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Spectroscopic characterization of extracellular polymeric substances from a mixed culture dominated by ammonia-oxidizing bacteria

Cuiqin Yin [a,b], Fangang Meng [a,b,*], Guang-Hao Chen [a,c,*]

[a] SYSU-HKUST Research Center for Innovative Environmental Technology, School of Environmental Science and Engineering, Sun Yat-sen University, Guangzhou 510275, China

[b] Guangdong Provincial Key Laboratory of Environmental Pollution Control and Remediation Technology (Sun Yat-sen University), Guangzhou 510275, China

[c] Department of Civil and Environmental Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China

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ABSTRACT

Extracellular polymeric substances (EPS) of aerobic (AerAOB) and anaerobic ammonium-oxidizing bacteria (AnAOB) are expected to have a significant impact on the performance of autotrophic nitrogen removal in engineered systems. However, there are a few investigations of the EPS of AerAOB and AnAOB, and the results are contradictory. In this study, photometric measurements indicated that the EPS of AerAOB- (31.74 ± 1.48 mg/g-VSS, volatile suspended solids) and AnAOB-enriched cultures (30.12 ± 1.52 mg/g-VSS) contained more polysaccharides than did conventional activated sludge from a municipal wastewater treatment facility (10.76 ± 0.83 mg/g-VSS). In addition, the EPS of the AnAOB-enriched culture was dominated by proteins, leading to a considerably higher protein/polysaccharide ratio (2.64 ± 0.12) than those of the AerAOB-enriched culture (0.56 ± 0.03) and conventional activated sludge (1.96 ± 0.09). Characterization using Fourier transform infrared spectroscopy (FTIR) revealed the dominance of amide bands and/or polysaccharide-associated bands in the EPS of AnAOB and AerAOB. These results corroborate the data from the photometric measurements. In addition, the EPS of AnAOB (23.1% ± 1.2%) and AerAOB (21.9% ± 1.1%) had a higher portion of α-helices, which is the key protein secondary structure that determines flocculation or cell aggregation, in the amide I band than that of activated sludge (16.7% ± 0.8%). X-ray photoelectron spectroscopy (XPS) characterization also revealed significantly different functionalities among the EPS of the three mixed cultures; e.g., O-(C,H), which indicates the presence of polysaccharides, was richer in the EPS of AerAOB, whereas protonated amines, which are commonly found in amino acids and amino sugars, accounted for a large portion of the EPS of AnAOB. The results of this study can potentially expand our knowledge of the microbial aggregates responsible for autotrophic nitrogen removal.

* *Corresponding authors*. SYSU-HKUST Research Center for Innovative Environmental Technology, School of Environmental Science and Engineering, Sun Yat-sen University, Guangzhou 510275, China.
E-mail addresses: mengfg@mail.sysu.edu.cn (F. Meng), ceghchen@ust.hk (G.-H. Chen).
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1. Introduction

Over the past several decades, autotrophic nitrogen removal has been increasingly applied for the treatment of nitrogen-rich wastewaters (Mulder et al., 2001; Sliekers et al., 2002; Windey et al., 2005). The autotrophic nitrogen removal is mainly achieved by aerobic ammonium-oxidizing bacteria (AerAOB) and anaerobic ammonium-oxidizing bacteria (AnAOB). AerAOB partially convert ammonium nitrogen into nitrite via nitritation, whereas AnAOB convert the remaining ammonium with nitrite into nitrogen gas through anaerobic ammonium oxidation (ANAMMOX) reactions (Windey et al., 2005). Indeed, the autotrophic nitrogen removal processes have shown great advantages over conventional nitrification–denitrification processes with respect to high nitrogen removal rates (Tang et al., 2011; Tsushima et al., 2007) and no requirement for organic carbon sources (Van der Star et al., 2007). However, autotrophic nitrogen removal processes are limited by the very slow growth rates of both AerAOB (doubling time is approximately 8 h to several days (Chen et al., 2013a)) and AnAOB (doubling time is as long as 11–20 days (Strous et al., 1998)).

