Bioresource Technology 271 (2019) 258–265

Contents lists available at ScienceDirect

Bioresource Technology

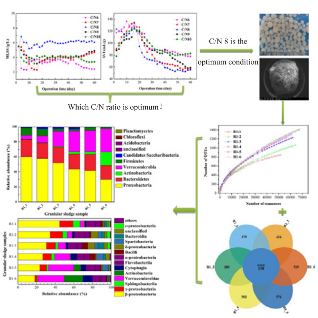
journal homepage: www.elsevier.com/locate/biortech

Impact of carbon to nitrogen ratio on the performance of aerobic granular reactor and microbial population dynamics during aerobic sludge granulation

Xiaochun Wang[a], Zhonglin Chen[a], Jimin Shen[a], Xia Zhao[b], Jing Kang[a,*]

[a] State Key Laboratory of Urban Water Resource and Environment, School of Environment, Harbin Institute of Technology, Harbin 150090, China
[b] College of Petrochemical Technology, Lanzhou University of Technology, Lanzhou 730050, China

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Aerobic granular sludge
Carbon to nitrogen ratio
Sequencing batch reactor
Decontamination efficiency
Microbial community

ABSTRACT

Carbon to nitrogen (C/N) ratio is one of the most important factor affecting aerobic granular sludge (AGS) growth and pollutant removal in aerobic granular sludge sequencing batch reactor (AGSBR). For stability of sludge granulation process, AGSs were domesticated in five sequence batch reactors (SBRs) with different C/N ratios (6, 7, 8, 9, and 10), which the ammonia nitrogen concentration of influent was 165 mg/L. The effects of C/N ratio on morphology and property of AGS were studied. The results showed that stable AGS was yielded with good settleability, high pollutant removal efficiency and rich microbial diversity when C/N ratio was 8. AGS yielded had stable structure due to higher protein in extracellular polymeric substances (EPS). High throughput 16S rDNA gene analysis revealed the microbial community diversity increased in AGS under the C/N ratio. The dominant microbes changed at the phylum, class and family three levels with the increasing operation time.

1. Introduction

Aerobic granular sludge (AGS) is a special self-immobilized microbial aggregate that possess the usual biofilm gradient. Compared with the conventional activated sludge floc, AGS has an excellent dense microbial structure for withstanding high-strength organic wastewater and shock loading. It also provides good settle-ability, a high concentration of biomass, and high removal efficiency for toxic pollutants (Bruin et al., 2004; Wu et al., 2018; Liu and Tay, 2004). AGS usually has large particle size, which limits the diffusion depth of oxygen molecules, and promotes the formation of stable anoxic/anaerobic zones in granular sludge. The structure provides an ideal place for facultative and aerobic microbial, which includes all kinds of ammonia-oxidizing bacterias, denitrifying bacterias and phosphate accumulating

* Corresponding author.
E-mail address: jingkanghit@163.com (J. Kang).

https://doi.org/10.1016/j.biortech.2018.09.119
Received 12 July 2018; Received in revised form 21 September 2018; Accepted 24 September 2018
Available online 24 September 2018
0960-8524/

bacterias. Therefore, the AGS has good removal efficiencies for nitrogen and phosphorus.

Activated sludge process is widely used to deal with stable wastewater. However, the conventional activated sludge floc process for industrial wastewater represents a great threat on the biota, and also a heavy economic burden on wastewater treatment plants (Sodhi et al., 2018). Some studies reported that AGS technology was more effective compared to the activated sludge for the removal of target contaminants from municipal wastewater (Balest et al., 2008; Kang et al., 2018). AGS system is widely considered to replace the activated sludge for treating both sewage and industrial wastewaters due to several advantages such as lower footprint, lower capital and operational costs, and possibilities for simultaneous occurrence of oxidative and reductive metabolic pathways in the aerobic, anoxic, and anaerobic micro-environments in the granules (Pronk et al., 2015; Derlon et al., 2016). Because of these advantages, AGS technology has been investigated for treating different kinds of wastewaters containing organics, nitrogen, phosphorus and toxic substances, such as livestock and poultry wastewater (Gao et al., 2011; Lee et al., 2010). However, loss of granular stability under the treatment of high concentration organic matter wastewater was a major obstacle for application of this technology (Lee et al., 2010; Wu et al., 2018). In this study, the AGS was domesticated in different C/N ratio under high nitrogen concentration for improving granular stability.

Carbon to nitrogen (C/N) ratio is known as a significant operational parameter in formation of AGS which affects the biomass growth, sludge settleability and pollutant removal (Wu et al., 2018). Previous study showed that the semi-starvation fluctuation C/N ratio condition was favorable to improving the performance of the AGS (Niu et al., 2017). The compact denitrifying granular sludge capable of denitrification at different C/N ratios was developed in sequencing batch reactors (SBRs), which C/N ratio of 1.5 was optimum for efficient and stable denitrification (Krishna Mohan et al., 2016a,b). It was also reported that AGS can be domesticated under high strength nitrates in SBR, and the final determination of the optimum C/N ratio was 3 (Krishna Mohan et al., 2016a,b). Some study reported that with the decrease of C/N ratio, the removal of total nitrogen (TN) and PO_4^--P gradually became worse, and the morphology of AGS was also affected. It can restore by increasing the C/N ratio from 100/10 to 100/5 (Chen et al., 2018). However, the effect of different C/N ratios on the growth mechanism of AGS, and microbial community structure in AGS of optimum C/N ratio are still not clear. Also there are few reports on the removal of high concentration organic compounds and total phosphorus (TP) by granular sludge.

