Journal of Environmental Management 183 (2016) 594–600

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Responses of soluble microbial products and extracellular polymeric substances to the presence of toxic 2,6-dichlorophenol in aerobic granular sludge system

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ARTICLE INFO

Article history:
Received 24 May 2016
Received in revised form
4 August 2016
Accepted 3 September 2016
Available online 9 September 2016

Keywords:
Aerobic granular sludge (AGS)
2,6 -dichlorophenol (2,6-DCP)
Soluble microbial products (SMP)
Extracellular polymeric substances (EPS)

ABSTRACT

The objective of this study was to evaluate the responses of soluble microbial products (SMP) and extracellular polymeric substances (EPS) to the presence of toxic 2,6-dichlorophenol (2,6-DCP) in aerobic granular sludge (AGS) system. Batch experiment showed that NH_4^+-N removal efficiency significantly decreased from 99.6% to 47.2% in the toxic 2,6-DCP of 20 mg/L. Moreover, the inhibition degrees of 2,6-DCP on $(SOUR)_H$, $(SOUR)_{NH_4}$ and $(SOUR)_{NO_2}$ were 7.8%, 32.1% and 9.5%, respectively. The main components of SMP, including protein (PN) and polysaccharide (PS) increased from 2.3 ± 0.74 and 16.8 ± 0.12 mg/L to 66.4 ± 0.56 and 18.0 ± 0.19 mg/L in the presence of 2,6-DCP. Three-dimensional excitation-emission matrix (3D-EEM) spectroscopy identified tryptophan PN-like, humic acid-like and fulvic acid-like substances in the control SMP, and their fluorescence intensities increased after exposure to 2,6-DCP. Synchronous fluorescence spectra suggested that the fluorescence quenching between EPS and 2,6-DCP was a static quenching process. The obtained results could provide insightful information on the responses of microbial products to AGS in the presence of toxic chlorophenols.

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1. Introduction

Aerobic granular sludge (AGS) is considered as a type of self-aggregation of microbial consortium with an approximately spherical external appearance, which has been paid extensive attention in the field of wastewater treatment (Mata et al., 2015). Compared to the conventional floc sludge, AGS has the advantages of excellent settle ability, diverse microbial species and capacity for simultaneous nitrogen and phosphorus removal (Giesen, 2013). Therefore, AGS is regarded as an environment-friendly and cost-effective biotechnology for wastewater treatment (Wei et al., 2012).

Although AGS has been wildly applied to municipal and industrial wastewater treatment, the performance of AGS system is easily influenced by the presence of toxic non-degradable pollutants (Zhu et al., 2013). Chlorophenol compounds are one of toxic chemical pollutants, which can cause serious effect to biological nitrogen removal process due to their low biodegradation, high toxicity and persistence (Gao and Wang, 2007). As one typical chlorophenol compounds, 2,6-dichlorophenol (2,6-DCP) is wildly used as an intermediate in making insecticides, herbicides, preservatives, antiseptics, disinfectants and other organic compounds. It is also been applied as a chemical uncoupler for reducing excess sludge production (Zhang et al., 2013). Therefore, it is desirable to explore the performance of AGS for the treatment of wastewater containing toxic 2,6-DCP. However, there have been few reports on the performance of AGS under the toxic stress of 2,6-DCP.

Especially, soluble microbial products (SMP) and extracellular polymeric substances (EPS) are regarded as two important microbial products in sludge, which directly relate to the operational performance of AGS system (Shaw et al., 2002). SMP are a kind of components in wastewater effluents originated from substrate metabolism and biomass decay, while EPS are one of complex high-molecular-weight mixture of polymers surrounding bacterial cells (Barker and Stuckey, 1999; Ni et al., 2011; Salama et al., 2015). It is generally accepted that the presence of SMP not only degrades the quality of effluents, but also causes adversely influences on the treatment efficiency (Li et al., 2013). In contrary, EPS usually act as the protective barrier of microbial cells to resist toxic substance and

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http://dx.doi.org/10.1016/j.jenvman.2016.09.014
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stabilize the sludge structure (Ramesh et al., 2006). Therefore, when AGS was exposed to the toxic chemical compounds, SMP and EPS may play significant different roles for influencing the performance of AGS system. However, litter information is available to this point.

Based on the above discussion, the objective of this study was to evaluate the response of microbial products from AGS in the presence of toxic 2,6-DCP by using the batch experiment and spectral approach. To achieve this purpose, SMP production was demonstrated by using three-dimensional excitation-emission matrix (3D-EEM) fluorescence spectroscopy and Fourier transform infrared (FTIR) spectroscopy. The interaction between EPS and 2,6-DCP was analyzed by means of synchronous fluorescence spectroscopy. The obtained results could provide insightful information on the response of microbial products from AGS in the presence of toxic compound.

2. Materials and methods

2.1. AGS and parent reactor operation

AGS was cultivated from a laboratory-scale sequencing batch reactor (SBR) for 1 year. Activated sludge was taken from a full-scale aeration unit as seeding sludge. The detailed composition of synthetic wastewater could be found elsewhere (Li et al., 2015). Influent pH of reactor was adjusted to 7.5 by addition of $NaHCO_3$ and HCl. The working volume of SBR and operating cycle were 17 L and 6 h, respectively. Each cycle of reactor consisted of 2 min feeding, 28 min anoxic process, 300 min aerobic reaction, 5 min settling, 10 min decanting and 15 min idle. The reactor was operated at a volumetric exchange ratio of 50%, giving a hydraulic retention time (HRT) of 12 h. Dissolved oxygen (DO) concentration was maintained above 5.0 mg/L through an air diffuser placed in the bottom of reactor.

