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Characterization of the extracellular polymeric substances and microbial community of aerobic granulation sludge exposed to cefalexin

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ABSTRACT

This study characterizes the extracellular polymeric substances and bacterial community composition of aerobic granules exposed to cefalexin (CLX). The presence of CLX potentially decreases granular stabilities, resulting in a lowered granule diameter. Chemical oxygen demand and NH_4^+–N removal efficiencies were slightly decreased and the denitrification process was inhibited with CLX addition. Extracellular polymeric substance contents were significantly increased in aerobic granules exposed to CLX. The shifts of fluorescence intensities and peak locations in 3D-EEM fluorescence spectra indicated changes of EPS components. High-throughput sequencing analysis showed aerobic granules with CLX addition in synthetic wastewater had superior diversity of microbial species, and this was the reason that the level and components of EPS changed. The species richness for bacteria was increased from 341 to 352, which was revealed by Chao1. The Shannon index of diversity rose slightly from 3.59 to 3.73 with CLX addition. The abundance of *Proteobacteria* significantly decreased, while the abundance of *Bacteroidetes* and *Chloroflexi* underwent a highly significant increase in aerobic granules exposed to CLX.

Introduction

Pharmaceuticals are used extensively to treat diseases in humans and animals. The growing occurrence of residues of human and veterinary pharmaceuticals, such as antibiotics, in the environment is causing increasing concern (Sarmah et al., 2006). The major sources of antibiotic residues in the environment include wastewater from hospitals, households, livestock farming, and pharmaceutical factories. Wastewater can contain extremely complex mixtures of various antibiotics and other drugs, such as cefalexin (CLX), chloramphenicol (CHL), carbamazepine (CAR), ibuprofen (IBU), and naproxen (NAP) (Sui et al., 2010; Krkošek et al., 2014). These kinds of pharmaceuticals can reach wastewater treatment plants (WWTPs) from different routes and are usually detected at levels ranging from ng L^{-1} up to mg L^{-1} not only in domestic and hospital effluents but also in effluents of pharmaceutical manufacturing facilities, which can present higher levels, reaching concentrations up to the mg L^{-1} range (Lin et al., 2009; Yang et al., 2014).

Cefalexin is one of the most prescribed antibiotics and is produced in great quantities, as indicated by its frequent occurrence and high concentrations in municipal sewage treatment plant influents (Evgenidou et al., 2015). Common wastewater treatments such as conventional activated sludge systems are unable to remove pharmaceuticals and personal care products efficiently (Zhou et al., 2009). Although relatively higher removal efficiencies of CLX were observed after treatment in an activated sludge system, the presence of CLX in effluents is a clear indication that CLX cannot be removed completely in current sewage treatment plant processes (Liu et al., 2011, 2012). Previous studies have already shown that aerobic granular sludge is extremely promising for the treatment of effluents containing toxic compounds (Miao et al., 2014). Compared with conventional activated sludge, the aerobic granulation sludge is less sensitive to fluctuation than activated sludge, due to a higher tolerance to toxicity as well as a higher biomass concentration. To date, treatment of wastewater containing CLX has been reported using physical–chemical processes such as the electro-Fenton oxidation process and sorption processes (Ledezma

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Estrada et al., 2012). However, there is limited information available on the interaction of CLX and aerobic granulation sludge.

The limited literature available seems to indicate that when aerobic granulation sludge is used for removal of antibiotics, sorption is an important mechanism due to the porosity and high amount of surface area; initial adsorption onto the surface is followed by intra-particle diffusion (Shi et al., 2011). The production of EPS is a response to the stress situation. Extracellular polymeric substances (EPS) secreted by cells could contribute to the adhesion, promoting microbial aggregation and the formation of matrix structure, and enhancing the communication between cells and the stability of granules (Kong et al., 2014). Characterization of EPS contents and components of aerobic granules exposed to CLX has not been carried out previously. In addition, microbes responsible for degradation and the toxicity of CLX on those microbes should be investigated, as should the qualitative and quantitative changes to the microbial community structure when using aerobic granulation technology to remove CLX and their effect on the granules.

The aim of this study was to evaluate the removal of CLX using aerobic granulation technology. The effect of CLX on COD and nitrogen removal was investigated. Additionally, changes in EPS and the characterization of microbes in aerobic granular sludge exposed to CLX were also evaluated with three-dimensional excitation–emission matrix (3D-EEM) and high-throughput sequencing techniques, respectively.

