Chemical Engineering Journal 251 (2014) 165–174

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

Effect of hexavalent chromium on extracellular polymeric substances of granular sludge from an aerobic granular sequencing batch reactor

Zichao Wang [a], Mengchun Gao [a,b,*], Sen Wang [a], Yanjun Xin [c], Dong Ma [c], Zonglian She [a], Zhe Wang [b], Qingbo Chang [b], Yun Ren [b]

[a] Key Lab of Marine Environment and Ecology, Ministry of Education, Ocean University of China, Qingdao 266100, China
[b] College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China
[c] College of Resource and Environment, Qingdao Agricultural University, Qingdao 266109, China

HIGHLIGHTS

- PN, PS and PN/PS ratios in LB-EPS and TB-EPS increase with addition of Cr(VI).
- FTIR showed functional groups of PN and PS in EPS clearly varied.
- 3D-EEM showed LB-EPS and TB-EPS contained protein- and humic acid-like substances.
- XPS results verified the presence of Cr(III) in LB-EPS and TB-EPS.
- RH showed a better linear correlation with PN/PS ratio in TB-EPS than in LB-EPS.

ARTICLE INFO

Article history:
Received 3 January 2014
Received in revised form 9 April 2014
Accepted 18 April 2014
Available online 26 April 2014

Keywords:
Extracellular polymeric substances
Hexavalent chromium
Three-dimensional excitation–emission matrix fluorescence spectroscopy
Fourier transform infrared spectroscopy
X-ray photoelectron spectroscopy

ABSTRACT

The effect of hexavalent chromium (Cr(VI)) on extracellular polymeric substances (EPS) of granular sludge was investigated in an aerobic granular sequencing batch reactor (GSBR). With the increase of Cr(VI) concentration from 0 to 30 mg L^{-1}, the polysaccharide (PS) in loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) increased from 1.68 to 5.05 mg g^{-1} VSS and 1.89 to 7.57 mg g^{-1} VSS, respectively, and the protein (PN) in LB-EPS and TB-EPS increased from 11.57 to 38.86 mg g^{-1} VSS and 12.76 to 62.98 mg g^{-1} VSS, respectively. The PN/PS ratios in LB-EPS and TB-EPS increased from 6.90 to 7.69 and 6.75 to 8.32, respectively, with the increase of Cr(VI) concentration from 0 to 30 mg L^{-1}. The five peaks identified by three-dimensional excitation–emission matrix (3D-EEM) fluorescence spectroscopy in LB-EPS and TB-EPS were attributed to protein-like and humic acid-like substances. Fourier transform infrared (FTIR) spectra indicated that the increase of Cr(VI) concentration had distinct effects on the functional groups of PN and PS in LB-EPS and TB-EPS. The X-ray photoelectron spectroscopy (XPS) results showed that a portion of Cr(VI) was reduced to Cr(III). The relative hydrophobicity (RH) of the granular sludge exhibited a better linear correlation with the PN/PS ratio in TB-EPS ($R^2 = 0.9782$) than that in LB-EPS ($R^2 = 0.9172$).

1. Introduction

Aerobic granular sludge is a novel technology for the biological treatment of wastewater, which has a stronger microbial structure, higher biomass retention and better sludge settleability than conventional activated sludge. In particular, the aggregations of microorganisms into compact aerobic granules provide them with resistance to damage from toxic substances, such as heavy metals [1]. Extracellular polymeric substances (EPS), which are secreted by microorganisms, play a major role in maintaining the compactness of granular sludge through complex multivalent cations and hydrophobic interactions [2,3]. Therefore, it is essential to understand the characteristics of EPS under the stress of exposure to heavy metals in the granular sludge system.

EPS are composed of polysaccharide (PS), protein (PN), small amounts of nucleic acids, lipids and other polymeric compounds, which have been observed outside the cell surface and in the intercellular space of microbial aggregates. EPS exhibit a dynamic double-layered structure, which consists of loosely bound EPS

* Corresponding author. Address: College of Environmental Science and Engineering, Ocean University of China, No. 238 Songling Road, Qingdao, Shandong Province 266100, China. Tel.: +86 532 66781061; fax: +86 532 66782810.
E-mail address: mengchungao@hotmail.com (M. Gao).

http://dx.doi.org/10.1016/j.cej.2014.04.078
1385-8947/

(LB-EPS) and tightly bound EPS (TB-EPS). The production and composition of EPS can be affected by many influencing factors, such as substrate type [4], nutrient content [5], solid retention time [6], shear rate of reactor [7], salinity [8] and heavy metal [9]. Among the above-mentioned influencing factors, heavy metal is regarded as one of the key factors due to the significant toxicity of heavy metal on microorganisms. Hexavalent chromium (Cr(VI)) is one of the common heavy metal pollutants in the effluents from many industries, such as leather tannery, electroplating and textiles, and is a well-known carcinogen that imparts lethal effects on the metabolism of almost all microbial strains (heterotrophic and autotrophic), protozoa and metazoan [10–12]. The change of microbial metabolism could cause the variation of EPS production and composition. Aquino and Stuckey [13] found that the biomass produced more EPS with the addition of Cr(VI), which might help them to cope with the stress of Cr(VI) in an activated sludge system. Sheng et al. [14] demonstrated that the addition of Cr(VI) stimulated the production of EPS produced by *Rhodopseudomonas acidophila* strain, and the PN/PS ratio of EPS in the presence of Cr(VI) was always higher than that under free Cr(VI). Ozturk and Aslim [15] illustrated that EPS secreted by *Chroococcus* sp. H4 increased with the increase of Cr(VI). They found that EPS were composed predominantly of glucose (99%) and a very small amount of galacturonic acid (1%) in the absence of Cr(VI) exposure, and EPS were composed primarily of xylose (75%) and small amounts of glucose (9%), rhamnose (14%) and galacturonic acid (2%) in the presence of 10 mg L^{-1} Cr(VI). Although some researchers have reported the effect of Cr(VI) on EPS production and component in pure cultures and activated sludge, as far as we know, little information has been found on evaluating the effect of Cr(VI) on PN and PS content, chemical compositions in LB-EPS and TB-EPS of granular sludge from an aerobic GSBR for a long-term operation, as well as analyzing the relationship between PN/PS ratio in EPS and relative hydrophobicity (RH) of granular sludge under different influent Cr(VI) concentrations.

