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Core-shell structured poly(vinyl alcohol)/sodium alginate bead for single-stage autotrophic nitrogen removal

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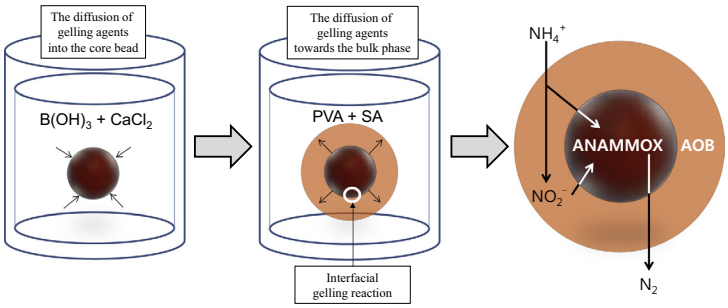
HIGHLIGHTS

- Interfacial gelling method was applied to fabricate core-shell structured gel beads.
- AOB and ANAMMOX bacteria were immobilized in separate locations.
- AOB protected the ANAMMOX bacteria from oxygen inhibition.
- Single-stage autotrophic nitrogen removal was validated in batch and continuous modes.

GRAPHICAL ABSTRACT



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ABSTRACT

A core-shell structured poly(vinyl alcohol)/sodium alginate gel bead was fabricated and the thickness of the outer layer was controlled. Immobilized ammonia-oxidizing bacteria (AOB) and ANAMMOX bacteria in outer and inner parts of the beads, respectively, cooperate to perform single-stage autotrophic nitrogen removal (SANR). As a critical designing factor, oxygen penetration depth according to the oxygen concentration in bulk phase and nitrifying biomass concentration in the outer layer were examined to protect strictly anaerobic ANAMMOX bacteria from oxygen inhibition. Oxygen penetrated up to a depth of 2350 ± 360 μm with the lowest nitrifying biomass of 703 mg-VSS/L at a dissolved oxygen concentration of 8 mg/L. However, a thick shell layer of more than 3 mm effectively protected the ANAMMOX bacteria from oxygen inhibition. The applicability of the core-shell structured gel bead for single-stage autotrophic nitrogen removal was validated in batch and continuous modes. A continuous bioreactor with a synthetic ammonia wastewater showed a maximum nitrogen removal efficiency of 80.4 ± 1.20% with a total nitrogen loading rate of 590 ± 12.1 g-N/m^3-d. Findings of this study suggest that start-up strategy of SANR using the core-shell structured gel bead can minimize the adaptation period without scarifying the ANAMMOX activity.

1. Introduction

The anaerobic ammonium oxidation (ANAMMOX) process is a proven technology for cost-effective biological nitrogen removal. ANAMMOX requires NH_4^+ and NO_2^- at a ratio of 1:1.32, and partial nitritation (PN) provides NO_2^- using ammonia-oxidizing bacteria

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(AOB) [1]. In the early stages of its implementation, the PN reaction was ideal to provide NO_2^- in a two-reactor configuration [2]. In the past decade, full-scale implementation of PN-ANAMMOX reactions has shifted to a single-stage configuration, which is called a single-stage autotrophic nitrogen removal (SANR) process [3]. In the SANR process, co-cultivation of AOB and ANAMMOX bacteria leads to a low concentration of toxic NO_2^- because the concurrent consumption of NO_2^- by the ANAMMOX bacteria has the same reaction rate as NO_2^- production by AOB [4]. In addition, the single-reactor system enables considerable simplification of the reactor configuration and lower capital costs than those of the two-stage system.

In the single-reactor system, a cell immobilization technology provides enhanced operational stability. For example, the dissolved oxygen (DO) gradient through the three-dimensional structure of biogranules offers aerobic and anaerobic zones, which are favorable for AOB and ANAMMOX bacteria, respectively [5,6]. In this biomass morphology, the AOB in the aerobic zone effectively protect the ANAMMOX bacteria from oxygen inhibition by depleting oxygen with a limited oxygen penetration depth of 100 μm [6]. The limited diffusion of DO is due to the dense distribution of the AOB on the surface of the biogranule. However, a low concentration of the AOB and oxygen overloading result in the expanded penetration of DO into the core ANAMMOX bead. In this regard, difficulties in regulating the DO could lead to incomplete nitrogen removal in the SANR process [7,8]. In addition, the biogranule is susceptible to low temperature and toxic chemicals [9,10] and may disintegrate when losing the selective pressures of a strong mechanical shear force and the presence of extracellular polymeric substances [11,12].