2.2. Batch experiment

Four 500 mL beakers were served as batch experimental reactors to evaluate the toxicity of 2,6-DCP to AGS. For each batch, 50 mL AGS was first added into the beaker, then 250 mL above mentioned synthetic wastewater and different volumes of predetermined 2,6-DCP stock solution were successively added into each beaker. Finally, deionized water was added into each beaker to ensure the mixed solution at 500 mL. As a result, 2,6-DCP concentrations in four beakers were 0, 5, 10 and 20 mg/L, respectively. DO concentration in each beaker was controlled at 2 mg/L. The beakers were then shaken at room temperature on a mechanical stirrer for 6 h. Samples were taken from beakers every 30 min for the determination of NH_4^+-N, NO_2^--N and NO_3^--N concentrations.

2.3. SMP extraction and spectra analysis

After batch experiment, supernatant of samples was collected and filtered through a 0.45 μm membrane and the filtrate was defined as SMP (Li et al., 2013). 3D-EEM spectra of SMP samples were measured and the range of corresponding scanning emission wavelength was set from 250 to 550 nm, while the excitation intensity was collected from 200 nm to 400 nm. A 290 nm cutoff filter was used to remove second-order Raleigh scattering for all samples.

2.4. EPS extraction and binding test

A modified heating method was used to extract EPS from AGS (Wei et al., 2015). More detailed, 10 mL AGS was first washed three times by deionized water, and then centrifuged in a 100 mL centrifugal tube at 4000 rpm for 5 min to remove the supernatant. After that, sludge pellet was re-suspended with 0.05% NaCl solution and heated at 80 °C for 1 h, and then EPS were separated from treated sludge via centrifugation of 8000 rpm for 15 min. The centrifuged supernatants were filtered through 0.45 μm acetate cellulose membranes and regarded as the EPS.

To conduct the binding test between EPS and 2,6-DCP, 0.5 mL extracted EPS was firstly added into 10 mL centrifugal tube, and then different volume of prepared 2,6-DCP solution and deionized water were added and mixed into a 10 mL tube. The test 2,6-DCP concentrations were finally controlled from 0 to 50 mg/L. Next, the mixed solutions were agitated on a shaker for 2 h for equilibrium before spectral analysis. Synchronous fluorescence spectra were measured by simultaneously scanning the excitation and emission wavelength from 250 to 360 nm with a constant offset ($\Delta\lambda$) at 60 nm.

2.5. SOUR assays

It was well reported that biological activity can be characterized by using specific oxygen uptake rates (SOUR) (Han et al., 2005). Generally, AGS includes three microbial communities, i.e. facultative and anaerobic bacteria exist in the core of the sludge, ammonium oxidizing bacteria (AOB) is in the middle and heterotrophic, aerobic bacteria grown on the outside (Gao et al., 2012). $(SOUR)_H$ by heterotrophic bacteria, $(SOUR)_{NH_4}$ by AOB, $(SOUR)_{NO_2}$ by nitrite oxidizing bacteria (NOB), were determined by using the method reported by Liu et al. (2004). The utilized substrates for determination of $(SOUR)_H$, $(SOUR)_{NH_4}$ and $(SOUR)_{NO_2}$ were $C_6H_{12}O_6$ (400 mg/L), NH_4Cl (20 mg/L) and $NaNO_2$ (20 mg/L), respectively. The detailed measurement process could be found in Supplementary material (Text S1).

2.6. Analytical methods

NH_4^+-N, NO_2^--N and NO_3^--N concentrations were measured by using a UV-spectrophotometer (TU-1901, Beijing Purkinje General Instrument Co., Ltd., China) according to the respective standard methods (APHA et al., 2005). Protein (PN) concentration was determined by using the modified Lowry method with bovine serum albumin as the standard (Frølund et al., 1995), while polysaccharide (PS) concentration was measured by using the anthrone-sulfuric acid method with glucose as the standard (Frølund et al., 1996). 3D-EEM spectra were measured by using a luminescence spectrometer (LS-55, Perkin-Elmer Co., USA). FTIR spectroscopy of SMP was recorded on a FTIR spectrometer (VERTEX70 spectrometer, Bruker Co., Germany) in the range of 4000–450 cm^{-1}.

3. Results and discussion

3.1. Toxic 2,6-DCP on the performance of biological nitrogen removal

Fig. 1 shows the effect of 2,6-DCP on the biological nitrogen removal, including the variations of NH_4^+-N, NO_2^--N and NO_3^--N concentrations. Compared with the control experiment (99.6%), NH_4^+-N removal efficiency decreased to 47.2% after exposure to 2,6-DCP of 20 mg/L. As a result, the effluent concentrations of NO_x^--N exhibited a significant difference. Meanwhile, an evident nitrite peak (23.26 mg/L) was observed at 4.5 h without the addition of 2,6-DCP, whereas no NO_2^--N accumulation appeared in the AGS system in the presence of different 2,6-DCP concentrations.

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