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Cell adhesion, ammonia removal and granulation of autotrophic nitrifying sludge facilitated by N-acyl-homoserine lactones

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HIGHLIGHTS

- AHLs addition enhanced granulation of autotrophic nitrifying sludge.
- AHLs improved microbial attachment of nitrifying sludge and ammonia degradation.
- Efficiencies of cell adhesion and ammonia removal related to AHLs chemical structure.
- AHLs increased biomass growth rate, microbial activity and extracellular protein.

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ABSTRACT

In this study, six N-acyl-homoserine lactone (AHL) molecules (C_6-HSL, C_8-HSL, C_{10}-HSL, 3-oxo-C_6-HSL, 3-oxo-C_8-HSL and 3-oxo-C_{10}-HSL) were each dosed into a bioreactor and seeded using autotrophic nitrifying sludge (ANS). The effects of the AHLs on cell adhesion, nitrification and sludge granulation were investigated. The results indicated that the efficiencies of cell adhesion and ammonia removal both had a close correlation with the side chain length and β position substituent group of the AHLs. The best-performing AHL in terms of accelerating bacterial attached-growth was 3-oxo-C_6-HSL, whereas C_6-HSL outperformed the others in terms of the ammonia degradation rate. The addition of 3-oxo-C_6-HSL or C_6-HSL increased the biomass growth rate, microbial activity, extracellular proteins and nitrifying bacteria, which can accelerate the formation of nitrifying granules. Consequently, selecting AHL molecules that could improve bacteria in attached-growth mode and nitrification efficiency simultaneously will most likely facilitate the rapid granulation of nitrifying sludge.

1. Introduction

Ammonium, which is abundant in many industrial and agricultural wastewaters, must be removed to prevent the oxygen depletion and eutrophication of surface water. Biological nitrogen removal (BNR) is highly promising for ammonium removal due to its various advantages, which include its high economic efficiency, lack of secondary pollution and facile operation. The nitrification process is normally the rate-limiting step of BNR due to the low proportion and slow growth of nitrifying bacteria. The granulation of nitrifying activated sludge can aid in avoiding the loss of nitrifying bacteria from suspended sludge and enable the reactor to have a high concentration of nitrifying bacteria even under short hydraulic retention times. However, sludge granulation is rather difficult to achieve in nitrification due to the slow growth rates of nitrifying bacteria (Belmonte et al., 2009). In several of the successful cases reported to date, a long cultivation time of 2 months or more was required to achieve nitrifying granular sludge, particularly for concentrated ammonia feeds with several organic substrates (Liu et al., 2008; Shi et al., 2010; Hosseini et al., 2014). Therefore, a method to achieve the rapid granulation of nitrifying activated sludge must be developed. Otherwise, it will hinder the actual application of granular sludge for nitrification or BNR on a large scale.

By exchanging chemical signals, a bacterial community may regulate its actions to coordinate the response of bacteria to environmental challenges. This phenomenon can be denoted as quorum sensing (QS) (Raina et al., 2010). Previous studies have shown that QS plays a significant role in coordinating biofilm formation, extracellular polymeric substance (EPS) production and microbial community structure (Valle et al., 2004; Wang et al.,

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2012), thus providing a new perspective for facilitating the adhesion and aggregation of nitrifying bacteria and reducing the loss thereof. N-Acyl-homoserine lactone (AHL) is a type of QS chemical, and many Gram-negative bacteria are able to produce AHLs. *Nitrosomonas europaea*, known as an ammonia oxidation bacterium (AOB), can produce AHL molecules, including 3-oxo-C_6-HSL, C_6-HSL, C_8-HSL and C_{10}-HSL, in wastewater treatment systems (Batchelor et al., 1997; Burton et al., 2005). Additionally, dosing with AHL molecules (e.g., 3-oxo-C_6-HSL, C_8-HSL) can increase the biomass of nitrifying biofilm and accelerate the recovery of damaged biofilm (Batchelor et al., 1997; Gamage et al., 2011). Although the QS effect of pure species of nitrifying bacteria on cell adhesion and biofilm formation has been characterized, the role of AHLs on the attached-growth and nitrification efficiency of activated sludge with a complex microbial community remains unknown. The AHLs with appropriate molecular structures will likely improve the microbial activity of nitrifying sludge, regulate the yield of EPSs for cell adhesion and strengthen the formation of nitrifying granular sludge, thus improving nitrification performance.

QS signal chemicals are often referred to as auto-inducers and can be classified based upon their molecular structures. All AHLs contain an acyl-homoserine lactonic ring, which can have different acylation branched chains in terms of the lengths of side chains and β position substituent groups (i.e., hydrogen, carbonyl or hydroxyl) (Fuqua et al., 1996). In this study, six AHL chemical molecules with different molecular structures (C_6-HSL, C_8-HSL, C_{10}-HSL, 3-oxo-C_6-HSL, 3-oxo-C_8-HSL and 3-oxo-C_{10}-HSL) were added to an autotrophic nitrifying sludge system to be tested separately. The influence of the AHLs on cell adhesion, nitrification efficiency and the granulation process of the sludge was investigated. In addition, the distribution of EPSs and the microbial community structure of nitrifying granules dosed with different AHLs were examined. The aims of the experimental study were to gauge the importance of AHL chemicals in the granulation of autotrophic nitrifying sludge and provide a strategy for achieving rapid sludge granulation.

