Process Biochemistry xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

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

Low frequency-low voltage alternating electric current-induced anoxic granulation in biofilm-electrode reactor: A study of granule properties

Edris Hoseinzadeh [a], Abbas Rezaee [a,*], Mahdi Farzadkia [b]

[a] Department of Environmental Health Engineering, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[b] Department of Environmental Health Engineering, School of Public Health, Iran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 22 November 2016
Accepted 15 February 2017
Available online xxx

Keywords:
Nuclear magnetic resonance
Granulation
Biofilm electrode reactor
Settling velocity
Apoptosis

ABSTRACT

We characterized anoxic nitrate granules produced by an alternating current biofilm electrode reactor operating at low voltage-low frequency under optimum conditions. Hydrodynamic results revealed that the settling velocity for the granules ranged from 0.12 to 2.85 cm/s, while that of settling velocities ranged from 0.07 to 3.42 cm/s. Granule diameter varied, with a mean mass of 3.65 ± 1.29 mm and corresponding dry mass ranging from 0.52 to 5.64 mg. Roundness ratio of the sampled granules was determined to be 0.78 ± 0.11. Integrity coefficient obtained from a shear strength test was $87.05 \pm 2.07\%$ after 2 min and $74.1 \pm 4.14\%$ after 5 min. An adhesion test revealed hydrophilic properties of bacteria. The Most probable number (MPN) value was 2.0×10^6 for HDB and 2.0×10^3 for ADB. An apoptosis assay by flow cytometry confirmed that the majority of cells (87.7%) were viable and non-apoptotic (Annexin V-PI) and dehydrogenase activity was 15.05 ± 1.76 μg TF/mg biomass cm^{-2} d. Comparison of seed and granules by ^{1}H NMR spectra showed different signals in the range of 0.279–1, 1–1.5, and 1.5–7.5 ppm. Therefore, the biofilm in ACBER can be easily granulated and used to generate dense and fast-settling sludge granules.

1. Introduction

Bio-Electrochemical System (BESs) or Biofilm Electrode Reactor (BERs) is a new technology in wastewater treatment that uses pure or mixed microorganisms as attached biofilm on the electrodes [1–3]. In these systems, oxidation and reduction reactions are catalyzed by biofilms-electrodes interaction [4–6]. BES/BERs is a flexible technology used in wastewater treatment, trace element removal, energy producing, CO_2 capture, and microbial biofilm [4]. In BES/BERs, microbial biofilm electric stimulation can improve the pollutant removal because of microbial metabolism enhancing [4,5]. An applied electric current promotes the ion migration rate, enhancing the reactions taking place on the electrodes surface [7]. The reactions formed in the microbial biofilm and the electrodes can be intensified and varied. Some studied have revealed the possibility of microbial granulation by inducing an electric field [7]. Huang et al. [8] tried to use a low DC electric field on sludge granulation and nitrification performing in SBR. Their results showed that an electrical field caused the granules to form more compact and denser granules in relation to the changing bacterial species population Nitrospira. In another study [7], a zero valent iron (ZVI) bed was used, with a voltage of 1.4 V and current 150 mA. An electric field produced by an UASB reactor was used to induce anaerobic sludge granulation. The results showed an increasing granule size (almost 4 times more than the control) with respect to the electric field, as well as greater EPS generation, leading to more stable granules, and ZVI reaction enhancing while improving UASB performance in terms of COD removal efficiency. Aerobic microbial granulation has been used successfully in the treatment of biological wastewater. Sludge granulation has achieved an even denser and stronger microbial community, promoting improved settleability rate, efficient clarifying, rich microbial diversity, thicker sludge and able to accept higher loading shock than biofilm or suspended activated sludge [8–12], yielding a high biodegradation efficiency. Results show the granulating sludge needs over three months to complete biodegradation, but that time can be shortened by changing physico-chemical operation parameters and hydraulic methods. Use of an electric field to induce sludge granulation is suggested as an efficient method of treating biolog-

* Corresponding author at: Environmental Health Engineering Department, Faculty of Medicine, Tarbiat Modares University, Jalal Ale Ahmad high way, Nasr bridge, P.O.Box: 14115-111, Tehran, Iran.
E-mail addresses: e.hoseinzadeh@modares.ac.ir (E. Hoseinzadeh), rezaee@modares.ac.ir (A. Rezaee), mahdifarzadkia@gmail.com (M. Farzadkia).

http://dx.doi.org/10.1016/j.procbio.2017.02.019
1359-5113/

Table 1
Results on sludge granulation in different processes.

