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Characterization of EPS compositions and microbial community in an Anammox SBBR system treating landfill leachate

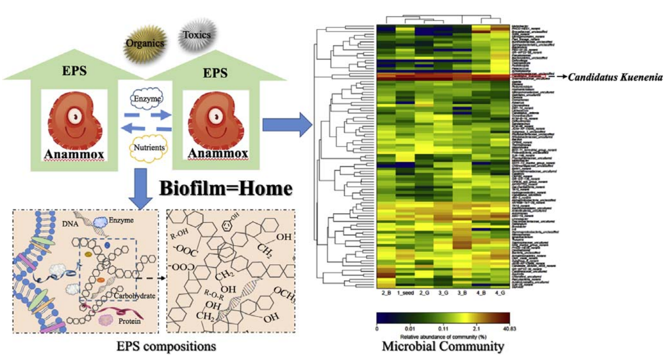
Lei Miao[a,b], Qiong Zhang[a], Shuying Wang[a], Baikun Li[a,c], Zhong Wang[a], Sujian Zhang[a], Man Zhang[a], Yongzhen Peng[a,*]

[a] *National Engineering Laboratory for Advanced Municipal Wastewater Treatment and Reuse Technology, Engineering Research Center of Beijing, Beijing University of Technology, Beijing, PR China*

[b] *School of Environmental Science and Engineering, Huazhong University of Science and Technology, Wuhan, PR China*

[c] *Department of Civil and Environmental Engineering, University of Connecticut, Storrs, CT, USA*

GRAPHICAL ABSTRACT



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ABSTRACT

The biofilm system is beneficial for Anammox process designed to treat landfill leachate. In this study, the composition of extracellular polymeric substances (EPS) and the microbial community in an Anammox biofilm system were analyzed to determine the functions driving the biofilm's ability to treat landfill leachate. The results demonstrated that increasing influent carbon oxygen demand (COD) could stimulate EPS production. EPS helped enrich Anammox bacteria and supplied them with nutrients and enzymes, facilitating effective nitrogen removal (approximately 95%). The variation in Anammox bacteria was similar to the variation in EPS composition. In the tested Anammox Sequencing Biofilm Batch Reactor (SBBR) system, *Candidatus Kuenenia* was dominant among known Anammox genus, because of its high substrate affinity and because it adapts better to landfill leachate. The relative abundance of *Candidatus Kuenenia* in the biofilm rose from 3.26% to 12.38%, illustrating the protection and enrichment offered by the biofilm in carrying out Anammox.

1. Introduction

Mature landfill leachate generally contains high concentration of ammonia (usually > 1000 mg N/L) and a low chemical oxygen demand (COD) to total nitrogen (TN) ratio (usually < 3) (Renou et al., 2008). This makes the leachate difficult to treat with a traditional nitrification-denitrification process. Anaerobic ammonium oxidation (Anammox) effectively treats mature landfill leachate, because it does

* Corresponding author.
E-mail address: pyz@bjut.edu.cn (Y. Peng).

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not require external carbon sources (Sri shalini and Joseph, 2012; Miao et al., 2015). However, biodegradable organics in mature landfill leachate can facilitate the growth of denitrifying bacteria, which then compete with Anammox bacteria for the organics. This significantly affects Anammox efficiency (Ni et al., 2012; Tang et al., 2010). Molinuevo et al. (2009) found that influent COD concentrations greater than 292 mg/L inhibited Anammox bacteria activity. A COD concentration above 150 mg/L adversely affected Anammox when treating landfill leachate using a three-stage Anammox system (Miao et al., 2014). Because Anammox process produces a small amount of nitrate, the nitrogen removal efficiency could be improved through the combination of Anammox and denitrification at certain COD concentrations (Li et al., 2015). Furthermore, Anammox bacteria grow more slowly than denitrifying bacteria (Miao et al., 2014). Therefore, it is important to control the influent COD concentration to accelerate Anammox while inhibiting denitrification.

A Sequencing Biofilm Batch Reactor (SBBR) refers to a Sequencing Batch Reactor (SBR) coupled with biofilms that grow on packing media. SBBR is particularly efficient at protecting slowly growing microorganisms from being washed out (Woolard, 1997). Biofilms also act as a buffer, reducing the concentration of toxic substances in bulk wastewater, and stabilizing the treatment of landfill leachate containing low biodegradable organics and recalcitrant compounds (Wen et al., 2016). One Anammox SBBR system has been successfully operated to treat mature landfill leachate with ammonia and COD concentrations of 3000 mg N/L and 3000 mg/L, respectively (Miao et al., 2016). This SBBR system improved nitrogen removal efficiency to 95% and could tolerate high concentrations of influent COD. The good nitrogen removal performance benefited from the biofilm protection. Compared with traditional activated sludge processes, the SBBR has a stronger resistance to adverse impact and a more stable ecological system for treating landfill leachate (Nicolella et al., 2000; Xiao et al., 2015). The important role of biofilm in wastewater treatment system elevate the need to study the mechanisms by which it works.