2. Methods

2.1. AHL reagents for dosing

N-Hexanoyl-L-homoserine lactone (C_6-HSL), N-octanoyl-L-homoserine lactone (C_8-HSL), N-decanoyl-L-homoserine lactone (C_{10}-HSL), N-(3-oxohexanoyl)-L-homoserine lactone (3-oxo-C_6-HSL), N-(3-oxooctanoyl)-L-homoserine lactone (3-oxo-C_8-HSL) and N-(3-oxodecanoyl)-L-homoserine lactone (3-oxo-C_{10}-HSL) were purchased from Sigma–Aldrich (mainland China) and stored at −20 °C.

2.2. Cultivation of autotrophic nitrifying sludge

The nitrifying activated sludge was cultivated in the sequencing batch reactor (SBR) for 300 days. The influent into the reactors was a synthetic inorganic nitrogen wastewater prepared with NH_4Cl and other nutrients without any organic substrates added. The compositions of the main nutrients and micronutrients in the synthetic wastewater were as follows (per liter): 4.89 mg of $MgSO_4$, 7.5 mg of $CaCl_2{\cdot}2H_2O$, 2.40 mg of $FeCl_3$, 8.75 mg of KH_2PO_4, 26.84 mg of $Na_2HPO_4{\cdot}12H_2O$, 12.5 μg of H_3BO_3, 15.85 μg of $CuCl_2{\cdot}2H_2O$, 12.5 μg of $MnSO_4{\cdot}H_2O$, 12.5 μg of $(NH_4)_6Mo_7{\cdot}4H_2O$, 12.5 μg of $AlCl_3$, 12.5 μg of $CoCl_2{\cdot}6H_2O$ and 12.5 μg of $NiCl_2$. The SBR column was 6 cm in diameter and 85 cm in height with a working volume of 2.4 L, which was operated in a fixed sequential mode for a 4-h cycle with 4 min of feeding, 182 min of aeration, 50 min of sludge settling and 4 min of effluent withdrawal from the middle port of the column. Aeration was conducted at an airflow rate of 1.0 L/min during the aeration phase. The experiment was performed at room temperature, and the water temperature was 20–22 °C. $NaHCO_3$ was dosed into the feed solution to maintain the pH of the reactor in the range between 7.5 and 8.0. The activated sludge from a full-scale sewage treatment plant (Xiaojia River, Beijing, China) was used as the seed sludge. Initially, the ammonia degradation efficiency was only approximately 80% at the initial low feeding load of 50 mg NH_4^+-N/L. Then, the concentration of ammonia nitrogen in the feeding wastewater was gradually increased to 500 mg/L during the 300 days of operation. The ratio of nitrifying bacteria in the cultured sludge increased, and nitrification gradually improved. Finally, the efficiency of the ammonia degradation was approximately 100% for the cultured nitrifying sludge.

2.3. Adhesion test of autotrophic nitrifying sludge dosing with different AHLs

The effect of the AHLs on the adhesion ability of autotrophic nitrifying sludge was investigated following a cell adhesion test protocol used in the literature (Gamage et al., 2011; Lv et al., 2014). First, the sludge was collected from the SBR and homogenized at 10,000 rpm for 30 s. Then, the sludge suspension was diluted with fresh phosphate buffer solution (PBS), and 5 mL of sludge suspension with an OD_{600} value of approximately 0.2 and a NH_4^+-N concentration of 100 mg/L was pipetted into each well of a 6-well flat-bottomed plastic plate (ϕ 34.8 mm × D 20 mm). Six AHLs (C_6-HSL, C_8-HSL, C_{10}-HSL, 3-oxo-C_6-HSL, 3-oxo-C_8-HSL and 3-oxo-C_{10}-HSL) were tested in the plates using a concentration of 2 μM. Six replicate wells were used for each analysis for each of the six AHLs. The negative control well did not contain AHL. All plates were statically incubated at 30 °C. The attached biomass in the well was quantified after incubation under two different conditions: (1) incubation for 12 h and (2) incubation for 24 h. Ammonia and AHLs were added to the well again simultaneously after the first 12 h for the 24-h sample. The attached biomass was quantified according to the method reported by Stepanovic et al. (2000) and Taweechaisupapong et al. (2005). Each well of the 6-well plates was washed with PBS repeatedly to remove all non-adherent cells. The plates were dried at 50 °C. A 5-mL aliquot of 99% methanol was used in each well to fix the remaining attached bacteria for 15 min. Then, the plates were emptied and dried again. A 3-mL aliquot of 2% Hucker crystal violet was added to each well for Gram staining and incubated for 30 min. Excess dye liquor was then removed under running tap water, and the plates were dried. Finally, 5 mL of 33% (v/v) glacial acetic acid was dosed into each well to dissolve the dye bound to the attached bacteria for 1 h, and the OD value was measured at 600 nm with an enzyme immunosorbent assay reader (OD_{600}).

2.4. Determination of the nitrification ability of cultured sludge dosing with different AHLs

To evaluate the effect of AHLs on the nitrification ability of the sludge, 100 mL of sludge suspension with an OD_{600} of 0.2 and a concentration of 120 mg/L NH_4^+-N was dosed into a 250-mL Erlenmeyer flask and then incubated at 130 rpm in an orbital shaker at 30 °C. One of the six AHLs (C_6-HSL, C_8-HSL, C_{10}-HSL, 3-oxo-C_6-HSL, 3-oxo-C_8-HSL and 3-oxo-C_{10}-HSL), each with a concentration of 2 μM, was also added to one of six separate flasks. The experiments were performed in duplicate. The sludge mixture was sampled at various time intervals, and the concentrations of nitrogen in various forms (i.e., NH_4^+-N, NO_3^--N and NO_2^--N) in the liquid phase of the sludge were measured. The mass-balance