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Characteristics of pellets with immobilized activated sludge and its performance in increasing nitrification in sequencing batch reactors at low temperatures

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ABSTRACT

Immobilized pellets obtained by means of entrapping activated sludge in waterborne polyurethane were successfully adapted in ammonium (NH_4^+–N) synthetic wastewater. Its physicochemical characteristics were determined using scanning electron microscope, pyrosequencing, and microelectrodes, and its influence on the nitrification process in sequencing batch reactors (SBRs) at low temperatures was evaluated. A large number of rod-shaped bacteria were observed on the surface of the immobilized pellet, in which *Rudaea* spp. (Xanthomonadaceae family) was an important bacterial component (23.44% of the total bacteria). The oxygen uptake rate of immobilized pellets reached 240.83 ± 15.59 mg O_2/(L·hr), and the oxygen was primarily consumed by the bacteria on the pellet surfaces (0–600 μm). The dosing of the pellets (30 mL/L) into an SBR significantly improved the nitrification efficiency at low temperatures of 7–11 °C, achieving an average NH_4^+–N removal of 84.09%, which is higher than the removal of 67.46% observed for the control group.

Introduction

Ammonium (NH_4^+–N) in wastewater is derived from the enzymatic breakdown of urea, proteins, and other nitrogen-containing materials. It is widely accepted that NH_4^+–N is toxic to several fish species in an aqueous solution even at low concentrations (0.1 mg/L) (Eshchar et al., 2006) and can be transformed into nitrite (NO_2^-–N) and nitrate (NO_3^-–N) in drinking water, which can confer risks to human health, such as infant methemoglobinemia and gastric cancer (Bruning-Fann and Kaneene, 1993; Ward et al., 2005). Thus, NH_4^+–N must be removed from wastewater before being discharged into a water body.

As an efficient and economical technology for NH_4^+–N removal, biological treatment is widely applied in wastewater treatment plants (WWTPs), but low temperatures (<15 °C) would sharply reduce the activity of microorganisms, such as nitrobacteria, leading to poor NH_4^+–N removal (Fdz-Polanco et al., 1994; Sudarno et al., 2011). Therefore, feasible methods to enhance NH_4^+–N removal at low temperatures are desired.

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Traditional practices have been carried out to improve NH_4^+–N removal by adjusting operating parameters, such as sludge load, return sludge, aeration rate, and hydraulic retention time (HRT) (Salem et al., 2003; Wu et al., 2007), but it is still difficult to achieve high treatment efficiency or reduced operation costs in biological systems at low temperatures. Another alternative method is bio-augmentation to enhance the nitrification process in a biological system *via* psychrotrophs (Ben et al., 2009; Chevalier et al., 2000; Huang et al., 2015), granular sludge (de Kreuk et al., 2005) or bacterial immobilization (Isaka et al., 2007, 2008). Due to the slow growth of microbes at low temperatures, it is hard to maintain responsible psychrotrophs as the predominant species or takes a long time to form stable granular sludge in biological systems. Therefore, a simpler and more effective method is still desired.

The immobilization technique entraps microorganisms in the interior of a porous material and has several advantages (Hashimoto and Sumino, 1998; Sumino et al., 1992; Qiao et al., 2010), such as easy preparation, a long biomass retention time and resistance to shock load, which are especially beneficial for the slow growth of nitrifying activated sludge. Thus, immobilization might be an effective method for enhancing NH_4^+–N removal at low temperatures. Isaka et al. (2007) reported using nitrifying bacteria entrapped in a polyethylene glycol gel carrier to obtain stable nitrification rates at 10 °C for high concentrations of NH_4^+–N in landfill leachates. Dong et al. (2011) also used entrapment of activated sludge pellets in waterborne polyurethane for the continuous treatment of micro-polluted water and achieved an NH_4^+–N removal rate of over 80%. Unfortunately, oxygen profiles and oxygen uptake rate have not been well characterized in these pellets. Recently, microelectrode measurements have been known as the most reliable techniques to directly measure the microenvironment and activity of microorganisms in their habitats with a high spatial and temporal resolution (Xiao et al., 2013; Hou et al., 2014; Ali et al., 2015). Furthermore, little work has been done to evaluate the nitrification activity for domestic sewage using immobilized pellets at low temperatures.

