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Anammox sludge immobilized in polyvinyl alcohol (PVA) cryogel carriers

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ABSTRACT

This study evaluated the use of PVA cryogels to encapsulate slow-growing anammox bacteria for deammonification treatment of wastewater. The cryogel pellets were prepared by freezing-thawing at −8 °C. On average, pellets contained 11.8 mg-TSS/g-pellet of enriched anammox sludge NRRL B-50286 (Candidatus *Brocadia caroliniensis*) in 4-mm cubes. They were tested with synthetic and partially nitrified swine wastewater using continuous stirred-tank reactors packed at 20% (w/v). The immobilized gel was retained inside the reactor by a screen that eliminated the need of sludge recycling. The stoichiometry of anammox reaction was maintained for more than 5 months under non-sterile conditions. The process was not limited by substrates availability unless quite low N concentration (<5 mg/L) achieving >93% removal efficiency. In mass balances, >80% of the potential N conversion activity was achieved (2920 mg-N/kg-pellet/d). In addition, the immobilized bacteria were resilient to inhibition at high nitrite concentrations (244–270 mg-N/L).

1. Introduction

Discovery in the early 1990's of the anaerobic ammonium oxidation (anammox) (Mulder et al., 1995) as a new pathway to biologically convert ammonium (NH_4^+) to dinitrogen gas (N_2) under absence of oxygen has arisen great expectations in the field of wastewater treatment. However, due to the low growth rate of anammox bacteria (doubling time in the range of 1.8–11 days; Isaka et al., 2006; Strous et al., 1998), one of the main challenges for implementing the anammox process is to ensure bacterial cells' retention inside the reactors. For this reason, immobilization of microbial cells has received increasing interest in wastewater treatment to minimize the risk of biomass wash-out from the reactors and provide stabilized treatment (Quan et al., 2011). These immobilization techniques include self-immobilization as granular biomass (Dapena-Mora et al., 2004; López et al., 2008), attachment on the surface of a carrier forming biofilm (Ni et al., 2010; Tsushima et al., 2007), and entrapment of the microbial biomass into gel pellets (Furukawa et al., 2009; Isaka et al., 2007).

The anammox process is especially suitable for the removal of nitrogen (N) from wastewaters containing high ammonium and low biodegradable organic carbon (Paredes et al., 2007). This process consists of a chemolithoautotrophic bioconversion mediated by Planctomycetes-like bacteria that under anoxic conditions oxidize NH_4^+ using nitrite (NO_2^-) as the electron acceptor. According to the anammox reaction proposed by Strous et al. (1998) (Eq. 1), NH_4^+ and NO_2^- are converted to N_2 and nitrate (NO_3^-) under stoichiometric molar ratios of 1.00:1.32:0.26:1.02 for NH_4^+ consumption, NO_2^- consumption, NO_3^- production and N_2 production, respectively.

$$1.00NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (1)$$

The use of synthetic polymers such as urethane, polyethylene glycol (PEG), and polyvinyl alcohol (PVA) for the entrapment of microorganisms was reported as advantageous in the field of wastewater treatment. These advantages include closely packed design of bioreactors, non-toxicity to microorganisms, mechanical strength and long life span of gels, enhanced process efficiency, and resilience to overloading rates as demonstrated in the studies of Sumino et al. (1992), Tanaka et al. (1996) and Vanotti and Hunt (2000) on immobilization of nitrifying biomass. Furukawa et al. (2009) demonstrated that both anammox and nitrifying biomass can be immobilized in PEG gel pellets and used in connected reactors for the successful deammonification treatment of anaerobic digested liquor containing high ammonium concentration (1400–1600 mg NH_4^+-N/L).

