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Nitrogen removal and microbial diversity of activated sludge entrapped in modified poly(vinyl alcohol)–sodium alginate gel

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ABSTRACT

We investigated nitrogen removal from secondary effluent via microbial entrapment with poly (vinyl alcohol)–sodium alginate gel modified with alumina nanoparticles. Fourier transform infrared spectroscopy (FT–IR) and Raman spectroscopy revealed changes in functional groups on the embedding beads with the modification and the Brunauer–Emmett–Teller (BET) demonstrated that the mechanical strength of beads improved. Batch experiments were conducted to investigate nitrogen removal in synthetic wastewater with initial total nitrogen concentrations of 10–45 mg L^{-1}. The maximum ammonia removal loads ranged from 9.63 to 59.58 mg L^{-1} h^{-1}. Scanning electron microscope (SEM) observations showed that the beads were highly porous and conducive for microorganism adhesion. Microbial diversity analysis (High throughput sequencing) demonstrated that the microbial community structure inside the beads changed significantly after acclimation to the reactor environment and the reaction process. *Alcaligenaceae_uncultured* and *Comamonadaceae_unclassified*, which can conduct both heterotrophic nitrification and aerobic denitrification, were identified. They may facilitate pathways for non-traditional biological denitrification inside embedding beads.

1. Introduction

Excessive nitrogen is a main driver of eutrophication (Conley et al., 2009; Huang et al., 2015; Wang et al., 2015). Discharge standards of China for industrial and municipal wastewater treatment plants have become increasingly stringent in an effort to reduce the input of ammonia nitrogen into water bodies. At present, total nitrogen (TN) concentration in secondary effluent is in the range of 15–20 mg L^{-1} according to the discharge standard of pollutants for municipal wastewater treatment plant and integrated wastewater discharge standard. Therefore, developing new methods for treating secondary effluent from wastewater (especially from industrial sources) treatment plants is necessary to lower TN concentration to safe level.

Biological aerated filters (BAF), membrane bioreactors (MBR), and moving bed membrane bioreactors (MBMBR) are widely used for advanced wastewater treatment (Pulido, 2016; Wang et al., 2016). However, these technologies have several limitations, such as high investment costs and complex operational procedures. Furthermore, the packing, energy consumption, and technology associated with these methods have yet to be optimized (Barwal and Chaudhary, 2014; Feng et al., 2015). Meanwhile, nitrifying bacteria are highly sensitive to certain environmental conditions and have slow growth rates (Munz et al., 2011; Yu and Zhang, 2012). In particular, the biological toxicity of secondary effluent from many industrial wastewater treatment processes greatly inhibits the activity of nitrifying bacteria, causing poor nitrogen removal efficiency.

Recently, gel entrapment techniques have been widely investigated for wastewater treatment (Ali et al., 2015; Li et al., 2009; Liu et al., 2016) owing to their ability to enhance the efficiency and tolerance of microorganisms (StrotmannL and Windecker, 1997; Yan et al., 2010), and facilitate the maintenance of high microorganism concentrations in reactors (Chen et al., 2015). However, only few studies have demonstrated the use of microbial entrapment for the treatment of low-strength ammonium wastewater and secondary wastewater (He and Xue, 2010; Qiao et al., 2010).

The choice of embedding carrier is an important aspect of gel entrapment techniques. A considerable number of studies have

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used polyvinyl alcohol (PVA) (Magri et al., 2012; Sheng et al., 2008), sodium alginate (SA) (Liu et al., 2012; Yan and Hu, 2009), waterborne polyurethane (WPU) (Chen et al., 2015), polyethylene glycol (PEG) (Isaka et al., 2012, 2013) as embedding carriers. In particular, PVA hydrogel beads are advantageous as embedding carrier owing to their low cost, suitable porosity, and non-toxicity to microorganisms (Bai et al., 2010). However, previous studies also have reported that PVA hydrogel beads can easily expand and agglomerate in water (Chen et al., 2015; Qiao et al., 2014). In addition, they may exhibit strong mass transfer resistance, thereby reducing the degradation efficiency of microorganisms. Many previous studies have concluded that the addition of alginate can reduce the mass transfer resistance and improve the network structure of PVA hydrogel beads (Khoo and Ting, 2001; Wang et al., 2006). Qiao et al. (2013) and Bae et al. (2015) used polyvinyl alcohol–sodium alginate (PVA-SA) as a matrix to immobilize nitrifying and anaerobic ammonia oxidation bacteria and obtained total nitrogen removal efficiencies of 77% and 88.9%, respectively. Moreover, some studies have demonstrated that microbial immobilization with inorganic nanoparticles can reduce mass transfer resistance and increase the functional specific surface area and loading rate of cells (Ansari and Husain, 2012; Shi et al., 2014). Owing to their high specific surface area and adsorption capacity, alumina nanoparticles have been widely used for reducing various contaminants in water and material modification (Deravanesiyan et al., 2015; Kumar et al., 2011; Qian et al., 2014). However, few studies have investigated the use of embedding beads modified with alumina nanoparticles for nitrogen removal from wastewater.

The microbial characteristics of embedding beads are also important for assessing the mechanisms of pollutant degradation. Although many studies have analyzed the features of a particular species in embedding beads, such as nitrifying bacteria, denitrifying bacteria, and anaerobic ammonia oxidation bacteria (Bae et al., 2015; Isaka et al., 2012, 2013; Qiao et al., 2013), few have evaluated the diversity of microbial communities within embedding beads.

