Journal of Environmental Management 173 (2016) 49–54

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Research article

Role and influence of extracellular polymeric substances on the preparation of aerobic granular sludge

Shuang Deng, Luxi Wang, Haijia Su*

Beijing Key Laboratory of Bioprocess, Beijing University of Chemical Technology, Beijing 100029, PR China

ARTICLE INFO

Article history:
Received 25 December 2015
Received in revised form
4 March 2016
Accepted 5 March 2016
Available online 11 March 2016

Keywords:
Aerobic granular sludge
Extracellular polymeric substances
Chemical oxygen demand
Sludge volume index
Food-to-microorganism

ABSTRACT

Due to the important role of the extracellular polymeric substances in the formation of aerobic granular sludge, the variation of the EPS contents in the process of cultivation and that in the one running cycle time were studied in this work. Aerobic granules with diameters between 0.8 and 1.1 mm were obtained within 30–35 days. The results suggested that the increase of EPS contents significantly contributed to the formation of aerobic granules. A linear relationship between the EPS and SVI was also developed, and it revealed that the aerobic granules had good settling property when the EPS exceeded 200 mg/g MLVSS. Two mainly components of EPS, protein (PN) and polysaccharides (PS), could act as the endogenous food for the microbes during the starvation period. The survival of the microbial population was jeopardized when the F/M ration was below 0.5 g COD/g SS d.

1. Introduction

The application of aerobic granular sludge is currently viewed as one of the promising wastewater treatment technologies, and has been extensively applied to treat the heavy metals wastewater (Liu et al., 2015), printing dye wastewater (Franca et al., 2015), sewage wastewater (Pronk et al., 2015), high-strength food wastewater (Jang et al., 2015), slaughterhouse wastewater (Pijuan et al., 2011), anilines wastewater (Dai et al., 2015) and others. An important priority and challenge remains how to rapidly cultivate the aerobic granules (Vymazal, 2011). The cultivation mainly could be associated with one or more of the characteristics of aerobic granules such as settling properties (Quan et al., 2015), microbial structure and diversity (Quan et al., 2015), stability (Liu and Liu, 2006; Zhang et al., 2015), and extracellular polymeric substance (EPS) content. It had also been reported that the addition of calcium, magnesium (Ni et al., 2013), poly aluminum chloride (Liu et al., 2014) and other metal ions with coagulation properties had produced a great increase in the amount of EPS, which accelerated the process of microbial aggregating and formation of the aerobic granules.

A good definition of composition and properties of EPS in the aerobic granular sludge would help in a better understanding of the formation of aerobic granules. Extracellular polymeric substances secreted by microbes during the growth and lysis, as the biomaterial (Lin et al., 2015) containing proteins, lipids, nucleic acid, α- and β-polysaccharides (Lee et al., 2010), is a class of metabolic products accumulating on the surface of microbes in the wastewater treatment system. The fluorescent staining and confocal laser scanning microscope (CLSM) experiments demonstrated that microbial aggregation had a loose structure, with protein and β-polysaccharides distributed in the granule interior and with lipids and α-polysaccharide at the rim region; core of dead cells was surrounded by live cells (Lee et al., 2010; Caudan et al., 2014). In addition, Zhu et al. (2015a) researched the proteins of EPS in the sludge flocs and aerobic granules using EEM and SDS-PAGE, and it indicated that the protein-like fluorophores (aromatic protein-like and tryptophan protein-like substances) and proteins with high molecular weight were the key components in the aerobic granular sludge, which probably contributed to the formation and structural stability of granular sludge. The PN and PS, as main components of EPS, contain large amounts of functional groups such as hydroxyl and electronegative carboxyl (Zhu et al., 2012a), which can change the sludge surface properties and hydrophobic forces (Lin et al., 2015). With the help of hydrophobic forces, the sludge flocs could be attached together and establish a special network structure to favor the formation of aerobic granules rapidly (Lee et al., 2010).

During the process of aerobic granules cultivation, EPS could maintain the structure of the granules (Caudan et al., 2014) and

* Corresponding author.
E-mail address: suhj@mail.buct.edu.cn (H. Su).

http://dx.doi.org/10.1016/j.jenvman.2016.03.008
0301-4797/

keep sludge stability and good settling properties. Wang et al. (2010) found that there was a correlation between EPS content and aerobic granular sludge characteristics in an aerobic granule membrane bioreactor to some extent. While, there were few researches on the relationship between the EPS and settling properties, especially the equation simulation in the SABR. Besides, it was discovered that the PN and PS as the nutrients were biodegradable by their own producers and by other microbes at the starvation period (Li et al., 2006). To certain extent, the starvation period (no external substrate supplied, also named famine period) also have a positive impact on the formation of aerobic granules (Zhang and Bishop, 2003; Li et al., 2006). So the food-to-microorganism (F/M), as an essential parameter that regulated the food for microbes' cultivation, was investigated by a few researchers. Lobos et al. (2008) found that a high food-to-microorganism (F/M) ratio would enhance microbial growth and hence facilitate the aerobic granulation process. However, the correlations between the F/M ratio and EPS content had not been well established (Li et al., 2011).

