Bioresource Technology 152 (2014) 1–6

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Partial nitrification of wastewaters with high NaCl concentrations by aerobic granules in continuous-flow reactor

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HIGHLIGHTS

- Partial nitrification with aerobic granules was tested under high NaCl stress.
- Reactor performance, granular characteristics, microbial community were monitored.
- High NaCl led to complete conversion from ammonium to nitrite.
- Mechanisms of high-salt tolerance of aerobic granules were discussed.

ARTICLE INFO

Article history:
Received 11 September 2013
Received in revised form 27 October 2013
Accepted 30 October 2013
Available online 7 November 2013

Keywords:
Aerobic granules
NaCl
Continuous-flow reactor
Osmoprotectants
Microbial community

ABSTRACT

Wastewaters with high salinity are yielded that need sufficient treatment. This study applied aerobic granules to conduct partial nitrification reactions for wastewaters with high NaCl concentrations in a continuous-flow reactor. The present granules revealed partial nitrification performances at nitrite accumulation rate >95% and chemical oxygen demand (COD) removal at >85% at salt concentration up to 50 g l^{-1}. High salinity led to compact and tough granules. The granules applied electrogenic ion pump and sodium–calcium exchanger to reduce intracellular Na^+ concentration; generated amino acids as osmoprotectants to resist the high osmotic pressure; produced excess extracellular polysaccharides and proteins with secretion of c-di-GMP; revised microbial community with halophilic strains. The present continuous-flow aerobic granule reactor (CFAGR) is a promising process to convert ammonium in highly saline wastewaters to nitrite, which can be applied with a subsequent Anammox process for efficient nitrogen removal.

1. Introduction

High saline wastewater is mainly discharged from chemical, pharmaceutical, paper-making, petroleum refinery or dyeing factories, and also as municipal wastewater. Biological treatment of high salinity wastewater, if feasible, would be environmentally friendly, relatively simple and cost-effective compared to physico-chemical clean-up options. However, excess ions such as K^+ or Na^+ induces high osmotic pressure, and causes desiccation through osmotic movement of water out of cytoplasm (Wood, 1999). Ludzack and Noran (1965) noted that organic removal or nitrification reactions were inhibited when salinity was higher than 20 g l^{-1}. Kincannon and Gaudy (1966) found that a noticeable decrease in BOD removal rate (about 75%) was discovered when sludge developed in non-saline water and then subjected to slug doses of 30 g l^{-1} NaCl.

Aerobic granules are self-aggregates coagulated by hydrodynamic shear, which have integrated spatial structure (Adav et al., 2008). Compared with activated sludge flocs, aerobic granules have significantly faster settling rate, good toxicity tolerance and resistance to hydraulic loading rate (Liu and Tay, 2004). Stable granules were noted to exist only in sequencing batch reactor (SBR) rather than in continuous-flow mode (Lee et al., 2010). Liu and Wang (2008) observed that aerobic granules were more slippery and regular in appearance under high salinity. Figueroa et al. (2008) proved that chemical oxygen demand (COD) and ammonium-nitrogen removal were not influenced at up to 10 g l^{-1} of Cl^-. However, further salinity increase to 2%, nitrification activities of biomass was severely blocked (Dincer and Kargi, 1999).

Anaerobic ammonium oxidation (Anammox) is an effective treatment process for wastewaters containing high concentrations

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http://dx.doi.org/10.1016/j.biortech.2013.10.112

of nitrogen (Jin et al., 2012). Fernández et al. (2008) and Dapena-Mora et al. (2010) noted that presence of 2–15 g l^{-1} NaCl could enhance formation of Anammox granules but >13.5 g l^{-1} NaCl would inhibit activities of Anammox bacteria (Dapena-Mora et al., 2007). Karta et al. (2006) successfully adapted Anammox bacteria to 30 g l^{-1} salt (90% NaCl + 10% KCl) without loss of activities. Yang et al. (2011) revealed that Anammox reaction can be achieved by Annamox bacterial community with fed $NH_4^+ + NO_2^-$ wastewater at 30 g l^{-1} NaCl. These studies revealed that Anammox reaction can be realized at high efficiency at high levels of NaCl. Restated, the partial nitrification and Annomax can be an effective treatment process for wastewaters containing high concentrations of salts and nitrogen.

Pronk et al. (2013) noted that their aerobic granules could stably exist without structural disintegration under up to 32.7 g l^{-1} NaCl in SBR mode. These authors noted that the ammonia oxidation capability of their granule did not deteriorate at high salinity, but nitrite oxidation did. Restated, nitrite accumulation was noted at high salinity for their aerobic granules. Wan et al. (2013) achieved rapid start-up of a continuous-flow aerobic granular reactor (CFAGR) with high partial nitrification efficiency. The partial nitrification by continuous flow reactor had such advantages on facilitating aeration control strategies and maintaining total biomass, comparing with SBR. Besides, the continuous flow reactor was simpler than SBR on operation and automatic control. However, no trial has been made up to date on the use of continuous-flow reactor to achieve partial nitrification under high NaCl concentrations. This study monitored the reactor performance, granular characteristics, microbial community, and the contents of polymeric substances of CFAGR under NaCl up to 50 g^{-1}. Mechanisms for high-salinity tolerance of the aerobic granules were discussed.