Abbreviations: ACR, ammonium conversion rate; BOD, biological oxygen demand; COD, chemical oxygen demand; CSTR, continuous stirred-tank reactor; DO, dissolved oxygen; EC, electrical conductivity; HRT, hydraulic residence time; NCE, nitrogen conversion efficiency; NCR, nitrogen conversion rate; NLR, nitrogen loading rate; NRE, nitrogen removal efficiency; NRR, nitrogen removal rate; PEG, polyethylene glycol; PVA, polyvinyl alcohol; SBR, sequencing batch reactor; SNCR, specific nitrogen conversion rate; SNRR, specific nitrogen removal rate; TSS, total suspended solids; VSS, volatile suspended solids.

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Two techniques have been proposed to entrap microorganisms using PVA; in both cases the microorganisms are first mixed with a PVA solution and then the PVA is polymerized. For the PVA cross-linking or hardening step, one technique uses chemicals and the other uses freezing. For example, microorganisms can be immobilized using PVA, alone or mixed with alginate, into spherical gel beads through dripping into a gelation solution. This chemical technique has been used with anammox (Hsia et al., 2008; Quan et al., 2011; Zhu et al., 2009). The other cell immobilization technique involves the PVA cryogels, which are prepared by physical cross-linking through the freezing/thawing method (Lozinsky and Plieva, 1998). In the field of wastewater treatment, Furukawa et al. (1994) used the PVA-freezing technique at −20 °C to immobilize marine nitrifying sludge, whereas Vanotti and Hunt (2000) applied the PVA-freezing technique at −4 °C to immobilize swine wastewater nitrifying sludge. Previous research showed that exposure to freezing temperatures (−20 °C during 2 months) may cause irrevocable inactivation of anammox bacteria (Vlaeminck et al., 2007), but the effect of short time exposure to freezing temperatures (needed for PVA polymerization and cell entrapment) has not been investigated.

While nitrite is an essential substrate for the anammox reaction, there is evidence that high nitrite concentration inside an anammox reactor may also become an inhibitor of the anammox reaction and, therefore, it can be an important parameter to control. Kimura et al. (2010), in their experiments with anammox entrapped in PEG gel pellets, determined that 274 mg NO_2^--N/L was the maximum concentration before nitrite inhibition occurred. In other cases, however, much lower nitrite concentrations (44–100 mg NO_2^--N/L) were found as inhibitory of the anammox (Egli et al., 2001; Strous et al., 1999).

The aim of this research was to evaluate the effectiveness of entrapping anammox biomass in pellets using PVA as gel carrier and the freezing polymerization technique. In addition, we investigated the following three aspects of the immobilized anammox process: the recovery time of the anammox pellets after immobilization; the stabilized anammox reaction using both synthetic wastewater and partially nitrified swine wastewater; and the effect of high nitrite concentration on the activity of the immobilized anammox.

2. Methods

2.1. Anammox sludge

The anammox bacteria used was Candidatus *Brocadia carolinien-sis* deposited under the provisions of the Budapest Treaty (WIPO, 1980) in the Agricultural Research Service Culture Collection (NRRL) at Peoria, Illinois, USA, with accession number NRRL B-50286 (Vanotti et al., 2011b). It was isolated from sludges of nitrification–denitrification systems treating liquid swine manure and cultivated in a 10-L jacketed up-flow reactor (120 cm) (hereafter called parent reactor) at the ARS laboratory in Florence, South Carolina, USA. The parent reactor contained a biomass support made of polyester non-woven material (Japan Vilene Co., Tokyo, Japan). At the time of sludge collection for this PVA immobilization study, the parent reactor was being fed with synthetic wastewater containing 152.7 mg NH_4^+-N/L and 152.7 mg NO_2^--N/L and operated with a hydraulic residence time (HRT) of 4 h, an N-loading rate (NLR) of 1735 mg N/L/d, and a water temperature of 30 °C. Under these conditions, the N-conversion efficiency (NCE) obtained was 94% and the total N-removal efficiency (NRE) was 85% (Vanotti et al., 2011b). Stoichiometric molar ratios for the anammox reaction (NH_4^+ consumption:NO_2^- consumption:NO_3^- production:N_2 production) obtained in the parent reactor during steady-state conditions (three years) were 1.00:1.30 ± 0.009:0.18 ± 0.006:1.06 ± 0.005, respectively (Vanotti et al., 2011b). The removal of NH_4^+ plus NO_2^- inside the 120-cm-long parent reactor was gradual as the wastewater passed through: 34% of the N removal occurred at the bottom, 33% at the middle, and 33% at the top. Therefore, the anammox sludge was collected from three different heights (bottom, middle, and top) of the parent reactor by means of a long probe and a peristaltic pump.

