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Partial nitrification using aerobic granule continuous-flow reactor: Operations and microbial community

Chunli Wan [a,b], Xue Yang [c], Duu-Jong Lee [a,b,c,d,*], Xiang Liu [b], Supu Sun [b]

[a] State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China
[b] Department of Environmental Science and Engineering, Fudan University, Shanghai 200433, China
[c] Laboratory of Environmental Pollution and Bioremediation, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, Xinjiang, China
[d] Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei 106, Taiwan

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ABSTRACT

Partial nitrification on ammonia laden wastewater using aerobic granule continuous-flow reactors was studied. Effects of temperature and carbon sources on performance of partial nitrification and characteristics of aerobic granules were reported. High nitrite accumulation ratio (>90%) was observed in all reactors after an acclimation phase of 6 days. Decrease in temperature or inorganic carbon concentration limited nitrite accumulation ratio; while nitrite accumulation was hard to recover once complete nitrification to nitrate was established in granules. The aerobic granules collected at continuous-flow reactor were stronger in strength than the seed granules from sequential batch reactor. Clone libraries revealed that *Nitrosomonas europaea* and *Nitrosomonas sp.* were the ammonia-oxidizing bacteria (AOB), whereas *Nitrobacter sp.* and *Nitrospira cf. moscoviensis* were nitrite-oxidizing bacteria (NOB). Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis revealed that temperature and carbon sources shifted the microbial community in granules.

1. Introduction

Ammonia laden wastewaters need intensive treatment before safe disposal of to receiving water bodies [1,2]. Partial nitrification reaction biologically converts ammonia to (NO_2^-–N) [3], which was studied in activated sludge systems during their start-up [4], pilot-scale tests [5], and reaction mechanisms [6]. Aerobic granular sludge is a novel biological wastewater treatment process that has advantages of high biomass retention, super settling capability, and low excess sludge production rates. Aerobic granule was applied for achieving nitrification [7,8] and partial nitrification [9,10].

Aerobic granules were cultivated in sequencing batch reactors (SBR) [11]. Long-term stability of aerobic granular processes is a challenge for its wide industrial applications [12]. In practice, the continuous-flow reactor is preferred to sequential batch reactor for wastewater treatment. Wan et al. [13] first achieved rapid start-up of partial nitrification in a continuous-flow aerobic granular reactor (CFAGR) *via* persistently excessive aeration. Partial nitrification requires enriching ammonia-oxidizing bacteria (AOB) and limiting growth of nitrite-oxidizing bacteria (NOB) in the granules. Various operational parameters such as temperature and carbon source may affect the reactor performance. For instance, Vázquez-Padín et al. [10] noted that generation lifetimes of AOB and NOB were significantly affected by operational temperature. The lifetime of AOB was shorter than NOB when the temperature was >15 °C. Specifically, in a sequential reactor, a relatively shorter operational time always benefited growth for AOB over NOB. As a consequence, the AOB was left in reactor and the NOB was discharged with excess sludge. The high free ammonia would poison bacteria. However, the AOB had better tolerance than NOB, and the ammonia was oxidized into nitrite by AOB, but no more oxidation was presented due to the severe inhibition on NOB. Thus, influent ammonia loading rates influenced the end products being nitrite or nitrate for ammonia laden wastewater treatments by sludge [4] or by granular sludge [10]. Controlling nutrient concentration could be used as a controlling parameter to partial nitrification [14,15]. Additionally, the presence of organic carbon determined the activities of heterotrophic denitrifier, hence affecting the end product distributions for the reactor [16].

This study investigated the effects of temperature and carbon sources on partial nitrification performance in an aerobic granule continuous-flow reactor. The microbial communities of aerobic

* Corresponding author at: State Key Laboratory of Urban Water Resource and Environment (SKLUWRE), Harbin Institute of Technology, Harbin 150090, China. Tel.: +86 0451 86286790; fax: +86 0451 86282100.
E-mail address: djleetw@yahoo.com.tw (D.-J. Lee).

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granules were identified with the help of denaturing gradient gel electrophoresis (DGGE) techniques and clone libraries. Cyclic diguanylate (c-di-GMP) molecules were the second messenger used by bacteria to regulate cellulose synthesis and regulate biofilm stability [17]. We also measured the extracellular polymeric substances (EPS) and concentrations of intracellular c-di-GMP concentrations subjected to changes in temperature and carbon sources.

2. Materials and methods

2.1. Cultivation of aerobic granules

The aerobic granules were cultivated in an SBR (6 cm × 180 cm) of 2.3 l working volume using the protocol modified from [13]. Specifically, the chemical oxygen demand (COD) of feed was at acetate: propionate = 2:1 rather than 3:1 in the previously proposed one. Mature aerobic granules were obtained after 16 d cultivation.

2.2. Continuous-flow tests

Two CFAGR were set up at the same geometry of [13]. In brief, the reactors were columns (6 cm × 105 cm) with a three-phase separator installed at the top to recover overflow granules. The seed granular sludge was 700 ± 30 mg/l, and the aeration rate was 5l min^{-1}. Experimental conditions were listed in Table 1. The synthetic wastewater was firstly prepared by basal media and carbon media. The compositions of basal synthetic media were NH_4Cl (0.2 g/l), KH_2PO_4 (0.026 g/l), $CaCl_2$ (0.01 g/l); $MgSO_4{\cdot}7H_2O$ (0.05 g/l), peptone (0.02 g/l). The carbon source tested was $NaHCO_3$ or organics composing of acetate and propionate. The testing temperature was controlled by circulating thermostatic water.