The objectives of this study were primarily to investigate the effect of C/N ratio on the stable operation of aerobic granular sludge sequencing batch reactor (AGSBR). Five parallel SBRs with different C/N ratios were set to investigate the physical and chemical properties of the AGS, including EPS and treatment efficiency. The results showed that the stable AGS finally yielded under the optimum C/N ratio. Besides, the microbial community succession of AGS at the phylum, class and family three levels was studied under the optimum C/N ratio, which to further understand the important role of microorganism in the granulation process.

2. Materials and methods

2.1. Wastewater composition and reactor setup

It is widely known that swine wastewater contains much organic matter and nutrients with high chemical oxygen demand (COD) of 2300–15,000 mg/L and ammonia nitrogen (NH_4^+-N) of 150–1400 mg/L (Cheng et al., 2018; Cao et al., 2018). The concentrations of COD, NH_4^+-N and TP in actual wastewater that from Sanyuan swine farm in Harbin, China, were 3000 mg/L, 214 mg/L, and 27.5 mg/L, respectively. According to the adaptability of granular sludge growth, with reference to the actual value of pollutants in the swine wastewater (Tremouli et al., 2013), five identical SBRs were set up for domesticate AGS by different C/N ratios (C/N ratios were 6, 7, 8, 9 and 10 respectively). Synthetic wastewater was prepared to simulate wastewater, which mainly consisted of glucose and anhydrous sodium acetate (as carbon sources). Ammonium chloride and potassium dihydrogen phosphate were used as nitrogen and phosphorus sources, respectively. The concentrations of the synthetic wastewater used were given in Table 1. The synthetic wastewater also included $CaCl_2$, $FeCl_3·6H_2O$, $MgSO_4·7H_2O$, $MnCl_2·4H_2O$, $ZnSO_4·7H_2O$, $CuSO_4·5H_2O$ and EDTA. The total effective volume and working volume of the SBR were 625 mL and 312.5 mL, respectively. The system was controlled by a programmable logic controller (PLC). The volumetric exchange ratio (VER) was 50% for each cycle. Every operated cycle of the reactor took 4 h. Generally each cycle included 6 min of substrate filling, 228 min of aeration, 2 min of settling and 4 min of effluent withdrawal. The aeration rate was 1.4 L/min.

Table 1
Concentrations of substance in the synthetic wastewaters.

C/N	COD (mg/L)	NH_4^+-N (mg/L)	TP (mg/L)
6	2100 ± 100	165 ± 10	27 ± 2
7	2500 ± 100	165 ± 10	27 ± 2
8	3000 ± 100	165 ± 10	27 ± 2
9	3300 ± 100	165 ± 10	27 ± 2
10	3600 ± 100	165 ± 10	27 ± 2

2.2. Analytical method

The morphology of AGS was observed by a digital camera, and the microstructure was observed with scanning electron microscopy (SEM; FEI Sirion SEM). The wastewater parameters, including the COD, NH_4^+-N, TP, mixed liquid suspended solids (MLSS), and sludge volume index (SVI), were measured using standard methods (APHA, 1998). EPS extraction was performed by heat treatment based on a referenced procedure (Gaudy, 1962), and the determinations of protein (PN) and polysaccharide (PS) were based on the Bradford method and anthrone sulfuric acid colorimetric assay (Fang and Jia, 1996; Kwang et al., 2014), respectively.

2.3. Collection of biological samples, DNA extraction and high-throughput sequencing process

Sludge samples were collected from the reactor that C/N ratio of 8 for the purpose of bacterial community analysis at experimental days 1 (R1-1), 15 (R1-2), 25 (R1-3), 35 (R1-4), 45 (R1-5), 60 (R1-6). For the collection of sludge sample 50 mL of mixed liquor was taken from the AGSBR. The DNA extraction of sludge sample was carried out using the EZ-10 genomic DNA extraction kit (Protocol-BS423, Shanghai Bioengineering, China) following the protocol provided by the manufacturer. The extraction of DNA was kept at −20 °C until further process. The sequencing was done in an Illumina MiSeq. Using the Illumina MiSeq Reagent Kit v3 by amplifying the regions V3-V4 of the domain Bacteria, and the V3-V4 universe primers 341F/805R, 341F Primers: CCTACACGACGCTCTTCCGATCTG (barcode) CCTACGGGNGG CWGCAG; 805R Primers: ACCCGAGAATTCCAGACTACHVGGGTATCT AATCC (Herlemann et al., 2011). Preheating at 94 °C for 3 min, which was followed by 5 cycles of: 94 °C during 30 s; 45 °C during 20 s and 65 °C during 30 s; and then 20 cycles of: 94 °C during 20 s; 55 °C during 20 s and 72 °C during 30 s; finally, elongation step at 72 °C during 5 min was conducted. More than 40,000 sequences with 400 bp length were obtained from each sample using the Miseq sequencing platform (Illumina, Inc., San Diego, CA, USA).