Therefore, the objectives of this study were to: (i) investigate the immobilization of activated sludge using PVA-SA modified with alumina nanoparticles via ultrasonication; (ii) investigate the nitrification and denitrification characteristics of embedding beads in synthetic secondary effluent wastewater with initial ammonium concentrations ranging from approximately 5 to 40 mg L^{-1}; and (iii) explore the diversity of microbial communities within embedding beads and their functional roles.

2. Materials and methods

2.1. Microbial carrier synthesis and immobilization

A polyvinyl alcohol–sodium alginate–alumina nanoparticles (PVA-SA-ALNPs) solution was prepared with 10% (w/v) polyvinyl alcohol (PVA; with a polymerization degree of 1750 ± 50; Shanghai, China), 0.8% (w/v) sodium alginate (SA; Chengdu, China), and 0.5% (w/v) alumina nanoparticles (ALNPs; purity = 99.99%; particle size = 50 nm; Shanghai, China). The PVA carrier was dissolved in deionized water at 90 °C, and the SA carrier was added to the PVA solution. ALNPs were added to the mixed polymer gel after cooling to 40 °C. The modified polymer gel was irradiated for 30 min using 40–kHz ultrasonic waves at 100% output power, and then left to cool completely.

The seed sludge consisted of activated sludge obtained from the aeration tank of the Yongchuan Municipal Wastewater Treatment Plant in Chongqing, China. The sludge was acclimated in synthetic wastewater with an initial ammonium concentration of 40 mg L^{-1} until the ammonium oxidation rate and ammonium concentration stabilized. After acclimation, the sludge was centrifuged at 3000 r min^{-1} for 15 min. After that, the residual was mixed with normal saline solution (0.9%) and centrifuged again. These steps were repeated for twice. And then added to the modified polymer gel (10% w/v). The mixture was transferred into a saturated boric acid solution containing 2% calcium chloride to induce the formation of beads (3.0 mm in diameter). Then, the beads were immersed in a sodium sulfate aqueous solution (0.5 mol L^{-1}) for 2 h at 4 °C. Finally, the beads were washed twice with normal saline solution and stored deionized water at 4 °C.

2.2. Synthetic wastewater

Synthetic wastewater used for the batch experiments contained (per L): NH_4Cl, 19–153 mg; $NaNO_3$, 30 mg; $NaHCO_3$, 117–526.5 mg; NaCl, 5.2–23.4 mg; KCl, 2.4–10.8 mg; $Na_2HPO_4{\cdot}12H_2O$, 11.6–52.2 mg; $MgSO_4{\cdot}7H_2O$, 8.4–37.8 mg; $CaCl_2{\cdot}2H_2O$, 2.4–10.8 mg; starch, 34 mg; and glucose, 56 mg. The initial ranges of ammonia (NH_4^+-N), total nitrogen (TN), and chemical oxygen demand (COD) in wastewater were 5–40 mg L^{-1}, 10–45 mg L^{-1}, and 80–100 mg L^{-1}, respectively.

2.3. Experimental setup

Batch experiments were conducted in reactors using synthetic wastewater with different ammonium concentrations. The working volume of the reactors was 0.5 L, the gel carrier packing ratio (volume ratio) was 10%, and the operation cycle was 6 h. During operation, the temperature, dissolved oxygen (DO), and pH of the wastewater were maintained at 10–15 °C, 2–4 mg L^{-1}, and 7.5–8.5, respectively. Nitrogen concentration was measured every 6 h.

The experiments were run in triplicate, and the arithmetic mean of replicates was used as the final nitrogen concentration value. All the experimental data were statistically analyzed by T–test to determine significant differences ($P < 0.05$).

2.4. Chemicals and analytical methods

All chemicals used in the experiments were of analytical grade. A dissolved oxygen optode (HACH–HQ30d, USA) was used to measure DO concentrations, and pH (PHS–3C; China) was measured daily. The concentrations of NH_4^+–N, NO_2^-–N, NO_3^-–N, TN, and COD were measured according to standard methods (SEPA, 2002). The concentration of NH_4^+–N was determined with Nessler's reagent spectrophotometry method (Spectrophotometer, 721E, Shanghai), NO_2^-–N was measured with N–(1–naphthyl) ethylene diamine dihydrochloride spectrophotometry method (Spectrophotometer, 721E, Shanghai), NO_3^-–N was determined with Ultraviolet spectrophotometry method (UVmin–1240, Japan), TN was determined with Alkaline potassium persulfate digestion–UV spectrophotometric method (UVmin–1240, Japan), and COD was determined with Dichromate method (DRB200, DR1010, HACH,USA).

The PVA–SA–ALNPs powder (post ultrasonication) was analyzed with FT–IR according to the KBr pellet method described by IRP vestige–21 (Shimadzu, Japan). Sixty–four scans with 0.5 cm^{-1} resolution were conducted to obtain spectra in the range of 4000 to 500 cm^{-1}. The PVA–SA and the PVA–SA–ALNPs powders were analyzed before and after ultrasonication using Raman Microscope (Renishaw, UK; 633 nm laser) to obtain spectra in the range of 4000 to 500 cm^{-1}. The beads were pretreated with the method described by Qiao et al. (2010), and then imaged using a scanning electron microscope (SEM; S-3400N; Hitachi, Japan). Samples of the embedding beads before and after modification were freeze–dried in a vacuum for 30 h, and specific surface area measurements were obtained with a Gemini VII 2390p surface area analyzer (Micromeritics, USA).

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