The objectives of this study were (1) to evaluate the variation of the PN and PS contents in the LB-EPS and TB-EPS of granular sludge with the increase of Cr(VI) from 0 to 30 mg L^{-1} in an aerobic granular sequencing batch reactor (GSBR); (2) to analyze the effect of Cr(VI) on the composition of LB-EPS and TB-EPS using three-dimensional excitation–emission matrix (3D-EEM) fluorescence spectroscopy, Fourier-transform infrared (FTIR) spectroscopy and X-ray photoelectron spectroscopy (XPS); and (3) to determine the respective relationship of the PN/PS ratios in LB-EPS and TB-EPS with the granular sludge RH under different Cr(VI) concentrations.

2. Materials and methods

2.1. Reactor set-up and operation

A lab-scale plexiglass aerobic granular sequencing batch reactor (GSBR) with a working volume of 5.7 L was used in the experiment. The GSBR had an internal diameter of 9 cm and an effective height of 90 cm. A peristaltic pump was used to feed the influent into the reactor. The effluent was drawn at a height of 45 cm from the bottom by a solenoid valve, and the volume exchange rate for every cycle was 50%. Air was supplied by an air diffuser at the bottom of the reactor with an airflow rate of 2 L min^{-1}. The aerobic GSBR was operated in a 6 h cycle. One cycle consisted of 0.2 h of influent addition, 5.6 h of aerobic stage, 0.1 h of settling and 0.1 h of effluent withdrawal. The system was operated at room temperature (20–30 °C).

2.2. Seed sludge and wastewater composition

The seed sludge was obtained from the recycled sludge of the secondary clarifier in Licunhe municipal wastewater treatment plant in Qingdao City, China. The initial mixed liquor suspended sludge (MLSS) in the GSBR was 3270 mg L^{-1}. The composition of synthetic wastewater was as follows (mg L^{-1}): glucose, 1536; NH_4Cl, 240; KH_2PO_4, 79; $MgSO_4$, 100; KCl, 20; $CaCl_2$, 50; $K_2Cr_2O_7$ from 57 to 170 (corresponding to Cr(VI) concentrations from 10 to 30 mg L^{-1}); and 0.3 ml L^{-1} trace element solution. The trace element solution contained (g L^{-1}) [16]: $ZnSO_4$, 0.12; $CoCl_2$, 0.15; $FeCl_3$, 1.5; H_3BO_3, 0.15; $CuSO_4$, 0.03; KI, 0.18; $MnCl_2$, 0.12; Na_2MoO_4, 0.06; and EDTA, 10.

2.3. Conventional index analysis

The measurements of chemical oxygen demand (COD), NH_4^+-N, NO_2^--N, NO_3^--N, MLSS, Cr(VI), Cr(III), total chromium and mixed liquor volatile suspended solids (MLVSSs) were performed according to the Chinese NEPA standard methods [17].

2.4. Morphology observation of granular sludge

The morphology of granular sludge was observed using a digital camera, fluorescence microscope (CKX41-A32PH, Olympus, Japan) and scanning electron microscope (SEM, S3400N, Hitachi, Japan). The granular samples examined by SEM observation were first washed with a phosphate buffer and fixed with 2% glutaraldehyde for 2 h at 4 °C. These fixed granules were washed with a phosphate buffer and dewatered with a graded ethanol series (30%, 50%, 70%, 80%, 90% and 100%). The dewatered samples were then dried using a critical point dryer. The samples were further sputter coated with gold for SEM observation.

2.5. EPS extraction and analysis

The EPS of the sludge samples were extracted and analyzed when the GSBR was operated at steady-state conditions under different Cr(VI) concentrations. A heat extraction method [4] was used to extract LB-EPS and TB-EPS from the granular sludge samples. The PN content in the LB-EPS and TB-EPS extractions was measured according to the Lowry method [18]. The PS content in the LB-EPS and TB-EPS extractions was determined using the anthrone–sulfuric acid method [19].

2.5.1. 3D-EEM fluorescence spectroscopy

The 3D-EEM spectra of the LB-EPS and TB-EPS extractions were measured using a luminescence spectrometry (F-4600 FL Spectrophotometer, Hitachi, Japan), and the corresponding scanning emission spectra from 200 to 500 nm at 5 nm increments were obtained by varying the excitation wavelength from 200 to 400 nm at 5-nm sampling intervals. The excitation and emission slits were kept at 10 nm, and the scanning speed was maintained at 1200 nm min^{-1} for all the measurements. The spectrum of deionized Milli-Q water (Millipore, USA) was recorded as the blank. Origin 8.1 software (Origin Lab, USA) was used to process the EEM data plotted as the elliptical shape of contours.

2.6. FTIR spectroscopy

The LB-EPS and TB-EPS extractions were completely dried to powder using a freeze dryer. After freeze drying, the LB-EPS (or TB-EPS) samples and dried KBr were mixed at a ratio of 1:100 and homogenized in an agate grinder. The mixture was then compressed and analyzed using a Tensor 27 FTIR spectrometer (Bruker Optics, Ltd., Germany). The FTIR spectra were recorded on KBr pellets between 4000 and 400 cm^{-1}.