In contrast, an "artificial biogranule", entrapping microorganisms in synthetic polymeric matrices, has distinguishable structural stability enduring unfavorable conditions [13,14]. Poly(vinyl alcohol) (PVA) is a repeating chain or polymer of the vinyl alcohol unit. The vinyl alcohol units link together and form chains of about 2000 units in the water solution. PVA forms the gel structure in an aqueous solution on-their-own when stored in the freezing condition and the mechanical strength is enhanced by the iterative freezing and thawing. By the boric acid, the networking between the PVA is facilitated. Boron bonds with four molecules of PVA and produced a monodiol type PVA-boric acid viscoelastic gel. The PVA-boric acid method is considered as a gentle procedure which prevents the inhibition of microbial activity. Thus, the PVA has been widely adapted to the organic carbon and nitrogen removal processes. For example, nitrogen removal processes have been conducted using co-immobilization system for nitrifying and denitrifying bacteria [15]. For the co-immobilized system, insignificant internal diffusion is verified in the PVA gel matrix [16].

A PVA/sodium alginate (SA) gel carrier has been also successfully applied for SANR by co-immobilizing AOB and ANAMMOX bacteria in a single structured bead [17]. To enhance the nitrogen removal performance, the DO concentration should be increased up to 2.7 mg/L along with an elevated nitrogen loading rate (NLR) [18]. In this case, the single structured bead sacrifices a portion of ANAMMOX bacteria, which are exposed to a relatively high oxygen concentration on the surface of the bead. Note that an oxygen concentration of 0.08 and 1.44 mg/L leads to reversible and irreversible inhibition of the ANAMMOX activity, respectively [19]. Thus, an immobilized co-culture system with a core-shell structure can be a better option because it provides a substantial consumption of oxygen in the shell layer and offers a safe anaerobic zone to protect the ANAMMOX bacteria without an activity loss.

However, the core-shell bead structure was adopted only for coupling autotrophic nitrification and heterotrophic denitrification [20]. This is an improved application of the co-immobilization technology of nitrifying and denitrifying bacteria in comparison to the single structured PVA/SA bead. A dynamic growth of nitrifying and denitrifying bacteria were modeled and evaluated in detail for the core-shell structure [21]. However, the DO profile according to the depth has not been investigated despite the importance of oxidative and reductive environments regarding to the ammonia-oxidation and denitrification capacities, respectively, for nitrogen removal. In addition, information on the oxygen penetration depth through PVA/SA gel is required to accomplish the rational design for the thickness of the outer layer focusing on the protection of anaerobic bacteria. In particular, thin outer layer performing ammonia-oxidation results in the irreversible inhibition of ANAMMOX bacteria by the exposure to oxygen and consequent failure of SANR. So far, the oxygen penetration depth has not been correlated to significant operational factors, such as nitrifying biomass concentration in the outer layer and DO concentration in the bulk phase.

In this study, interfacial crosslinking reaction on the surface of the core bead was used to fabricate a shell layer on the PVA/SA lattice. To our best knowledge, this study is the first application of the core-shell structure to SANR. Because heterotrophic conditions caused by the organic carbon as an electron donor for denitrifiers result in rapid heterotrophic growth on the surface of the beads and a reduced nitrification efficiency, combination of core-shell structure and ASNR provides improved process stability. For more successful application, this study aims to investigate the critical design factor of oxygen penetration depth through PVA/SA gel lattice. The information of the oxygen penetration depth can be utilized to decide the optimal thickness of the outer layer for SANR. Second, the interfacial crosslinking reaction was modified to control the targeted thickness of the outer layer. Third, a fully autotrophic nitrogen removal process was performed with AOB and ANAMMOX bacteria entrapped in a core-shell structured gel. In a batch mode, the density of the nitrifying biomass in the shell layer was tested for the total nitrogen (TN) removal efficiency. In a continuous mode, the TN removal performance was evaluated during the start-up period.

2. Materials and methods

2.1. Preparation of the ANAMMOX core beads

The seed ANAMMOX sludge was collected from an upflow anaerobic sludge bioreactor (UASB) [22]. After the activity enrichment for more than one year, the final ANAMMOX activity was 0.51 mg-N/mg-volatile suspended solids (VSS)-day. The aggregated biomass was homogenized by a homogenizer (IKA, T10 Basic, Germany) equipped with a rotor-stator disperser (S10 N-19G, steel). *Candidatus* Jettenia sp. was dominant among the Planctomycetes phylum in the seed sludge. The ANAMMOX seed sludge was composed of 75.4% of VSS and 24.6% of fixed suspended solids (FSS). The mixture of 15% PVA and 2% SA was autoclaved at 121 °C for 30 min and cooled to 40 °C. After that, an 800 mg-VSS /L of the pre-cultured ANAMMOX biomass was mixed with the solution of PVA and SA. The mixture was then dropped into a solution of $B(OH)_3$ and $CaCl_2$ gelling agents using a silicon tube with a diameter of 2.0 mm. To enhance mechanical strength of the gel beads, the fabricated PVA/SA gel beads were phosphorylated in a solution of 0.5 M KH_2PO_4 for 1 h and washed with a large amount of distilled water. The PVA/SA beads were stored in distilled water for 2 days at 4 °C before the enrichment reaction in a bioreactor. The fabrication procedures for the PVA/SA gel beads and the enrichment process with a defined medium were previously described in detail [23]. A completely stirred tank reactor (CSTR) with a working volume of 500 mL was used to enrich the ANAMMOX activity with a packing