exchange resin (CER) technique (Dowex Marathon C, 20–50 mesh, sodium form) [14]. The sludge samples were centrifuged at 3,000 rpm for 15 min, after which the sludge pellets were rinsed twice in a 0.1 M NaCl solution. Next, the sludge pellets were resuspended to their initial volume using a buffer solution that consisted of 4 mM NaH_2PO_4, 2 mM Na_3PO_4, 1 mM KCl, and 9 mM NaCl at pH 7.0 [14]. After resuspension, the sludge was transferred to an extraction beaker and the CER was added at a dose of 60 g g^{-1} SS [15]. The suspensions were stirred for 12 h at 4°C. To remove the CER, the suspensions were allowed to settle for 5 min. The EPS was harvested by centrifugation for 30 min at 12,000 rpm and 4°C. The supernatant was filtered through 0.45 μm acetate cellulose membranes to acquire the EPS solution. The EPS was used for chemical and EEM fluorescence spectral analysis.

The protein (PN) content was measured using the modified Lowry Folin method [16]. The polysaccharide (PS) content was determined with the anthrone carbohydrate method [17]. Standard methods were used to measure the SS and VSS of the sludge, as well as the COD and the ammonium and phosphate concentrations [18].

The fluorescence spectroscopy of the EPS was recorded with a fluorescence spectrophotometer (F-4600, Hitachi High-Technology Corporation, Japan). To obtain the EEM spectra, emission spectra from 300 to 550 nm were obtained at sequential 0.5 nm increments by varying 10 nm increments of the excitation wavelength from 250 to 450 nm. In this study, an emission cutoff filter of 290 nm was used to remove second order Raleigh light scattering. The scanning speed was 1,200 nm/min [15]. The software MatlabR2009a (MathWorks Inc., USA) was used to process the EEM data.

3. Results and discussion

3.1. SBR performance

The SBR was operated for a total of 140 d. During the operational period, the sludge volume index (SVI_{30}) decreased from an original value of approximately 100 mL g^{-1} to 33.6 mL g^{-1} (by day 105), after which it stayed between 40 and 50 mL g^{-1}. During the operational period, the COD concentrations in the influent of the reactor varied from 607.5 to 1,495.0 mg L^{-1}, and the COD removal efficiency was 83.4%–97.9%, with an average removal efficiency of 91.9% (Fig. 1(A)). During the granulation, to rapidly cultivate AGS, COD was at high level. After that, to slow the growth of AGS, the COD concentration in the SBR decreased from about 1,500 mg/L to about 600 mg/L. The COD in effluent may consist of microbial metabolites and inert substance, such as soluble microbial products (SMP), utilization associated products (UAP), and biomass associated products (BAP) [19,20].

The NH_4^+-N concentration in the influent ranged from 47.6 to 84.0 mg L^{-1}; the NH_4^+-N removal efficiency varied from 32.8% to 63.5% with an average removal efficiency of 51.8% (Fig. 1(B)). This low removal efficiency was likely related to the short sludge retention time, meaning there were not enough nitrobacteria to remove NH_4^+-N completely [21]. As shown

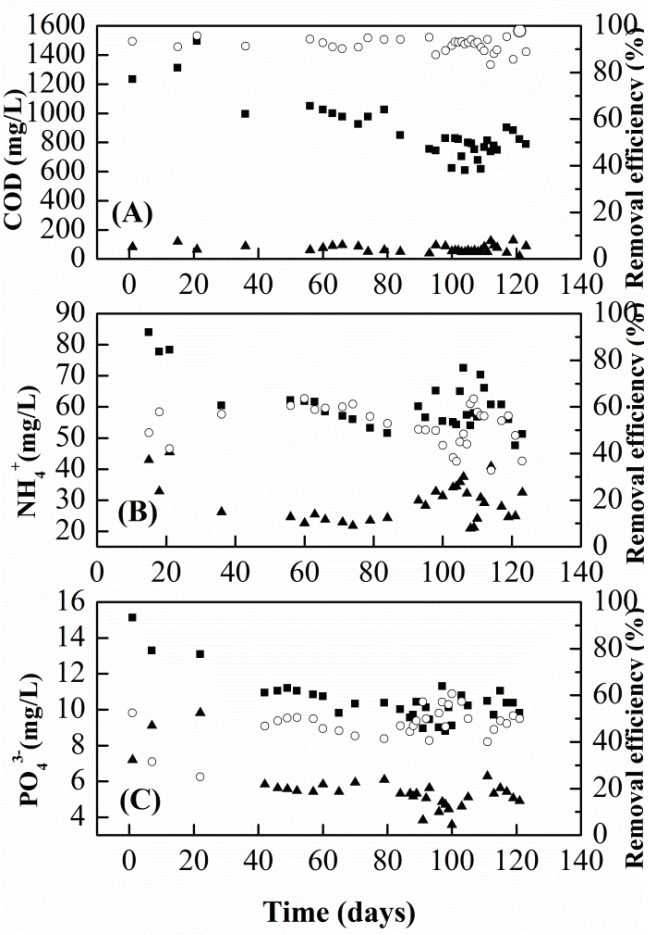

Fig. 1. Operating profiles of the SBR: (A) COD, (B) NH_4^+-N, and (C) PO_4^{3-}-P concentrations. Influent (■), effluent (▲), and removal efficiency (○) are shown.

