Bioresource Technology 241 (2017) 945–950

Contents lists available at ScienceDirect

Bioresource Technology

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

Rapid start-up of denitrifying granular sludge by dosing with semi-starvation fluctuation C/N ratio strategy

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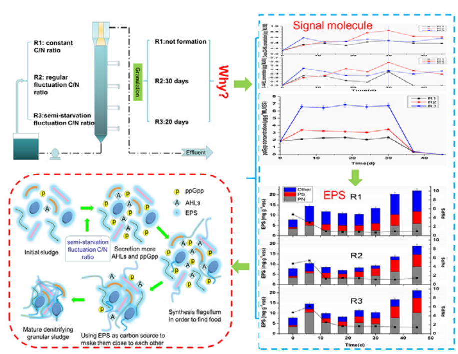
HIGHLIGHTS

- Semi-starvation fluctuation C/N ratio (SSF) could promote ppGpp and AHLs content.
- The ppGpp, AHLs, EPS and denitrifying sludge granulation process are correlated.
- The denitrifying granular sludge was rapid formation by dosing with SSF strategy.
- A mechanism for denitrifying sludge granulation with SSF strategy was proposed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 April 2017
Received in revised form 27 May 2017
Accepted 30 May 2017
Available online 1 June 2017

Keywords:
Semi-starvation fluctuation C/N ratio
ppGpp
AHLs
Denitrifying sludge granulation
Performance

ABSTRACT

This study cultivated denitrifying granular sludge in three UASB reactors by the semi-starvation fluctuation C/N ratio strategy (reactor 1 (R1): constant C/N ratio; R2: regular fluctuation C/N ratio; and R3: semi-starvation fluctuation C/N ratio (SSF)). Microbial aggregates appeared in R1, R2 and R3 on days 28, 14 and 6, respectively. Compared with the results in R1 and R2, the guanosine tetraphosphate (ppGpp) concentration was highest, the acyl homoserine lactones (AHLs) concentration quickly reached a certain threshold, and more protein (PN) of extracellular polymeric substances (EPS) secretion resulted in the rapid formation of denitrifying granular sludge in R3. The SSF strategy enhances microbial diversity, and denitrifying granular sludge has a better nitrogen removal performance. The result demonstrates that ppGpp, AHLs, EPS and the denitrifying sludge granulation process are associated. A mechanism for denitrifying sludge granulation with SSF strategy was proposed from the aspect of quorum sensing (QS).

1. Introduction

Nitrate pollution is widely distributed in surface and ground water, which can cause eutrophication, carcinoma, malformation and mutation (Liu et al., 2012). Compared with a variety of physicochemical methods to remove nitrogen from water, biological denitrification processes have thus far shown broad application prospects in nitrate removal due to their low cost and environmentally friendly products (Xing et al., 2016). The overall development of biological wastewater treatment is directed towards high efficiency, energy conservation and equipment miniaturization. Moreover, granular sludge is one of the important means of achieving

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http://dx.doi.org/10.1016/j.biortech.2017.05.206
0960-8524/

these goals (Xue et al., 2016). Therefore, the denitrification process has been widely studied to optimize the factors that could accelerate the granulation process. Zhao et al. (2013) showed that the C/N ratio was the primary parameter significantly affecting the denitrification rate in many bioreactors. Wu et al. (2012) showed that different C/N ratios had different effects on the formation of granular sludge, when the influent C/N mass ratio was lower than 2 or greater than 8, the aerobic granules were unsuccessfully cultivated in the sequencing batch reactors. Theoretically, without considering microbial growth (the carbon source content is just enough to degrade the nitrate, and the microbes are in a starvation state), 1 mg NO_3^--N hypothetically demands 2.86 mg biodegradable chemical oxygen demand (COD) to accomplish the denitrification process (Zhang et al., 2016). Previous research results noted that if considering microbial growth (the carbon source content can be used to degrade nitrate, as well as can be utilized by microbes), the calculated optimal COD/NO_3^--N (C/N) ratio is 3.7 (Chiu and Chung, 2003). Thus, the C/N ratio for the microbes in a semi-starvation state is 3.28:1. However, an intermittent feeding strategy had a critical role in granule formation (Yang et al., 2014). Still, few studies have investigated the effect of denitrification performance and granulation of denitrifying sludge under a semi-starvation fluctuation C/N ratio condition.

Besides, Sun et al. (2016) provided an intermittent feeding strategy that can seriously affect the quorum sensing (QS) system and comprehensive evidence that the QS system is closely associated with the granulation process. Another previous study showed that QS from activated sludge flocs could regulate the enzyme activity in specific cells through the response to changes in the concentration of acyl homoserine lactones (AHLs) such as lipase and cellulose (Chong et al., 2012). Additionally, recent studies indicated that guanosine tetraphosphate (ppGpp) is not only involved in the inhibition of RNA synthesis by cells during stringent stress (Traxler et al., 2008) but also in the synthesis of the flagellum and the effects of bacteria self-immobilization (Magnusson et al., 2007), which are closely relevant to denitrifying granulation. Overall, these results suggest that QS plays a major role in the ecological environment of denitrifying granular sludge. While, we are well-informed on the signaling molecules behavior in denitrifying granular sludge, research on the relations between the mechanism of denitrifying granulation and signaling molecules are far from sufficient.