The major components of biofilm are the microorganisms and the extracellular materials, known as extracellular polymeric substances (EPS). EPS are produced by the microorganisms embedded in biofilms (Flemming and Wingender, 2010). Essentially, EPS are complex high molecular-weight extracellular polymers, and mainly include polysaccharides, proteins, nucleic acids, and humic acids (Zhang et al., 2014). EPS play an important role in microbial adhesion and aggregation, and benefit the formation and stability of microbial community structure (Flemming and Wingender, 2010). Usually, the EPS of sludge are divided into three parts: soluble EPS (S-EPS), loosely-bound EPS (LB-EPS), and tightly-bound EPS (TB-EPS). The LB-EPS and TB-EPS are the dominant components of total EPS. Each EPS fraction contains different components and represents different chemical properties (Wei et al., 2017). LB-EPS and TB-EPS contribute to microbial adhesion and aggregation. In particular, TB-EPS exhibits a high flocculating capability and bridges cells together in clusters (Pellicer-Nacher, et al., 2013). EPS also support microbial survival in many ways (Li and Yang, 2007; Zheng et al., 2016). Therefore, EPS are significant components of SBBR systems.

The amounts and constituents of EPS may respond to different physical and biological conditions (Shammi et al., 2017), affecting treatment performance. Miqueleto et al. (2010) found that the type of carbon source and a high C/N ratio favored EPS production in an anaerobic sequencing batch biofilm reactor fed with synthetic wastewater. Jin et al. (2014) evaluated EPS variation during bio-augmentation treatment; activating additional sludge led the Anammox consortia to secrete more EPS. These outcomes highlight the need to study how EPS variations are influenced by landfill leachate characteristics in the biofilm system. Furthermore, microbial communities can also impact EPS components and characteristics, and should be analyzed to better understand the correlation of biofilm function and microbial community structure.

Characterizing EPS compositions and variations could help explain the potential drivers behind biofilm functions. Most recent EPS studies have used synthetic wastewater, and have focused on EPS production and impacts on sludge aggregation and bioflocculability (Liang et al., 2010; Jin et al., 2014; Zhang et al., 2014; Meng et al., 2016). Zhang et al. (2014) evaluated EPS in biofilm and suspended sludge fed with synthetic wastewater; that study showed that aggregation potential mainly depended on the EPS for S-sludge cells and on the cells themselves for biofilm cells. Liang et al. (2010) investigated the extraction and structural characteristics of EPS in autotrophic nitrifying biofilm and activated sludge using synthetic wastewater. They found that the quantities of proteins, carbohydrates, humic substances, and DNA significantly varied in different layers, but dominated the TB-EPS. However, no known research studies have explored EPS composition in the biofilms used to treat landfill leachate and the relationships among the EPS, microbial community, and nitrogen removal performance.

In this study, the variation and composition of EPS in an Anammox SBBR system (containing both biofilms and floccular sludge) treating landfill leachate were analyzed to describe the effect of EPS on nitrogen removal performance. The study discusses the variations in EPS components and the microbial community structure during different system start-up periods. Characterizing EPS compositions and microbial communities can help demonstrate the biofilm's function in the Anammox system when treating landfill leachate.

2. Materials and methods

2.1. Experimental setup and operational procedure

A two-stage Anammox SBBR system, comprised of a nitritation SBR (SBRni) and an Anammox SBBR (ASBBR), was used in this study. The exchange volumetric rates of SBRni and ASBBR were both 50% (Miao et al., 2016). The start-up of the Anammox SBBR system had four periods (Table 1). During Period 1, the inoculas were added into ASBBR, and synthetic wastewater with low concentrations of nitrite and ammonium was used to make the inocula adapt to the new environment. The inoculums of ASBBR were in a mixture of granular and flocculent forms and the MLSS was 2000 ± 100 mg/L after inoculation. During Period 2, the carriers with a packing ratio of 10% were added into ASBBR to form the biofilm. To promote biofilm culturing, synthetic wastewater with high concentrations of nitrite and ammonium was used as influent for the ASBBR. During Period 3 and 4, the effluent of SBRni and real mature landfill leachate, respectively, were gradually added into ASBBR. The operational temperatures for the SBRni and ASBBR were maintained at 25 and 35 °C, respectively, across all periods using a temperature controller.

Table 1
Experimental procedure.

Experimental Phases	Influent Composition of ASBBR	Substances Concentrations of ASBBR influence (mg/L)		
		NH_4^+	NO_2^-	COD
Period 1	synthetic wastewater	300–450	400–600	—
Period 2	synthetic wastewater	500–1000	600–1400	—
Period 3	The mixture of synthetic wastewater and effluent of SBRni	300–600	400–800	100–200
Period 4	The mixture of raw mature landfill leachate and effluent of SBRni	200–350	250–450	500–1000