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Start-up of granule-based denitrifying reactors with multiple magnesium supplementation strategies

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ABSTRACT

In the present work, the effect of Mg^{2+} supplementation on the start-up of a denitrification process and the granulation of denitrifying sludge was investigated in three upflow anaerobic sludge blanket (UASB) reactors. The reactors R_1 and R_2 were continuously and intermittently, respectively, supplied with 50 mg L^{-1} Mg^{2+}, whereas R_0 was used as the control. The nitrogen loading rate (NLR) and organic loading rate (OLR) gradually increased, and extremely high values were obtained (36.0 kgN m^{-3} d^{-1} and 216.0 kgCOD m^{-3} d^{-1}, respectively). Granulation occurred in R_1 first, but the reactor capacities were comparable. Suffering from starvation, the R_0–R_2 performances were comparable. At the end of the experiment, the average diameter of the granules in R_0, R_1, and R_2 were 1.67, 1.72 and 1.68 mm, respectively, and the settling velocities of the granules in R_1 and R_2 were 1.14-fold the speed of R_0. The specific denitrifying activity (SDA) of the sludge from the reactors supplied with Mg^{2+} was greater than the reactor without Mg^{2+}. Intermittent Mg^{2+} supplementation was identified as the best choice to be utilized to cultivate denitrifying granules, which was consistent with kinetic analysis.

1. Introduction

Nitrogen- and carbon-rich compounds are major pollutants that cause a series of environmental problems (Guo, 2007; Lettinga et al., 1980). Denitrification, an essential step in biological nitrogen removal processes, has been prevalent for decades (Li et al., 2014a; Mateju et al., 1992; Sahinkaya et al., 2013; Shen et al., 2013), and the simultaneous removal of nitrogen and carbon is obtained during denitrification processes (Franco et al., 2006; Lew et al., 2012; Li et al., 2013). Commonly, the effectiveness of bioprocesses depends on biomass concentration, and granular sludge enables the maintenance of a high loading rate.

Anaerobic granules are self-immobilized aggregates and have been reported to be enriching agents in various types of reactors such as the upflow anaerobic sludge blanket (UASB), anaerobic baffled reactor (ABR) and expanded granular sludge bed (EGSB) (Lant and Hartley, 2007; Puyol et al., 2009; Uyanik et al., 2002). Biomass granulation depends on the source of inoculums, the type of organic source, water hardness, ratio of organic substrate to nitrate-nitrogen and other operational conditions (e.g., hydraulic loading, pH, temperature, and metal ions) (Ahmad et al., 2011; Bhuvanesh et al., 2013; Sheng et al., 2010). As previously reported, when lactate was used as the carbon source for denitrification, floatation appeared independently of the applied nitrogen-loading rate (NLR). However, with glucose, operating at high NLR, reactor instability occurred due to foam production (Cuervo-López et al., 1999). Additionally, divalent metal ions, e.g., Ca^{2+}, promoted the granulation of denitrifying sludge (Liu and Sun, 2011); however, research using Mg^{2+} was ambiguous. Mg^{2+} has been proven to play an essential role in enzyme activity and to stimulate enzyme reactions associated with the synthesis of cell materials (Brdjanovic et al., 1996); therefore, the effect of Mg^{2+} on sludge granulation in denitrification processes with glucose as the carbon resource is worth exploring.

The aims of the present study were (i) to investigate the differences in the performances of denitrifying reactors with multiple Mg^{2+} supplementation strategies during the start-up period, (ii) to distinguish the characteristic diversities of the biomass and (iii) to confirm the effectiveness of Mg^{2+} augmentation during sludge

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granulation.

2. Materials and methods

2.1. Experimental setup and operation

The experiment was conducted in three identical UASB reactors (R_0, R_1 and R_2) with a working volume of 1 L and each of the reactor was designed with an internal diameter of 60 mm. R_1 was operated with a constant dose of 50 mg L^{-1} Mg^{2+} in the influent, R_2 was operated with periodic supplementation of 50 mg L^{-1} Mg^{2+}, and R_0 was used as the control. Each cycle of R_2 was 14 d, and in the first 7 d (half cycle), the cycle was run with a supply of 50 mg L^{-1} Mg^{2+}, whereas Mg^{2+} was absent during the rest of the cycle. Otherwise, all of the reactors were placed in a thermostatic room at 35 ± 1 °C with identical operating conditions.

The entire experiment was divided into the following five periods: start-up (d 1-65, P1), granulation (d 66-188, P2), mature (d 189-266, P3), starvation (d 267-306, P4) and recovery (d 307-369, P5).

2.2. Seed sludge and synthetic wastewater

Each reactor was inoculated with 1 L flocculent-activated sludge from a local wastewater treatment plant to testify if this sludge type is appropriate for the star-up process in this study. Suspended solids (SS) and volatile suspended solids (VSS) in the sludge of the reactors were similar after inoculation (14.3 and 8.8 g L^{-1}, respectively), with a VSS/SS ratio of 61.6%.