Materials and methods

Experimental setup and operation

Two double-walled cylindrical column sequencing batch airlift reactors (SBARs) in parallel with a height of 100 cm and internal diameter of 8 cm were used in this study. The SBARs contained an internal riser with a height of 80 cm, internal diameter of 5 cm, and bottom clearance of 1.5 cm. A detailed description of the SBARs was given in Kong et al. (2013). The SBARs were operated in 6 h successive cycles with 10 min of influent filling, 310 min of aeration, and 5 min of settling, plus 5 min of effluent withdrawal and 30 min of idling, with a volumetric exchange ratio of 50%, resulting in a hydraulic retention time of 12 h.

During the study, an ambient temperature of 21 ± 3 °C was maintained. Air was introduced via a fine bubble aerator at the bottom of the reactor through a porous stone diffuser with an airflow rate of 200 L h^{-1} during the aeration phase. The operating conditions in both SBARs were the same.

Seed sludge and synthetic wastewater

Aerobic granulation sludge with an initial concentration of 3430 mg L^{-1} in mixed liquor suspended solids (MLSS) collected from SBARs were used in this research (Kong et al., 2013). The reactor (R1) to which CLX was not added in synthetic wastewater served as a control, while 20 mg L^{-1} CLX in synthetic wastewater was added to the other reactor, R2.

The composition of synthetic wastewater and trace element solutions have been described in Kong et al. (2013). The $NaHCO_3$ was dosed into the feeding solution to maintain the reactor at neutral pH between 7.0 and 7.8 throughout the experiment.

Analytical methods

Water samples were filtered by syringe nylon membrane filters (0.45 mm pore size) for removing biomass. Ammonium (NH_4^+–N), nitrite (NO_2^-–N) and nitrate (NO_3^-–N) were measured by the spectrophotometric method according to standard procedures (APHA, 2005). Chemical oxygen demand (COD) was measured regularly with a Thermo Orion COD165 (Thermo Orion, USA) and MLSS concentration was measured regularly according to standard methods (APHA, 2005). The size distribution of granule samples was analyzed using the wet sieving method as described by Laguna et al. (1999). Cefalexin concentrations were measured regularly at 268 nm using a UV spectrophotometer according to Liu et al. (2011).

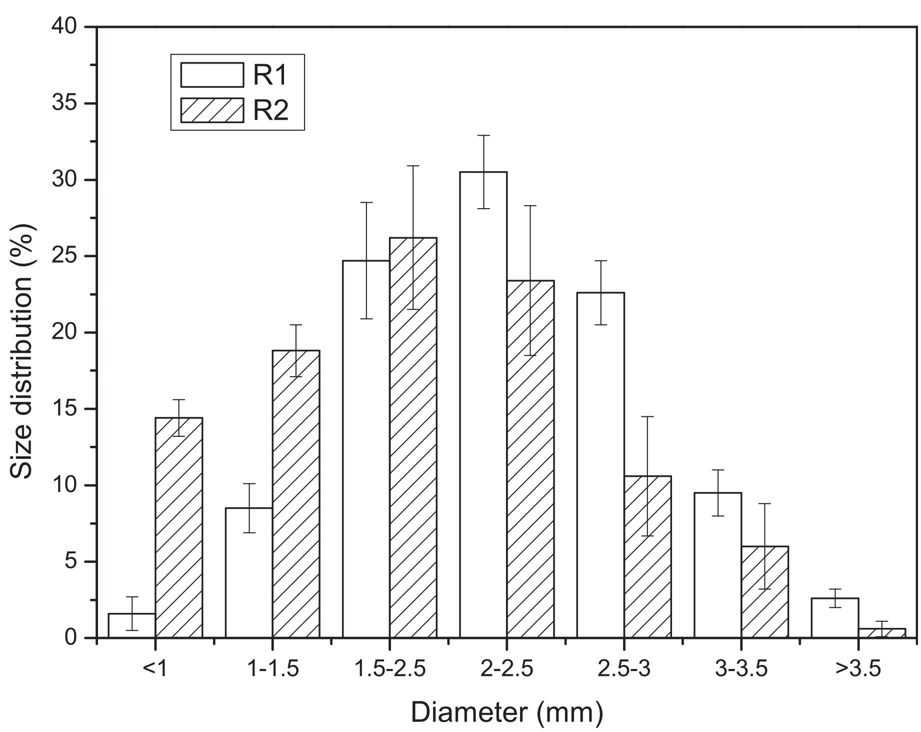

Fig. 1. Size distribution of aerobic granulation in R1 (control) and R2 (with CLX) on day 52 (n = 3).