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Magnesium carbonate precipitate strengthened aerobic granules

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HIGHLIGHTS

- Magnesium carbonate was precipitated inside aerobic granules.
- Treated granules have enhanced stability in 220 days test in continuous-flow reactors.
- Carbonate salt did not change much granule morphology or microbial communities.
- $MgCO_3$ precipitation is a low cost and easy process for granule modification.

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ABSTRACT

Aerobic granules were precipitated internally with magnesium carbonate to enhance their structural stability under shear. The strengthened granules were tested in continuous-flow reactors for 220 days at organic loadings of 6–39 kg/m^3/day, hydraulic retention times of 0.44–19 h, and temperatures of 10 or 28 °C. The carbonate salt had markedly improved the granule strength without significant changes in granule morphology or microbial communities (with persistent strains *Streptomyces* sp., *Rhizobium* sp., *Brevundimonas* sp., and *Nitratireductor* sp.), or sacrifice in biological activity for organic degradation. $MgCO_3$ precipitated granules could be used in continuous-flow reactor for wastewater treatment at low cost and with easy processing efforts.

1. Introduction

Aerobic granular sludge is an emergent biological technology for industrial wastewater treatments (Adav et al., 2008). Aerobic granules have compact interior and large hydraulic diameter so they exhibit much better settleability and higher resistance to toxicity of wastewaters than conventional activated sludge. However, the structural stability of aerobic granules in long-term operation is generally poor (Lee et al., 2010). Ways of enhancing granule stability during long-term storage are desired (Whiteley and Lee, 2015; Lv et al., 2014; Yang et al., 2014).

Ren et al. (2008) noted that calcium carbonate was accumulated at core of their aerobic granules. These authors claimed that the calcium accumulated granules have high structural strength. Lin et al. (2013) observed accumulation of $CaPO_4$ minerals in their anammox granules and commented that the presence of minerals could enhance strength of the granules. Winkler et al. (2013) measured the density of their aerobic granules and noted that increase in 1–5% v/v of precipitates in granular interior could strongly increase the granule density and thereby the settling velocity. Angela et al. (2011) noted mineral clusters concentrating calcium and considerable amount of phosphorus at their granules' core. Metal precipitates commonly noted in mature granules were reported to be able to enhance granule strength and stability in long-term operation (Lee et al., 2010).

To stimulate mineral precipitation inside granules, Juang et al. (2010) applied enriched denitrifying bacteria as seed sludge and high organic loading rate wastewater with concentrated phosphate salts to cultivate their aerobic granules. As the granules grew to finite size to limit intra-granular oxygen mass transfer, anaerobic denitrification reaction would occur to yield alkalinity to increase local pH at core, hence inducing calcium and iron precipitates inside the granules. These authors confirmed the enhancement of granule stability by successfully operating the so-yielded granules in continuous-flow stirred reactors for 216 days without granule disintegration. Wan et al. (2014) claimed that calcium precipitate formed at alkaline core can serve as nuclei for subsequent cell attachment and granule growth.

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Biologically induced mineral precipitation has been adopted in many fields of practice (Achal et al., 2013; Kumari et al., 2014). However, biological processes are slow and are difficult to control considering the very different bacterial groups co-existing in the granules. We proposed in this study the use of direct chemical precipitation to enhance the granule stability. Mature granules were first cultivated then were dipped in the supersaturated solution for precipitation. The modified granules were then tested on their strength and the bioactivity on wastewater treatment in continuous-flow reactor. The sparsely soluble precipitate, once formed inside the granules, should be persistent for a long time. However, owing to their low solubility in water, the amount of precipitates to be brought into the granules would also be low. We tested an intermediate soluble precipitate, magnesium carbonate ($MgCO_3$), on granules modification. The treated granules were tested in continuous-flow reactors for confirming their structural stability during long-term operations (220 days).

2. Experimental

2.1. Cultivation of aerobic granules

The aerobic granules were cultivated in a column sequencing batch reactors (SBR) (6 cm × 180 cm) with 2.3 L of working volume. At the beginning of each cycle, 1.6 L of synthetic wastewater was fed at the following composition (per liter): propionate, 57.3 g, ethanol 9.6 g, NH_4Cl 6 g, K_2HPO_4 40 g, KH_2PO_4 20 g, $CaCl_2$ 1.2 g, $MgSO_4{\cdot}7H_2O$ 0.75 g, $FeSO_4{\cdot}5H_2O$ 0.6 g, $NaHCO_3$ 0.4 g, peptone 6 g, meat extract 3.75 g, pH 7.2 ± 0.1. The seed sludge was collected from sludge recycling tank with mixed liquor suspended solids (MLSS) of 6000 mg l^{-1}. The aeration rate was 5 l min^{-1}. The SBR was operated at 4 h cycles, each comprising filling (3 min), aeration-settling (227 min), decanting (5 min) and idle (5 min). Mature granules were cultivated in 14 d of SBR operation (U3).