2.2. Immobilization procedure

The anammox sludge extracted from the parent reactor was encapsulated in polymer gel pellets of PVA according to the freezing method described by Vanotti and Hunt (2000). The anammox sludge had a granular structure with granules measuring 2–4 mm and had a reddish color (Fig. A.1). It was concentrated in a beaker to 14.9 ± 0.9 g TSS/L (11.3 ± 0.7 g VSS/L; VSS/TSS = 0.76) by rapid settling (5 min) and decanting. The decanting was not straightforward because about 25% of the anammox sludge floated in the beaker. A sieve 0.25-mm pore size that covered the beaker was used and retained all the anammox sludge during decanting. The PVA polymer used for the preparation of the pellets was PVA-HC in powder form (100% saponification, 2000 polymerization, Kuraray Co., Tokyo, Japan). The PVA powder was sprinkled on warm water and mixed by hand to form a polymer suspension of 20% (w/v). The suspension was then autoclaved at 121 °C during 50 min to obtain complete PVA dissolution. After cooling to 37 °C, 400 mL of the polymer solution were mixed with 440 mL of the concentrated anammox sludge (Fig. A.1). The mixture was transferred into four non-stick aluminized steel pans (23 × 23 cm) to make four sheets approximately 4-mm thick. Subsequently, the pans were leveled and cooled for 2 h at 8 °C, and frozen for 17 h at −8 °C. After fast thawing for about 15 min inside a warm water bath (Fig. A.2), immobilized anammox pellet cubes of about 4-mm length were prepared using a sushi knife. The immobilized pellets were washed for 30 min under N_2 bubbling with synthetic medium (section 2.7.1) until foaming produced by unpolymerized PVA stopped. Wet weight of the pellets was determined after draining the pellets during 1 min over a 1-mm sieve. Corresponding volume was determined using water displacement in a 1-L graduated cylinder. The pellets density was 1.035 g/mL. Conditioning of immobilized anammox pellets was performed in closed glass vessels during 2 days at 33 ± 1 °C under fill-and-draw procedure using the same synthetic medium and applying a NLR of 230 mg N/L/d. Thereafter, the activity of the anammox pellets was tested using continuous stirred-tank reactors (CSTR).

2.3. Immobilized reactors set-up and operation

The pelletized anammox biomass obtained above (557 g wet) was split into two cell culture glass flasks (Celstir, Wheaton, Millville, NJ, USA) that were operated as CSTR (Fig. 1). Each reactor had a working volume of 1.4-L and a pellet packing ratio of 20% (w/v). Process temperature was controlled at 33 ± 1 °C using a heated water bath. Magnetic stirring speed was set to 100 rpm. The influent was supplied by an inflow peristaltic pump (model 7553-80, Cole-Parmer, Vernon Hills, IL, USA) from a 55-L tank through a feed line Tygon tubing (3.2-mm i.d.). The influent liquid was bubbled with N_2 each time the influent tank was refilled and covered with a floating polystyrene foam sheet to reduce gas exchange with the atmosphere. The feed line was introduced inside the reactor through a rubber stopper using Chemfluor PTFE tubing (4.8-mm i.d.) up to the bottom. The effluent line used the same Chemfluor PTFE tubing that exited the reactor through a rubber stopper. To retain the pellets inside the reactor, an outlet port was assembled at the beginning of the effluent line. The port consisted of a plastic