Bioresource Technology 142 (2013) 517–522

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Partial nitrification using aerobic granules in continuous-flow reactor: Rapid startup

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

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

HIGHLIGHTS

- Nitritation granules were cultivated in this study.
- Cultivation period of nitritation granules noted was 52 days.
- The *Pseudoxanthomonas mexicana* strain were enriched enhance granule stability.
- *Nitrosomonas europaea* was enriched in granules to achieve 85–90% nitritation.

ARTICLE INFO

Article history:
Received 29 March 2013
Received in revised form 28 April 2013
Accepted 29 April 2013
Available online 9 May 2013

Keywords:
Continuous flow reactor
Aerobic granules
Partial nitrification
PCR-DGGE
Colony library

ABSTRACT

This study applied a novel strategy to rapid startup of partial nitrification in continuous-flow reactor using aerobic granules. Mature aerobic granules were first cultivated in a sequencing batch reactor at high chemical oxygen demand in 16 days. The strains including the *Pseudoxanthomonas mexicana* strain were enriched in cultivated granules to enhance their structural stability. Then the cultivated granules were incubated in a continuous-flow reactor with influent chemical oxygen deamnad being stepped decreased from 1500 ± 100 (0–19 days) to 750 ± 50 (20–30 days), and then to 350 ± 50 mg l^{-1} (31–50 days); while in the final stage 350 mg l^{-1} bicarbonate was also supplied. Using this strategy the ammonia-oxidizing bacterium, *Nitrosomonas europaea*, was enriched in the incubated granules to achieve partial nitrification efficiency of 85–90% since 36 days and onwards. The partial nitrification granules were successfully harvested after 52 days, a period much shorter than those reported in literature.

1. Introduction

Partial nitrification reaction converts ammonia to nitrite, which, when combined with a subsequent Anammox step, achieves effective treatment for ammonium-laden wastewaters (Feng et al., 2007). The implementation of partial nitrification reaction is achieved by ammonia-oxidizing bacteria (AOB) while the formed nitrite can be further oxidized into nitrate by the nitrite oxidizing bacteria (NOB). The AOB generally grow slowly hence are not easily maintained at high population densities in the conventional activated sludge systems (Belmonte et al., 2009), especially when the formed nitrite is to be further oxidized to nitrate by nitrite-oxidizing bacteria (NOB). Consequently, sufficient accumulation of nitrite in a partial nitrification reactor from ammonia-laden wastewaters requires the enrichment of AOB and the suppressed activities of NOB (Ciudad et al., 2005).

Nitrifying bacteria are difficult to be maintained in conventional activated sludge systems owing to their low growth rates (Ni et al., 2008). Feng et al. (2007) applied a membrane bioreactor (MBR) to enrich the AOB in the partial nitrification reactor in order to achieve highly efficient nitrite accumulation. Since membranes can retain impurities including AOB in the reactor, membrane fouling is inevitable in MBR operations (Yang et al., 2012). Li et al. (2013) applied activated carbon to enrich AOB in the attached biofilms, reaching sufficient nitrite accumulation with granular activated carbon being the added carrier. Aerobic granulation is novel in the biotechnology field for wastewater treatments (Adav et al., 2008; Lee et al., 2010). Functional consortia are immobilized in aerobic granules hence the granular reactors can be applied to multiple target reactions (Lee et al., 2013). The autotrophic partial nitrifying granules were successfully cultivated (Bin et al., 2011; Vázquez-Padín et al., 2010).

* Corresponding author at: Department of Environmental Science and Engineering, Fudan University, Shanghai 200433, China. Tel.: +886 2 23625632; fax: +886 2 23633040.
E-mail address: djlee@ntu.edu.tw (D.-J. Lee).

0960-8524/$ - see front matter
http://dx.doi.org/10.1016/j.biortech.2013.04.120

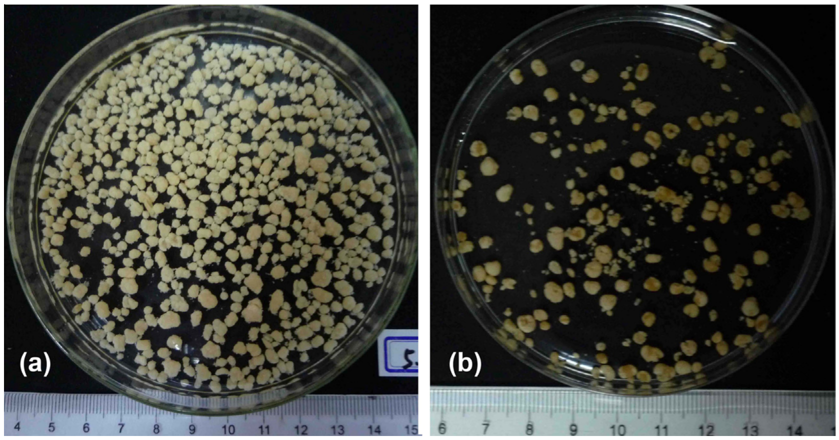

Fig. 1. Appearances of aerobic granules (a) Seed granules; (b) granules on 50 days continuous-flow operation.