Given that the cultivation of aerobic granular sludge was still an issue of investigation, the objectives of in this work was to study how the EPS made a significantly impact on the formation of aerobic granules, and the relationship between the EPS and SVI, and relationship between the EPS and F/M. The findings were essential to the development of an effective start-up strategy for the cultured rapidly aerobic granules in wastewater treatment.

2. Materials and methods

2.1. Reactor set-up and operation

The experiment was performed in a sequential air-lift bioreactor with a working volume of 3 L, an internal airlift diameter of 50 mm and an external diameter of 80 mm, and the height was 130 mm. Effluent was discharged from the middle of the reactors (6-outlet in Fig. 1) at a volumetric exchange ratio of 50%. Fine air bubbles for aeration were supplied through the dispenser at the reactor bottom, at an air flow rate of 300 L/h. The reactor configuration was shown in Fig. 1. The reactor reference one running cycle time was set at 6 h.

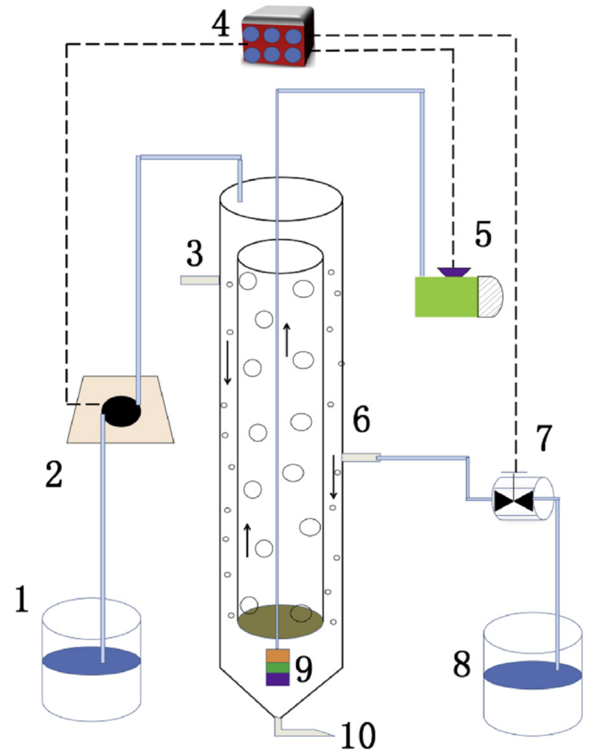

Fig. 1. The sequential air-lift bioreactor (SABR) set-up (1-influent bucket; 2-peristaltic pump; 3-sampling port; 4-PLC controller; 5-air compressor; 6-outlet; 7-solenoid value; 8-effluent bucket; 9-aeratior; 10-sludge discharge port).

Table 1
The influent composition during the cultivation in the SABR.

COD of influent (mg/L)	Sodium acetate (mg/L)	NH_4Cl	K_2HPO_4	KH_2PO_4	$MgSO_4$
500	641.0	95.6	8.5	22.7	25.0
750	961.4	143.3	12.8	34.1	37.5
1000	1281.9	191.1	17.0	45.4	50.0

2.2. Influent components and seed sludge

The seed anaerobic sludge had sand-like appearance, and the mixed liquor volatile suspended solid (MLVSS) was about 4800 mg/L at the beginning of the experiment. A synthetic wastewater with sodium acetate as carbon source was used as influent nutrients. The components of the synthetic wastewater were shown in Table 1.

2.3. Extraction and analysis of EPS

Sludge sample pretreatment: 20 mL of slurry mixture taken from the SABR and centrifuged (8000 rpm, 10 min) at 4 °C. When the supernatant was discharged, the remainder was washed twice with phosphate solution. After being shaken for 1 min, the obtained sludge sample was refrigerated at 4 °C before use.

Extraction and analysis of EPS: The sludge was brought up to a given volume with 20 mL of ultra-pure water and was placed in a water bath at 80 °C for 30 min, then centrifuged at 5000 rpm for 15 min. The supernatant were filtered through a 0.22 μm filter (Millipore, USA), then the polysaccharides (PS) and protein (PN) in the supernatant were obtained. Total extractable EPS was defined as the sum of protein and polysaccharide. PS was detected using phenol/sulfuric acid with 0–100 mg/L of glucose solution as the standard, and PN was detected using the coomassie brilliant blue method with 0–250 mg/L of bovine serum albumin as the standard (Liu and Tay, 2002).

2.4. Analytical methods of sludge properties and water quality

The sludge morphology and structure in the different phases were microscopically observed (Chong Qing Aote SMZ-DV320). The raw samples from the reactors are periodically analyzed for COD, total nitrogen (TN), biomass concentration (mixed liquor volatile suspended solids [MLVSS]) and sludge volume index (SVI) in accordance with standard methods during the aerobic sludge granulation (APHA, 1998).

3. Results and discussion

3.1. Variation of EPS content during the cultivation

3.1.1. The cultivation of aerobic granular sludge

The biomass was significantly influenced by the different COD contents (Thanh et al., 2009) because of the shortage or surplus of nutrients. In this work, the sequential air-lift bioreactor was used to cultivate aerobic granules rapidly under different organic loading conditions, and it was found that the mature granular sludge could