Chemosphere 205 (2018) 88–97

Contents lists available at ScienceDirect

Chemosphere

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

Effective seeding strategy using flat type poly (vinyl alcohol) cryogel for anammox enrichment

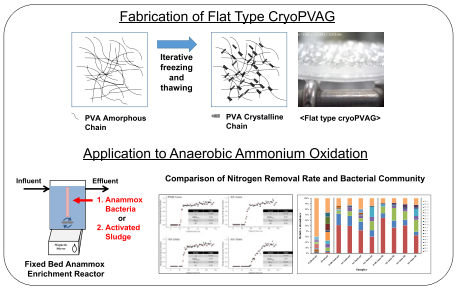
Minkyu Choi [a, b], Kyungjin Cho [a], Seockheon Lee [a], Yun-Chul Chung [a], Joonhong Park [b, **], Hyokwan Bae [c, *]

[a] Center for Water Resource Cycle Research, Korea Institute of Science and Technology (KIST), 5 Hwarang-ro 14-gil, Seongbuk-gu, Seoul 02792, Republic of Korea
[b] Department of Civil and Environmental Engineering, Yonsei University, 50 Yonsei-ro, Seodaemun-Gu, Seoul 03722, Republic of Korea
[c] Department of Civil and Environmental Engineering, Pusan National University, 63 Busandeahak-ro, Geumjeong-Gu, Busan 46241, Republic of Korea

HIGHLIGHTS

- Flat type cyroPVAG was utilized for anammox enrichment.
- Four anammox reactors with different inocula and thicknesses were used.
- The start-up period of anammox reactors was evaluated by a modified Gompertz model.
- Substrate diffusion limitation of cryoPVAG was verified at different gel thicknesses.
- Candidatus Brocadia sinica was the predominant species after anammox enrichment.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 January 2018
Received in revised form
29 March 2018
Accepted 6 April 2018
Available online 13 April 2018

Keywords:
Anaerobic ammonium oxidation
Poly (vinyl alcohol) cryogel
Inoculum source
Substrate diffusion limitation
Illumina MiSeq

ABSTRACT

In this study, anammox enrichment reactors were operated using flat type poly (vinyl alcohol) cryogel (cryoPVAG) with precultured anammox bacteria (PAB) and activated sludge (AS) from an anoxic tack of the A2O process to evaluate the effect of different seeding sources on anammox enrichment. In addition, cryoPVAGs with different thicknesses (1, 2, and 3 mm) were used to investigate the effects of the thickness on anammox enrichment. The regression analysis with a modified Gompertz model showed that the start-up period of the anammox enrichment using PAB inoculum was approximately 14 days earlier than that of AS inoculum at a nitrogen loading rate of approximately 1 kg-N m^{-3} day^{-1}. Substrate diffusion was limited in 3-mm cryoPVAG with respect to trend in nitrogen removal rate. Quantitative PCR analysis indicated that in the initial phase, the 16S rRNA gene copy numbers of anammox microorganism in cryoPVAG were significantly different according to the seeding source, but finally converged to a similar level after anammox enrichment. The anammox reaction was initially promoted by cryoPVAG. Next, anammox biomass detached from cryoPVAG and enriched in the bulk phase to maximize NRR. Illumina MiSeq sequencing revealed that *Candidatus* Brocadia sinica led to the active anammox reaction, and its relative abundance decreased with increasing gel thickness.

* Corresponding author.
** Corresponding author.
E-mail addresses: parkj@yonsei.ac.kr (J. Park), hyokwan.bae@pusan.ac.kr (H. Bae).

https://doi.org/10.1016/j.chemosphere.2018.04.055
0045-6535/

1. Introduction

Eutrophication in water environments derived from excessive nitrogen disposal is a serious environmental problem (Smith et al., 1999). As a solution for nutritional pollution, conventional biological nitrogen removal (BNR) consisting of nitrification and denitrification is the preferred biological technology for use in wastewater treatment plants. However, these BNR processes have limitations, such as the requirement for excessive aeration for nitrification and an external carbon source for denitrification. Anaerobic ammonium oxidation (anammox) shown in Eq. (1) is a promising alternative solution as a cost-effective nitrogen removal process (Mulder et al., 1995; Van de Graaf et al., 1995; Strous et al., 1998). To ensure successful nitrogen removal, stable partial nitritation (PN) is required prior to the anammox process as a pretreatment rather than complete nitrification (Eq. (2)).

$$1\ NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \quad (1)$$

$$1\ NH_4^+ + 0.79\ O_2 + 1.14\ HCO_3^- \rightarrow 0.10\ C_5H_7NO_2 + 0.42\ NH_4^+ + 0.56\ NO_2^- + 1.08\ CO_2 + 1.68\ H_2O \quad (2)$$

Compared to conventional BNR processes, aeration is reduced by 56.8% in the PN process based on the stoichiometric calculation, and an external carbon source is not required because the PN-anammox process is completely autotrophic. Additionally, the reduction in CO_2 emission is another advantage (Tian et al., 2015). However, anammox bacteria have a long doubling time of 11 days and low sludge output of 0.11 g volatile suspended solids (VSS) per g NH_4^+–N (Van Dongen et al., 2001). The slow growth rate of anammox bacteria positively affects the treatment of excess sludge production, but the start-up period for activating anammox reaction significantly increases.