Feeding was synthetic, glucose and sodium nitrate were chosen as the C and N sources, respectively, and the chemical oxygen demand (COD) to nitrate-nitrogen ($COD/NO_3^- - N$) ratio of 6.0 was higher than the theoretical stoichiometric ratio (4.9) for complete denitrification (including bacterial growth) (Franco et al., 2006). Therefore, nitrate was the limiting substrate. The synthetic wastewater composition is presented in Table 1. Additionally, 50 mg L^{-1} Mg^{2+} was independently added into the wastewater as needed in the form of $MgSO_4 \cdot 7H_2O$, and the initial COD and inorganic $NO_3^- - N$ concentrations were set at 300 and 50 mg L^{-1}, respectively. The pH of the synthetic wastewater was in the range of 6.9–7.3 and was adjusted by the addition of 1 mol L^{-1} sodium hydroxide or hydrochloric acid solutions.

2.3. Analytical methods

Influent and effluent samples were taken and analyzed every 2 d. The values for the sludge volume index (SVI), SS, VSS, pH, $NH_4^+ - N$, $NO_2^- - N$, $NO_3^- - N$ and COD were determined according to standard methods (APHA, 2005). The granule diameter was determined according to the method described by Jin et al. (2013). Extracellular polymeric substances (EPS) were extracted according to the 'heating' method, and extracellular protein (PN) and polysaccharide (PS) concentrations were determined as described by Ma et al. (2012). The shape of the granule was observed using a stereoscope (EZ4HD, LEICA, Germany). Mineral content, including potassium, calcium, magnesium, iron, copper and zinc, was detected in the denitrifying granules. A 50-mL sample of mature granules was taken from the reactors, and a 50-mL sample of seed sludge was also tested at the beginning of the experiment. The samples were ground after air-drying and pushed through a 100 mesh sieve. Then, 0.45 g of the sieved powder was digested with 64–66% HNO_3 and 70–72% $HClO_4$ (w/v). The digested biomass was further diluted to 50 mL with deionized water, and the diluted samples were analyzed by flame atomic absorption spectrophotometry (AA-6300C, Shimadzu, Japan).

Table 1
Composition of the synthetic wastewater.

Composition	Concentration (mg L^{-1})	Composition	Concentration (mg L^{-1})
Na_2HPO_4	2655	Yeast extract	100
NaH_2PO_4	2375	Trace element I[a]	1.25*
$NaHCO_3$	400	Trace element II[b]	1.25*
$CaCl_2$	0.5	Nitrate	add as required
Peptone	240	Glucose	add as required

* Milliliter per liter wastewater.
[a] The ingredient of the trace element I (g L^{-1}): 5.000 EDTA, 9.14 $FeSO_4 \cdot 7H_2O$.
[b] The ingredient of the trace element II (g L^{-1}): 15.000 EDTA, 0.430 $ZnSO_4 \cdot 7H_2O$, 0.240 $CoCl_2 \cdot 6H_2O$, 0.990 $MnCl_2 \cdot 4H_2O$, 0.250 $CuSO_4 \cdot 5H_2O$, 0.220 $NaMoO_4 \cdot 2H_2O$, 0.210 $NiCl_2 \cdot 6H_2O$, 0.014 H_3BO_4.

2.4. Specific denitrification activity (SDA) batch assays

SDA batch assays were performed in serum bottles with a liquid volume of 120 mL. The wastewater was prepared as described above, the pH was adjusted as needed (6.9–7.3), and $COD/NO_3^- - N$ was fixed at 6.0. The bottles were flushed with pure argon gas (99.99%) for 15 min and firmly closed with 4-mm-thick butyl rubber septa to maintain an anaerobic environment. Then, the bottles were placed in a vibrator at 35 ± 1 °C and on a shaker at 180 rpm. $NO_3^- - N$ and COD concentrations were monitored every 30 min, and SDA was estimated according to Li et al. (2014b).

2.5. Determination of the settling velocity (V_S)

V_S was determined using the method described by Mu et al. (2008). A measuring cylinder with a height of 40.0 cm and an internal diameter of 10.0 cm, to minimize the wall effect on granule settlement, was used in the test.

2.6. Kinetic analysis

Kinetic experiments were conducted over a wide range of substrate concentrations, and the Haldane model which was widely applied to predict the substrate degradation kinetic was used to describe the reactions (Zhang et al., 2013). The kinetic model was described using

$$r = \frac{r_{max} S}{K_S + S + S^2/K_{IH}} \quad (1)$$

where r is the substrate degradation rate (mgN h^{-1} g^{-1}VSS), S is the substrate concentration, r_{max} is the maximum substrate degradation rate (mgN h^{-1} g^{-1}VSS), K_S is the half-saturation coefficient (mg L^{-1}), and K_{IH} is the Haldane inhibition coefficient (mg L^{-1}). The kinetic parameters were estimated by nonlinear curve fitting using Origin (V. 8.5).

3. Results and discussion

3.1. Performance

The experiment was divided into five periods according to the progress of granulation. Granules appeared during the start-up period (d 1-65, P1), and larger aggregations were formed during the granulation period (d 66-188, P2). The potential of the reactors was explored during the mature period (d 189-266, P3), and the ability of resisting and recovering from starvation was tested during the starvation (d 267-306, P4) and recovery (d 307-369, P5) periods.

The performances of $NO_3^- - N$ and COD removal by the reactors