The high pH, high NH_4^+-N concentration, low dissolved oxygen (DO), high temperature with short solid retention time (SRT) could inhibit NOB and lead to nitrite accumulation for a nitrification–denitrification system (Lemaire et al., 2008). Liu et al. (2008) noted that nitrite could be accumulated in their aerobic granular SBR at feed carbon/nitrogen (C/N, in g COD/g N) ratio of 5/1 and 3/1. With longer aeration time the formed nitrite would be further oxidized to nitrate. Shi et al. (2009) cultivated granules within 120 days of operation at C/N of 5/1 in an SBR and successfully cultivated AOB enriched aerobic granules with high COD removal and high nitrite accumulation capability. Shi et al. (2010) also cultivated autotrophic nitrifying granules in SBR after 120 days of operation with C/N (in g HCO_3^-/g N) of around 8/1. These authors noted that AOB and NOB co-existed in their nitrifying granules while in an SBR cycle the nitrite accumulated during 25–180 min and then declined over time to nitrate in 240–370 min.

Lopez-Palau et al. (2012) noted minimum changes in microbial communities during sludge granulation, even with sufficient partial nitrification activities. The supply of additional COD at the completion of ammonia oxidation stage in an anoxic/oxic SBR treated ammonia-laden wastewater benefits the growth of AOB rather than the NOB in the granular sludge (Wang et al., 2012). At a C/N ratio of around 6.5/1, Lemaire et al. (2008) proposed that since the nitrite was consumed by the nitrite pathway in denitrification so the NOB in the sludge was gradually eliminated from the sludge systems owing to nutrient insufficiency. When in a granular form and at C/N ratio of 6.7/1, the NOB/AOB ratio was increased over several testing cycles so excess nitrite was produced by the immobilized denitrifiers to furnish growth of NOB (Winkler et al., 2012). The availability of NO_2^- appears as a key factor for the growth rate of NOB. However, in a partial nitrification reactor, the NO_2^- concentration is expected to be high, hence the suppression of activities of both NOB and denitrifiers in the granules are preferred.

The cultivation period of nitrifying granules from autotrophoc medium was found to be 300 days (Tsuneda et al., 2003) and 120 days (Shi et al., 2009, 2010). Mature aerobic granules could be rapidly cultured in a high COD medium (Adav et al., 2008). Additionally, as in other aerobic granular systems the nitrifying granules could be operated only by a SBR mode (Adav et al., 2009; Chen et al., 2013). In industrial applications, the continuous-flow mode is preferred (Juang et al., 2010). Stable granules that could be operated in a continuous-flow reactor were cultured with intra-granular precipitation of inorganic salts (Lee et al., 2010). The present study applied a novel strategy for a rapid startup of partial nitrification reactor with nitrifying aerobic granules for ammonium-laden wastewaters. First the aerobic granules were rapidly cultivated at high COD and high DO in order to eliminate most denitrifying bacteria from granulation. Then the mature granules were fed into the continuous-flow reactor at reduced C/N ratio and increased quantities of inorganic carbon for enriching AOB over NOB in the granules. The interior strength of partial nitrification granules were confirmed using ultrasonication tests. The shift in microbial community in partial nitrification granules was noted by denaturing gradient gel electrophoresis (DGGE) and cloning.

2. Methods

2.1. Cultivation of aerobic granules

The aerobic granules were cultivated in a SBR reactor (6 cm × 180 cm) of 2.3 l working volume. For each SBR cycle, 1.6 l of synthetic wastewater was pumped in at the compositions of (per liter): NH_4Cl 0.2 g, KH_2PO_4 0.66 g, K_2HPO_4 1.22 g, $CaCl_2$ 0.03 g, $MgSO_4{\cdot}7H_2O$ 0.025 g, $FeSO_4{\cdot}5H_2O$ 0.02 g, $NaHCO_3$ 0.013 g, peptone 0.4 g, yeast extract 0.25 g, pH 7.2 ± 0.1, and chemical oxygen demand (COD) at acetate:propionate = 3:1. The COD of the fed wastewater was adjusted according to Table S1. The seed sludge at suspended solids (SS) of 6000 mg l^{-1} was collected from the recycled sludge stream in a wastewater treatment plant in Shanghai, China. The aeration rate was 5 l min^{-1}. Six SBR cycles were conducted daily, with each cycle consisting of 3 min feed, 227 min aerobic and settling, 5 min decanting and 5 min idle phase. The settling time was adjusted according to Table S1, and the aerobic time was correspondingly adjusted. In this study, mature aerobic granules were obtained after 16 days cultivation (Fig. 1a).

2.2. Continuous-flow aerobic granular reactor operation

The schematic diagram of the continuous-flow aerobic granular reactor (CFAGR) is shown (Fig. 2). The reactor was a column (6 cm × 105 cm) with a three-phase separator installed at the top to recover overflow granules. The aerobic granules cultivated in Section 2.1 were fed into the CFAGR with suspended solids (SS) of 820 ± 30 mg l^{-1}. The aeration flow was 5 l min^{-1}, yielding a dissolved oxygen (DO) value of 7 mg l^{-1} in all tests. During first 30 days of operation, the chemical compositions of fed wastewater were the same as those for cultivation medium except for the dosed COD values. In the next 20 days, the composition of wastewater fed was (g l^{-1}): NH_4Cl 0.2, KH_2PO_4 0.026, $CaCl_2$ 0.01, $MgSO_4{\cdot}7H_2O$ 0.05, $NaHCO_3$ 0.5, peptone 0.02, and prescribed COD. The COD in the continuous-flow operation was acetate: propionate = 3:1 and the operation temperature was maintained at