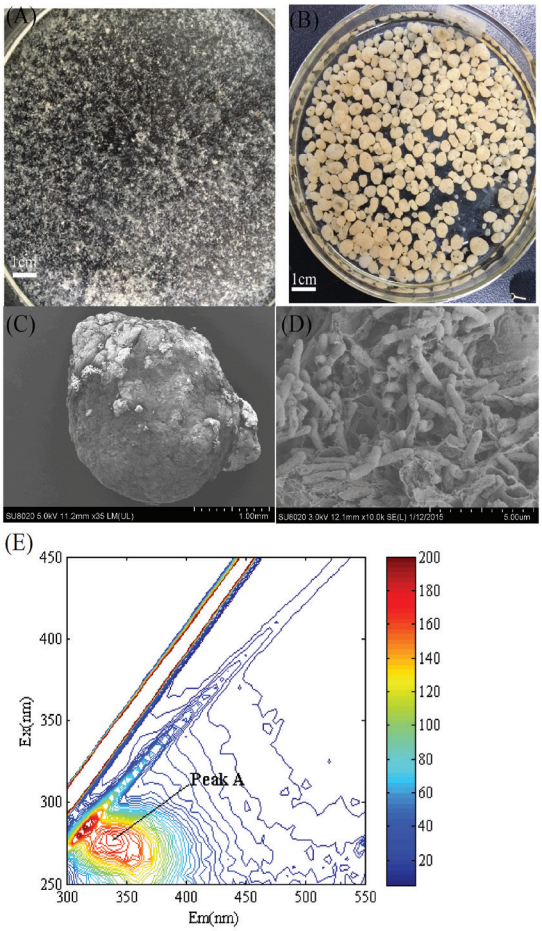

Fig. 2. Images illustrating the reaction components: (A) Seed sludge, (B) Mature aerobic granular sludge, (C) Low-magnification SEM image of AGS, (D) SEM image of a granule, and (E) EEM fluorescence spectrum for AGS-EPS.

in Fig. 1(C), the phosphate removal efficiency varied from 25.0% to 60.7% and the phosphate concentration in the influent ranged from 8.8 to 15.1 mg L^{-1}. The average phosphate removal efficiency was 47.8%. The low phosphate removal efficiency was probably related to the types of microorganisms in the SBR: there were too few phosphorus-accumulating organisms in the SBR to remove phosphate [22].

3.2. Evolution of sludge characteristics

The seed sludge used in the SBR was typical activated sludge with loose, shaggy and irregular morphology (Fig. 2(A)). After 105 d of operation, the flocculent sludge gradually took the shape of mature granules with average diameter of 3.3 mm (Fig. 2(B)). The granules were irregular spheres (Figs. 2(B) and 2(C)). High magnification SEM images reveal the cluster architecture of the AGS in detail (Figs. 2(D)). The granules consisted of bacterial populations with rod and coccus-like morphologies. Fewer filamentous bacteria were discovered at the surface of the sludge (Fig. 2(D)).

In this study, three-dimensional EEM spectroscopy was used to characterize the EPS from AGS. The EEM fluorescence spectrum of the AGS-EPS shows one peak (Fig. 2(E)). The intense region has excitation/emission wavelengths (Ex/Em) of 275–280/335–345 nm (peak A). The peak A is a protein-like peak, suggesting that the fluorescence is related to the aromatic amino acid tryptophan [23,24]. Compared with the fluorescence peak region of proteins reported previously (280–285/335–340 nm) [15], the location of peak A shows a blue shift, which could be due to a decrease in certain functional groups such as aromatic rings [13,25]. The fluorescent intensity of humic substances was not obvious and even absent in the EPS. These results suggest that protein-like substances were the major component in sludge EPS and that these play a significant role in the structure of the AGS [13].

3.3. Comparison of COD removal efficiency in the presence of TMX

In the batch experiments, COD concentrations in the influent remained at 1,000 mg L^{-1}. COD removal efficiency decreased as the TMX concentrations increased from 0 to 500 mg L^{-1} (Fig. 3). After 3 h of operation without TMX, the COD removal efficiency of AGS and AFS was 90.9% and 83.6%, respectively. With TMX concentrations ranging from 50 to 500 mg L^{-1}, the COD removal efficiency decreased from 85.2% to 57.9% for AGS and 73.2% to 18.6% for AFS.