Accordingly, the objectives of this study were primarily (1) to investigate the effect of semi-starvation fluctuation C/N ratio strategy on the performance of the UASB in biological nitrate reduction using methanol as the carbon source; (2) to determine whether ppGpp, AHLs, EPS and the denitrifying granulation process are associated and explore the mechanism of denitrifying granular sludge; and (3) to identify and phylogenetically analyse the primary functional denitrifiers in the UASB operating under semi-starvation fluctuation C/N ratio conditions.

2. Materials and methods

2.1. Reactor setup and operation

Three identical Plexiglas UASB reactors were set up to cultivate the denitrifying granular sludge by different feeding methods (R1, constant C/N ratio; R2, regular fluctuation C/N ratio; and R3, semi-starvation fluctuation C/N ratio). The reactor was 60 cm in height with an internal diameter of 6.8 cm, and it was equipped with a three-phase separator with a working volume of 3.5 L. The influent was synthetic wastewater, which contained the following compounds (mg L^{-1}): chemical oxygen demand (COD) by methanol and NO_3^--N by sodium nitrate, and 0.1 mL microelement solution. The microelement solution contained the following compounds (g L^{-1}): 0.43 $ZnSO_4{\cdot}7H_2O$, 0.014 H_3BO_4, 0.99 $MnCl_2{\cdot}4H_2O$, 0.25 $CuSO_4{\cdot}5H_2O$, 0.19 $NiCl_2{\cdot}6H_2O$, and 0.24 $CoCl_2{\cdot}6H_2O$. Flocculent seed sludge was collected from the return sludge-thickening tank at the XianYang Road Wastewater Treatment Plant, TianJin, China. The mixed liquor suspended solids (MLSS) and the mixed liquor volatile suspended solids (MLVSS) were 40.21 ± 2.01 g L^{-1} and 25.64 ± 1.28 g L^{-1}, respectively.

The experimental process lasted 45 days, which were divided into two periods (i.e., the acclimatization (1–30 days) and examination periods (31–45 days)) according to the different feeding methods (Fig. 1). The hydraulic retention time (HRT) remained at 3.5 h throughout the whole operation. The reactors were operated at room temperature (16–25 °C).

2.2. Signal molecules analytical method

All samples were chromatographed by an Agilent Acquity UPLC (Agilent, USA) at a 0.3 mL min^{-1} flow rate. The column dimensions were 4.6 × 50 mm, and it was filled with BEH C18 packing material with 1.8 μm particle size. The column was thermostated at 35 °C; the sample system was at 4 °C. The mobile phase consisted of a linear gradient (70–90%) of solvent B (methanol) and solvent A (water with 0.1% formic acid). The effluent was ionized by electrospray ionization in positive mode and was analysed using the multiple reactions monitoring approach by Agilent 6430B MS (Agilent, USA). The matrix-matched multiple reaction monitoring experiments were conducted for two standard AHLs.

In this study, HPLC (Agilent1100, Agilent Co. Ltd., USA) was performed with a C18 column and a diode array detector (DAD). The initial conditions of HPLC analysis for ppGpp were as follows: mixed solvent (95% solvent A as 20 mM Tris with pH 8.0 and 5% solvent B as 20 mM Tris + 1.5 M sodium formate with pH 8.0) at 1 mL min^{-1}, detection at 260 nm, and a column temperature of 40 °C. The level of sodium formate buffer was ramped up to 60%. The standards of AHLs and ppGpp were purchased from Biolog (America) and TriLink BioTechnologies (America), respectively.

2.3. Other analysis methods

The COD, NO_3^--N, MLSS and MLVSS were measured using standard methods (APHA, 1998). The average particle size of denitrifying granular sludge was measured by sieve method. Heat EPS extraction procedures were used, and the extracellular polysaccharides (PS) and extracellular proteins (PN) were assayed by the anthrone-sulfuric acid method (Gaudy and Gaudy, 1962) and Coomassie brilliant blue assay (Frølund et al., 1995), respectively.

3. Results and discussion

3.1. Effect of semi-starvation fluctuation C/N ratio on the characteristics of denitrifying granular sludge

3.1.1. Denitrifying sludge granulation

Initially, seed sludge in R1, R2 and R3 was black in color and flocculent in morphology. Nevertheless, sludge in the three reactors showed diverse appearances after cultivation for 30 days when the acclimatization period finished. In the acclimatization period, the sludge in R1 (constant C/N ratio) formed tiny aggregates after operation for 28 days at the C/N ratio of 9:1. Comparably, the sludge in R2 (regular fluctuation C/N ratio) and R3 (semi-starvation fluctuation C/N ratio) formed numerous tiny aggregates developed on day 14 and day 6, respectively. On day 20, the sludge in R3 had achieved complete granulation, and on day 30, the sludge in R2 had achieved complete granulation. The MLSS and MLVSS of