The technique for securing a large amount of anammox bacteria is important for promoting the anammox reaction and reducing the start-up period in wastewater treatment processes. Diverse types of bioreactors with efficient retention of anammox bacteria have been designed. A batch system using suspended cells is used to initiate the anammox reaction and enrich anammox bacteria under anoxic conditions, however, the slow growth yield prevented massive cultivation (Van de Graaf et al., 1995). More suitable reactors in continuous mode have been developed using suspended sludge and attached-growth biomass including sequencing batch reactor, membrane bioreactor, up-flow anaerobic sludge blanket reactor, fixed bed reactor, rotating biological contactor and upflow biofilter (Strous et al., 1998; Egli et al., 2001; Fux et al., 2004; Trigo et al., 2006; Jin et al., 2008; Bae et al., 2016). Previous studies have also attempted to enrich anammox activity by using conventional activated sludge (AS), which is a useful seeding source when pre-cultured anammox bacteria (PAB) are not available for start-up (Gutwiński et al., 2016; Cho et al., 2017).

Among anammox enrichment systems, whole cell immobilization is a promising approach to overcome the slow growth rate of anammox bacteria. Whole cell immobilization techniques are advantageous because they show enhanced retention of biomass by preventing washout through easy solid-liquid separation and consequent reduction in the lag period (Chen et al., 1996; Hsia et al., 2008). Poly (vinyl alcohol) (PVA) has been widely used for whole cell immobilization because it is relatively inexpensive and has excellent tensile strength without causing toxicity to the microorganism (Hsia et al., 2008; Chou et al., 2012). Particularly, PVA-sodium alginate (PVA-SA) gel beads using bifunctional reagent of boric acid ($B(OH)_3$) and calcium chloride ($CaCl_2$) are representative gel material for environmental remediation. Using PVA-boric acid reaction, boric acid acts as the crosslinker, and four molecules of PVA are linked with boron. Consequently, monodiol type of gel lattice by PVA-boric acid is produced (Bae et al., 2017; Tang et al., 2017). PVA-SA gel beads have been also used for anammox enrichment (Chen and Lin, 1994; Ali et al., 2015; Bae et al., 2017). However, because a dense layer is formed on the surface during the PVA-SA fabrication process, gas permeability is decreased and the gel expands by the accumulation of gas contents produced by microorganisms (Chen et al., 1996; Chen and Houng, 1997).

From this perspective, PVA cryogel (cryoPVAG) is attractive material for wastewater treatment including in the anammox process due to the enhanced gas permeability and structural stability (Asano et al., 1992; Sheng et al., 2008; Magrí et al., 2012). CryoPVAG is fabricated by gelation through repetitive freezing and thawing. Freezing process initiates the phase separation of PVA solution into the frozen solvent crystal (i.e., water crystal) and unfrozen liquid microphase of PVA. The gel-forming constituents are concentrated in the unfrozen liquid microphase while accelerating the rate of PVA cyrogel formation. After thawing, the water crystals melt but the formed gel remains. As a result, macroporous hydrogel, i.e., cryogel, is produced (Lozinsky et al., 2003; Lozinsky and Okay, 2014). In terms morphology, only the cube-type of cyroPVAG has been used previously. In this study, flat type cryoPVAG was applied to start-up the anammox process. Effective diffusion of NH_4^+ and NO_2^- was limited by up to 1.3 mm (Ni et al., 2009). However, a typical PVA-SA gel bead is 3–5 mm in diameter, indicating that it has a large inactive area, also known as a dead space (Zhang and Ye, 2011; Ali et al., 2015; Bae et al., 2015). Thus, we predicted that minimizing the thickness of the cyroPVAG may rapidly increase anammox activity, even with less seeding source. In addition, stable structure of a flat type cryoPVAG was expected to prevent abrasion and loss of PVA gel during anammox enrichment.

In this study, flat type cryoPVAGs with thicknesses of 1, 2 and 3 mm were applied in anammox enrichment to determine the optimum thickness. Two seeding sources of PAB and AS were used. Nitrogen removal performance was evaluated according to seeding source and thickness of the cryoPVAG. A continuous reactor was inoculated with fixed bed cryoPVAG with minimized packing ratio. Real-time quantitative polymerase chain reaction (qPCR) was applied to verify the growth of total and anammox bacteria, and changes in bacterial community structure were identified by high-throughput sequencing method.

2. Materials and methods

2.1. Seeding sludge

PAB were obtained from an up-flow continuous bioreactor and AS was taken from a domestic wastewater treatment plant in Daejeon, South Korea (Bae et al., 2010a). The dominant anammox bacteria species of PAB and AS was *Candidatus* Jettenia sp. in the phylum Planctomycetes (Bae et al., 2017). The inoculum of PAB was composed of 63.2% and 36.8% of VSS and fixed suspended solids (FSS), respectively; AS consisted of 79.9% and 20.1% of VSS and FSS, respectively. Two inoculum sources were homogenized using a homogenizer (IKA, T18 digital ULTRA-TURRAX®, Staufen, Germany) including a dispersing element (S18N-10G). The VSS concentration of inoculum injected into the flat type cryoPVAG were 5271.7 ± 498.6 and 7116.7 ± 40.4 mg-VSS L^{-1} of PAB and AS, respectively.

2.2. Preparation of flat type cryoPVAG

The cryoPVAG was fabricated using a 10% PVA solution. The 10%