2.2. Granule modification

The cultivated granules were subjected to chemical precipitation modification using $MgCO_3$, whose solubility in water is 0.39 g l^{-1} at 20 °C. Ten grams of $MgCO_3$ salt was added to vigorously stirred 100 ml distilled water at 90 °C. The so-yielded suspension was filtered to remove solids and the filtrate was then slowly cooled to 60 °C. The mature granules cultivated in Section 2.1 were first collected with surface moisture gently removed by tissue papers. Then granules were put into each of the 60 °C salt solution with gentle stirring for 30 min. Then the granules were collected and dipped into iced water for 5 s. This process was repeated for three times to allow mineral precipitation to occur inside the granules. The $MgCO_3$ modified granules were named U1 granules. The original granules were termed U3 granules.

As a comparison, some cultivated granules from Section 2.1 were first dipped into the stirred 60 °C 15 g l^{-1} NaCl solution for internal precipitation. Then the NaCl modified granules were put to $MgCO_3$ coating as stated before, named U2 granules in this work.

2.3. Continuous-flow reactor tests

Continuous-flow reactors of volume 0.8 L were applied for granule testing. The feed flow rate of synthetic wastewaters as used in Section 2.1 ranged 0.7–6 ml/min, giving hydraulic retention time (HRT) of 19–2.2 h. The corresponding organic loading rates (OLR) were 6–39 kg chemical oxygen demand (COD) m^{-3} d^{-1}. The temperature of reactors was 28 °C during day 1–124, and was reduced to 10 °C on day 125 and onward. The experimental conditions were listed in Table 1. Air was pumped into reactor bottom at 5 l/min. Initially 50 granules were put into the reactors. The number of granules was counted at fixed time intervals.

Table 1
Experimental conditions for continuous-flow reactors.

Day	OLR (kg COD/m^3/day)	HRT (h)	Temperature (°C)
1–19	6.0	19.0	28
20	12.0	19.0	28
21–35	6.0	19.0	28
36–40	12.0	9.50	28
41–48	18.0	9.50	28
49–62	18.0	6.34	28
63–70	24.8	6.34	28
71–77	24.8	4.44	28
78–79	0 (cease feeding)	–	28
80–82	24.8	4.44	28
83–89	27.3	4.44	28
90–111	27.3	3.33	28
112–116	31.3	3.33	28
117–118	31.3	2.66	28
119	31.3	0.44	28
120–121	31.3	2.66	28
122–124	34.3	2.66	28
125–132	31.3	2.66	10
133–163	34.3	2.66	10
164–179	34.3	2.22	10
180–189	39.0	2.22	10
190	19.5	4.44	10
191–192	26.0	3.33	10
193–220	32.5	2.66	10

2.4. Characterization and analyses

The strength of aerobic granules was characterized using ultrasound method (Wan et al., 2013). The tested aerobic granules were randomly picked and placed into a 25 mLconical flask loading with 15 mL deionized water. The flask was placed in an ultrasonic bath at 20–25 kHz, 65 W. The ultrasonic was intermittently applied at 2.5 s (on)–3 s (off)cycles. After the full spectral scan (190–900 nm) for aerobic granules with filamentous structure, 290 nm was the best wavelength to judge the turbidity of treated samples.

Determination of COD and NH_4-N concentrations in samples were in accordance with the Standard Method (APHA et al., 1998). The pH of suspension was measured using a pH meter (Multi 340i-WTW, Germany). Concentrations of Mg^{2+} were measured using atomic absorption spectroscopy (Hitachi-Z 5000, Japan). The appearance and size of granules were analyzed by SEM methods, according to Lv et al. (2014).

Genomic DNA was extracted and purified using the Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacture's instructions. Electrolysis in 1% agarose gel with staining by ethidium bromide was applied to assure successful DNA extraction. The forward and reverse primers for 16S rDNA amplification were 968f-GC (5′-GCGGGCGGCGCGGGGCGCGGGCGGGCGGCGGGGGCGGGCAACGCGAAGAACCTTAC-3′) and 1401r (5′-CGGTGTGTACAAGACCC-3′). The extracted DNA samples were amplified in a Biosystems (Perkin–Elmer, Foster City, USA) GenAmp PCR System 9700 programmed as follows: initial denaturation of DNA for 5 min at 95 °C; 35 cycles of 1 min at 95 °C, 1 min at 58 °C, and 90 s at 72 °C; and extension of incomplete products for 10 min at 72 °C. The denaturing gradient gel electrophoresis (DGGE) was performed with a DCode™ universal mutation detection system (Bio-Rad, Hercules, CA, USA). The polymerase chain reaction samples were applied to polyacrylamide gels in a TAE buffer with a denaturing gradient ranging from 45% to 65%. Denaturation of 100% corresponds to 7 M urea and 40% (v/v) formamide. The gradient gel was cast with a gradient delivery system (Model 475, Bio-Rad, Hercules, CA, USA). Electrophoresis was run at a constant