2. Methods

2.1. Granules and reactor

The aerobic granules were cultivated in an SBR and the experimental procedures are referred to a previous study (Wan et al., 2013). The CFAGR has two principal parts: the bottom-structure was 6 × 105 cm column; the top-structure resembled with triphase separator of anaerobic equipment, and a two-decker stabilizer was set to prevent the loss of aerobic granules.

The aerobic granules were cultivated from the SBR and were fed to the CFAGR at volatile suspended solids (VSS) 844.3 ± 50 mg l^{-1}. The influent was simulated municipal wastewater at low C/N ratios with compositions as follow: NH_4Cl 0.2 g l^{-1}; KH_2PO_4 0.026 g l^{-1}; $CaCl_2$ 0.01 g l^{-1}; $MgSO_4{\cdot}7H_2O$ 0.05 g l^{-1}; $NaHCO_3$ 0.5 g l^{-1}; peptone 0.02 g l^{-1}, pH 7.2 ± 0.1. The chemical oxygen demand (COD) was supplied using mixed sodium acetate and propionate (2:1). During 1–23d, 24–54d, and 55–75d, the NaCl concentrations in influent were 15 g l^{-1}, 30 g l^{-1} and 50 g l^{-1}, respectively. The column temperature was at 28 ± 1 °C. The hydraulic retention time (HRT) of the CFAGR was 0.5d; the applied aeration flow rate was 5 l min^{-1}.

2.2. Analysis

2.2.1. Extracellular polymeric substrate and granular strength

The EPS of granules was extracted using formaldehyde and NaOH (Liu and Fang, 2002). The contents of extracted proteins and polysaccharides were measured using Folin reagent (Lowry et al., 1951) and phenol-vitriol (Herbert et al., 1971), respectively. The granular strength was evaluated by ultrasound (20–25 kHz, 65 W at 2.5 s (on) −3 s (off) cycles), with the supernatant turbidity being measured spectrophotometrically at 600 nm (Wan et al., 2013).

2.2.2. Microbial community

The microbial community was investigated by polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) technique according to Wan et al. (2013). The genic DNA (100 μl) was extracted by Mo-bio kit (Mobic Inc., USA), and then PCR and DGGE (with 30–60% denaturant) were sequentially conducted as refers. The gene sequences were compared on the GenBank to identify the closest genes using the BLAST alignment tool. The phylogenetic tree was portrayed by MEGA4.1.

2.2.3. Extraction and measurement of intracellular c-di-GMP

The extraction of c-di-GMP was amended referred to procedures by Simm et al. (2004). Restated, the granules were firstly freeze dried at −60 °C and 0.2 g of the dried granule and 15 ml ddH_2O were loaded in a 50 ml tube. Lysozyme was added with terminal concentration of 1 mg ml^{-1} and some glass beads (0.1 mm) were added and vortex for 15 min. Then the suspension was incubated at 37 °C for 1 h. The mixture was centrifuged at 9000 rpm for 15 min. The supernatant was transferred to a new 50 ml tube with double volumes of ethanol and vortex for 10 s. The tube was incubated at 4 °C for 1 h and shaken every 5–10 min, then was centrifuged at 9000 rpm for 15 min at 4 °C. The precipitate was incubated at 37 °C for 3 h, and then 3 ml of ddH_2O was added and vortex for 10 s. The mixture was transferred to a 5 ml tube and centrifuged at 12,000 rpm for 10 min. Finally, 1 ml supernatant was loaded into chromatogram vial for high-performance liquid chromatography (HPLC) analysis to measure concentration of c-di-GMP. The HPLC (Aglient 1260, Aglient Co. Ltd., USA) was performed with a C18 column at 40 °C, detection at 254 nm by diode array detector (DAD). Runs were performed in mixed solvent (95% of solvent A as 0.9% NaCl and 5% solvent B as 100% acetonitrile) at 1 ml min^{-1} (Hyodo et al., 2005).

2.2.4. Extraction and measurement of cation and free amino acids

The extraction procedures of cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) and free amino acids (proline and glutamine) were the same as the extraction of intracellular c-di-GM&P (Section 2.2.4). The supernatant was analyzed by HPLC (Aglient 1260, Aglient Co. Ltd., USA) using pre-column derivatization, equipped with refractive index detector and ZoRBAX eclipse AAA column (4.6 × 150 mm, 3.5 μm). The mixed solvent (40 mM Na_2HPO_4 as solvent A and 45:45:10 (v/v/v) of acetonitrile:methanol:H_2O as solvent B) at 2 ml min^{-1}. All the standards were purchased from Agilent Co., Ltd.

2.2.5. Other analyses

The contents of mixed liquor suspended solids (MLSS), COD, NH_4^+-N, NO_2^--N, and NO_3^--N were measured according to the Standard Methods (APHA, 1998). Concentrations of acetate and propionate were determined using a 7890A gas chromatograph (Agilent, USA) fitted with HP-FFAP capillary column (30 m × 0.25 mm × 0.25 μm) and flame ionization detector. The temperature was programed as follow: 80 °C for 5 min, then increasing to 200 °C at 10 °C min^{-1}. The ion concentration was analyzed by inductively coupled plasma mass spectrometry (HITACHI, P-4010).

3. Results and discussion

3.1. Reactor performance

Removal efficiencies of COD were all exceeding 85% in continuous-flow aerobic granular reactor with high-salt stress (Fig. 1a). On