2.3. Analytical methods

2.3.1. Strength of aerobic granules

The strength of aerobic granules was determined by ultrasound method. Three aerobic granules and 15 ml of ddH_2O were loaded in a 25 ml erlenmeyer flask which was placed in an ultrasound bath at 20–25 kHz, 65 W. The ultrasound was intermittently applied at 2.5 s (on)–3 s (off) cycles. Ultrasound treated samples were collected and analyzed spectrophotometrically at 600 nm.

2.3.2. Microbial communities

The microbial community was analyzed by PCR-DGGE technique. Details of the DNA extraction, purification, PCR and DGGE were available in [13]. The diversity was expressed by Shannon–Wiener index [18]. The subjective bands were recovered by reamplification using primers 8F-GC:CGCCC GCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGAGAGTTTGA TCCTGGCTCAG; 518R: ATTACCGCGGCTGCTGG. The purified amplicons were ligated into pMD-18T and transformed into *E. coli* DH5α cells. Then, the positive colonies were randomly picked for sequencing by Sangon Co., Ltd (Shanghai, China). The gene sequences were compared on the GenBank to identify the closest genes using the BLAST alignment tool.

The specific PCR primers for AOB and NOB were synthesized according to Nicolaisen and Ramsing [19] and Purkhold et al. [20]. The PCR products were purified, ligated into pMD-18T, and transformed into *E. coli* DH5α cells. Fifty positive colonies were randomly selected for sequencing by Sangon Biotech Co., Ltd. (Shanghai, China). The gene sequences were aligned to Genbank by BLAST tool.

2.3.3. Other analyses

The concentrations of COD, NH_4^+–N, NO_2^-–N, NO_3^-–N, mixed liquid suspended solids (MLSS) were determined according to [21] Standard Methods. The pH and DO were measured by WTW 340i (Germany). All measurements were in triplicate with average values being reported. The nitrite accumulation ratio (NAR) was calculated as follow: $NAR = [NO_2^-–N]/[NO_x^-–N]$, with $[NO_x^-–N] = [NO_2^-–N] + [NO_3^-–N]$.

The intracellular c-di-GMP was measured based on protocols of Wan et al. [22]. In brief, the granules were first freeze dried and then vortexed with glass beads and added lysozyme (with buffer). The liquor phase was added with ethanol to collect precipitate, which was then extracted by water for analysis.

3. Results

3.1. Partial nitrification and transformation of nitrogen

3.1.1. Effects of temperature on partial nitrification

In reactor 1, phase I at 25 °C, HRT12h (1–20 d) had rapid accumulation of nitrite up to 30 mg/l (Fig. 1A) and NAR greater than 90%, with concentrations of both NH_4^+–N and NO_3^-–N in effluent being less than 5 mg/l. In phase II during 21–39 d with decrease in temperature to 15 °C, the NH_4^+–N removal rate was around 80%, whereas NAR was dramatically decreased. This occurrence should be attributed to the fact that AOB is more active at high temperature than NOB. At the end of phase II, nitrite and nitrate in effluent were 16.2 and 8.9 mg/l, respectively.

Further decrease the temperature to 6 °C (40–102 d) led to severe inhibition on NH_4^+–N oxidation, with concentration of NH_4^+–N in effluent rapidly increased to 30 mg/l and NO_2^-–N in effluent decreased to 6 mg/l (Fig. 1B). Prolonged HRT to 24 h reduced NH_4^+–N in effluent from 30 to 25 mg/l. Decrease again HRT back to 12 h during 129–144 d deteriorated again NH_4^+–N removal with 35 mg/l of NH_4^+–N in effluent. In phase VI during 145–167 d with temperature back to 12 °C, the NH_4^+–N concentration in effluent remained at around 35 mg/l. In phase VII during 168–183 d with temperature being increased back to 25 °C, NH_4^+–N removal rate gradually recovered, and at the end the test the concentration of NH_4^+–N in effluent was 2.7 mg/l. However, the partial nitrification capability could not be recovered. The NAR was only 10%, and NO_2^-–N in effluent was lower than 5 mg/l, whereas, NO_3^-–N in effluent was greater than 20 mg/l.

3.1.2. Effects of carbon source on partial nitrification

Fig. 2 indicates the influences of carbon sources on CFAGR performance. In phase I (1–24 d) the CFAGR was fed with 500 mg/l of $NaHCO_3$ and 400 mg/l of COD, after 10-day acclimation phase,

Table 1
Experimental conditions for the CFAGR reactors.

Reactor	Phase	Duration (d)	Temperature (°C)	HRT (h)	Carbon source(g/l)	
					$NaHCO_3$	COD[c]
1[a]	I	1–20	25	12	0.5	0.2
	II	21–39	15			
	III	40–102	6			
	IV	103–128		24		
	V	129–144		12		
	VI	145–167	12			
	VII	168–183	25			
2[b]	I	1–24	25	12	0.5	0.4
	II	25–44			0.5	0.2
	III	45–65			0.25	0.2
	IV	66–84			0.1	0.2

[a] Tests of influences on partial nitrification by temperature.
[b] Tests of influences on partial nitrification by carbon sources.
[c] The COD were composed of acetate and propionate at molar ratio of 3:1.