Currently, most of the AerAOB and AnAOB exist in the form of sludge flocs (Jeanningros et al., 2010; Trigo et al., 2006), biofilms (Egli et al., 2001; van den Akker et al., 2010; Zekker et al., 2012) and anaerobic granules (Tang et al., 2009). Content and compositions of extracellular polymeric substances (EPS) are of great importance to the formation of these bacterial aggregates and related with their performance in reactors. On one hand, a high EPS level would benefit the formation and stabilization of granular sludge (Liu et al., 2009; Liu and Tay, 2002; Pol et al., 2004; Tang et al., 2011) because it can support bacterial surface adhesion and cell aggregation (Vlaeminck et al., 2010; Zhang et al., 2011). On the other hand, a high content of EPS could result in poor reactor performance, e.g., increased rate of membrane fouling in membrane bioreactors (MBRs) (Jiang et al., 2013; Wang et al., 2012). In addition, mass transfer could be a rate-limiting step for nitrogen removal (Hwang et al., 2010; Manz et al., 2003) due to the high density and abundance of EPS in the granules or biofilms (Sheng et al., 2010; Vlaeminck et al., 2010). Hence, the reactor performance would rely upon the amount and characteristics of the EPS in the microbial aggregates responsible for autotrophic nitrogen removal. A better understanding of EPS production in autotrophic nitrogen-removing sludge is necessary for optimizing reactor performance.

To date, a few studies have mentioned the EPS content in AnAOB- or AerAOB-dominated mixed culture. For instance, Tang et al. (2011) reported that the EPS from AnAOB granules contained 164.4 ± 9.3 mg/g-VSS of proteins and 71.8 ± 2.3 mg/g-VSS of polysaccharides, respectively, with a protein/polysaccharide ratio of 2.3. Recently, Jiang et al. (2013) reported high protein/polysaccharide ratios (6.6 and 10.1 on two different operating days) the EPS from AnAOB-dominated sludge in an ANAMMOX-based MBR. In comparison, the EPS from AerAOB seemed to have a higher content of polysaccharides than that of proteins (Shen et al., 2014; Zhang et al., 2011); particularly the polysaccharide/protein ratio of EPS increased significantly with increasing nitritation loading rates of AerAOB (Shen et al., 2014). However, contradictory results were also obtained, e.g., a previous study by Liang et al. (2010) reported that the EPS from AerAOB-enriched culture contained higher proteins than polysaccharides. In fact, the contradictory results of various investigations could be due to the different methods used for the extraction of EPS and the different protocols used for the qualification of polysaccharides and proteins (Chen et al., 2013b; Liang et al., 2010). Thus, a comparative study of EPS from different cultures should be conducted based on given extraction and measurement protocols. The aim of the present study is to characterize the EPS in the sludge of two such reactors, i.e., one lab-scale ANAMMOX reactor and one lab-scale nitritation membrane bioreactor.

EPS can be excreted from microorganisms, which are mainly composed of proteins and polysaccharides (Comte et al., 2006; Frølund et al., 1996). The physicochemical characteristics of EPS are complex and are greatly related to different functional groups, such as carboxyl, hydroxyl, phosphate and amide groups (Liu and Fang, 2002; Phoenix et al., 2002). In recent years, X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), fluorescence spectroscopy and nuclear magnetic resonance (NMR) have been used to determine these functional groups or element compositions in EPS (Badireddy et al., 2008; Sheng et al., 2006). With these analytical methods, considerable information regarding the functional and structural groups of EPS extracted from AerAOB- or AnAOB-enriched culture can be obtained. In this study, XPS and FTIR will be extensively applied, while a detailed analysis of the amide I region in EPS will also be conducted based on the decomposition of protein secondary structures. In addition, the EPS in sludge collected from a municipal wastewater treatment facility will be analyzed as a reference of a non-autotrophic nitrogen-removing mixed culture.

2. Materials and methods

2.1. Source of the mixed culture

The AnAOB-enriched mixed culture was obtained from a lab-scale ANAMMOX reactor fed with nitrite and ammonium (Meng et al., 2014), with a ratio of ammonium nitrogen to nitrite nitrogen of approximately 1. The reactor was operated using a hydraulic retention time (HRT) of 12 h. The pH of the feed wastewater was maintained at 7.5 ± 0.3. The temperature of the reactor was maintained at 33 ± 2 °C. The reactor was continuously operated for approximately 400 days, and it finally reached a nitrogen removal rate of approximately 1.6 kg-N/(m^3·d). The AnAOB-enriched mixed culture was composed of *Candidatus* Kuenenia-like species, *Candidatus* Jettenia-like species and *Candidatus* Brocadia-like species (Meng et al., 2014). EPS of suspended sludge from the reactor collected on three different days during the continuous operation was extracted.

The AerAOB-enriched mixed culture was collected from another lab-scale nitritation membrane bioreactor that treated synthetic wastewater containing $(NH_4)_2SO_4$, $NaHCO_3$ and trace elements (Shen et al., 2014). In brief, this reactor was