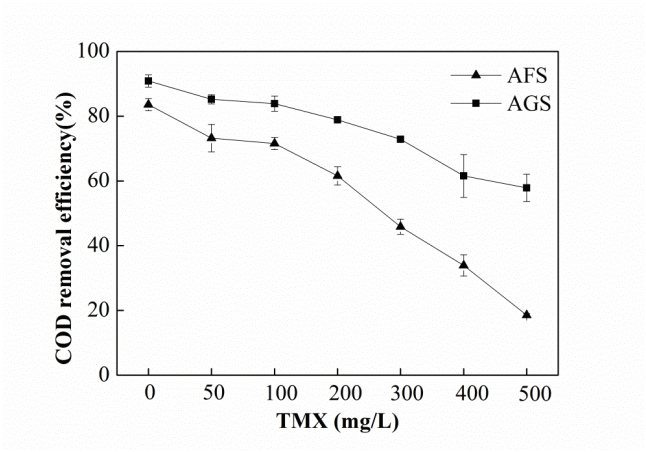

Fig. 3. COD removal efficiency of AFS and AGS during the treatment of synthetic wastewater in the presence of TMX.

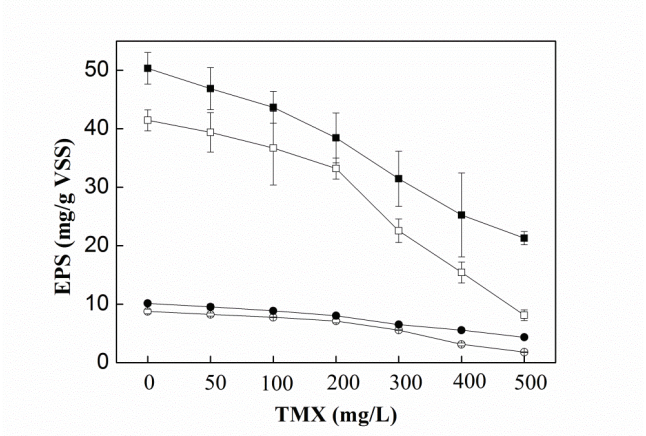

Fig. 4. Variations in EPS content during the experimental process. Profiles of PN and PS are shown as follows: PN of AGS (■) and AFS (□), PS of AGS (●) and AFS (○).

These results were consistent with previous reports [26], which showed that the COD removal efficiency decreases with increasing bisphenol A (BPA) concentration. A possible explanation for this behavior could be associated with the capability of biomass to deal with the inhibitory substrate [27]. The COD removal efficiency decreased more for AFS than for AGS as the TMX concentrations varied from 100 to 500 mg L^{-1}; this is likely related to the structure of AGS vs. AFS. Because TMX has low toxicity, low concentrations of TMX had a small effect on COD removal efficiency. However, at high concentrations of TMX, AGS, which had a larger particle size, could inhibit the diffusion of TMX, and thus protect its internal structure from toxicity. In contrast, the smaller particle size of AFS meant that it could not protect itself from toxicity. Thus, because AGS has larger particles than AFS, the diffusion resistance of AFS is lower than that of AGS, and toxic compounds could exert greater effects on AFS than AGS.

3.4. Inhibition analysis of TMX on EPS components

EPS constituted an important component, playing a significant role in sludge flocculation and granulation [13,28]. The contents of PN and PS are shown in Fig. 4. The PN and PS contents of AGS without TMX were 50.3 and 10.1 mg g^{-1} VSS, and those of AFS were 41.5 and 8.8 mg g^{-1} VSS. In the batch experiments, the PN and PS contents decreased as the TMX concentrations increased from 0 to 500 mg L^{-1}. These results suggest that TMX had an inhibitory effect on the EPS. Such changes could be related to the deterioration of the sludge at the high TMX loads. However, it has been reported that PN concentrations increase with exposure to BPA [26]. In the short batch experiments, the bacteria could not secrete enough EPS to resist the toxicity of TMX. The PN and PS contents were higher in the AGS than in the AFS; this is consistent with previous reports that more EPS occurs in granules than in flocculent sludge [28,29]. In both the AGS and the AFS, the PN contents were higher than the PS of all of the EPS for the same concentrations of TMX, which agrees with previous reports [1,13,28,30]. These results are consistent with the EEM result, which suggested that PN played an important role in EPS.

When the TMX concentrations were higher than 200 mg L^{-1}, the PN and PS decreased more in the AFS than in the AGS, which was probably related to structural differences between AGS and AFS. Due to the diffusion limitation, the size of AGS is bigger than AFS, and then the inhibition of TMX in AFS was more obvious than that in AGS when exposed to high concentration of thiamethoxam (TMX). Given the larger particle size and greater resistance to diffusion of the AGS, the AGS was better able to protect itself from toxicity.

4. Conclusions

Based on the results of this study, we draw the following conclusions:

COD was effiently removed in a SBR reactor, but NH_4^+-N and PO_4^{3-} can't be removed efficiently. According to the results of three-dimensional EEM spectroscopy and the inhibition analysis of TMX on EPS components, they showed that proteins were the major component of EPS in the AGS, and played a significant role in the structure of AGS.

AGS, in comparison with AFS, removed COD more efficiently when exposed to TMX. The contents of EPS in AGS were higher than that in AFS. When exposed to high concentrations of TMX, the inhibition of TMX was greater for AFS than for AGS.

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