Hence, the aim of this work is to reveal the physicochemical characteristics of pellets obtained from entrapped activated sludge in waterborne polyurethane by means of scanning electron microscopy (SEM), pyrosequencing and microelectrode measurements. Furthermore, immobilized pellets were added to a sequencing batch reactor (SBR) to evaluate the performance in increasing nitrification for the treatment of artificial wastewater at low temperatures.

1. Materials and methods

1.1. Immobilized pellets

Elastic gel immobilized pellets (cubes with 3-mm-long sides, black, unscented, density of 1.02 g/cm^3) were obtained by means of entrapping activated sludge (20 g/L) in waterborne polyurethane, as described in previous reports (Dong et al., 2011, 2012). Artificial wastewater was used for acclimation of immobilized pellets in order to activate and produce more nitrobacterium. The compositions contained (per liter) NH_4Cl, 306.0 mg; $NaHCO_3$, 936.0 mg; KCl, 18.9 mg; NaCl, 41.0 mg; $Na_2HPO_4{\cdot}12H_2O$, 92.6 mg; $CaCl_2{\cdot}2H_2O$, 18.9 mg and $MgSO_4{\cdot}2H_2O$, 67.2 mg. The acclimation experiments were conducted in an up-flow inner circulation aerated reactor (Dong et al., 2011). The working volumes of the reactor and pellets were 18 L and 1.8 L, respectively (packing ratio of 10%). To ensure sufficient dissolved oxygen (DO: 3–4 mg/L) and mixing, air was supplied from the bottom of the reactor using a stone air diffuser. The initial pH was in the range of 7.2–7.4, and the temperature was 18 °C in the reactor during the acclimatization period. The HRT was controlled at 4 hr by adjusting the feed flow rate. About 15 days, the acclimatization was deemed to finish when the average NH_4^+–N concentrations in the effluents were less than 5 mg/L.

1.2. Morphological observation by SEM

The surface and cross-sectional morphological characteristics of the immobilized pellets were examined by SEM (S-3000N SEM, Hitachi, Japan). Pellets sampled from the up-flow inner circulation aerated reactor on day 0 and day 20 represent un-acclimated pellets and acclimated pellets, respectively. The collected samples were rinsed with 0.1 mol/L phosphate buffer three times, fixed with 2.5% glutaraldehyde solution for 12 hr at 5 °C, dehydrated through a graded ethanol series up to 100%, and dried with a critical point dryer (K850, Quorum Technologies Ltd., UK). These samples were cut in half with a sterile scalpel for observing cross-sectional images, and the preparative surfaces and cross sections of the immobilized pellets were sputtered with gold for SEM observations.

1.3. Microbial community analysis by pyrosequencing

Microbial communities in un-acclimated and acclimated immobilized pellets were analyzed by pyrosequencing. Sample pretreatment and DNA extraction were performed according to a report by Isaka et al. (2012). Sequences of the 16S rRNA gene including the variable V3 region were amplified with two primers: tP2 (5′-acgtacatATTACCGCGGCTGCT-3′) and tP3 (5′-acgtacatCCTACGGGAGGCAGCAG-3′) (Zhang et al., 2011). Polymerase chain reaction (PCR) amplification was performed using a thermal cycler PCR system (PCR Sprint, Thermo electron, UK). The PCR products were evaluated by 1.2% (W/V) agarose gel electrophoresis and purified with a Gel/PCR DNA Fragment Extraction Kit (Geneaid, UKAS). Amplicon libraries were prepared using a mixture of three independent PCR products for each sample. The concentration of the PCR amplicons was measured using a Fluoroskan Ascent with a Quant-iT PicoGreen dsDNA reagent (Invitrogen, USA). Samples for 454 pyrosequences were sent to the Chinese National Human Genome Center in Shanghai, which performed amplicon pyrosequencing using a standard Roche 454/GS-FLX Titanium (McKenna et al., 2008).

Sequences obtained from pyrosequencing reads were processed to remove short sequences with lengths less than 100 nucleotides, primer mismatches, or average quality scores lower than 25. The sequences were verified in Ribosomal Database Project II (RDP Release 10) using Chimera Check (http://rdp.cme.msu.edu/index.jsp), and all chimeric sequences were discarded. The taxonomic identities of sequences were assigned using the Classifier program of the RDP-II at a confidence level of 80% (Zhong et al., 2014).