Process	Concluded of granulation inducer	Main result	Ref.
Denitrifying Sulfur conversion-associated Enhanced Biological Phosphorus Removal (DS-EBPR) process	Superficial up-flow velocity and lowering the OLR led to granulation accelerating.	Granules in 375–400 μm diameter formed after 200 day.	[20]
High rate hybrid anaerobic reactors (HAR)	start-up SVI would act as a sludge granulation inducer and selector for granule microbial communities	Granulation led to decrease SRT time, microbial community similarity and Methanosaeta and Methanosarcina presence affected granulation.	[21]
Up-flow anaerobic sludge blanket (UASB) reactor with installation of zero valent iron (ZVI) bed with a pair of electrodes.	ZVI in combination with an electric field accelerated sludge granulation.	By supplying voltage the mean granule size reached almost 9 times greater in 60 days (723.5 μm VS. 88.9 μm) as well as settling velocity was as high as of 56.1 m/h.	[7]
Sequencing batch reactor (SBR) with piggery wastewater and anaerobic condition.	Raw piggery wastewater with high contents of Ca and Fe ions in and SBR operating mode resulted in speed garnulation.	After 18 days the granules formed with average size of 200 μm and high settling velocity.	[22]
Biofilm electrode reactor supplying with low voltage-low frequency alternating current to denitrifying.	Low voltage-low frequency alternating current and presence of Ibuprofen in the synthetic wastewater led to biofilm granulation.	The granules formed after 45 days operation. The granules size were in rage of 1.65–7.29 mm with settling velocity of 0.12–2.85 cm/s. As a result of granulation the HDB and ADB count and cell wall charge of bacteria were changed.	Present study

ical wastewater [7], assisted by the variety of heterotrophic and autotrophic reducer bacteria present in the formed sludge granules. However, the population density can affect the removal efficiency and stability. As seen in other studies [13], the changing GSBR operation parameters, such as non-aeration and non-continuous feeding, may result in predominating some species of bacteria in the formed aerobic granules. Liu et al. [14] studied the biological removal of sulfide, nitrate and phenol using an expanded granular sludge bed (EGSB) reactor. They found bacterial population biodiversity in both heterotrophic and autotrophic denitrifies located in granules. They showed the autotrophic denitrifying population was decreased because of nitrite accumulation. Lochmatter et al. [15] found heterotrophic denitrifying predominant in the aerobic granular sludge in sequencing batch reactors. Some authors emphasized better sludge granulation in the presence of electron acceptors such as nitrate [16–19]. Most synthetic wastewater contains electron acceptors along with organic compounds. Li et al. [19] showed that sludge granulation in the acidogenic process would be induced by denitrification conditions, which improve the organic and nitrate removal capacity. Furthermore, the contribution between autotrophs and heterotroph denitrifies in the granules improved the efficiency of nitrogen removal. Table 1 summarizes the results of sludge granulation as seen in the selected studies.

However, based on our knowledge, no studies have used electrical current to enhance anoxic granulation. In this study, an alternating current based on a low voltage-low frequency current is used to form anaerobic granulation. The heterotroph and autotroph nitrate reducer's population of the granules, surface properties of the granules microorganism, settling and shear strength property of granules, solid-state NMR analysis, apoptosis assay by flow cytometry analysis and dehydrogenase activity were investigated.

2. Materials and methods

2.1. Bioreactor and start-up

All chemicals were of an analytical reagent grade and used without further purification. Microbial biofilm and granules were synthesized in a batch biofilm-electrode reactor that was supplied with alternating current named ACBER. This bioreactor had a working volume of 5 L with 304 stainless steel mesh (SS) and carbon cloth (CC) electrodes in cylindrical cross-section with a distance of 2-cm between electrodes. Applied current (in terms of peak-to-peak voltage (Vpp)) supplied by an AFG-2000 function generator (GW INSTEK; 0–10 Vpp, 0.056 A, 50 Ω). The biofilm electrode reactor was seeded by activated sludge from a municipal wastewater treatment plant in Tehran, Iran. The reactor operated in batch mode with a contact time of 6.5 h, an influent nitrate of 600 mg/L with Ibu as an organic carbon source with a C/N ratio of 4.2; an alternating current of 8 Vpp with a sinusoidal waveform frequency of 10 Hz; and maintaining an ambient temperature of about 20 °C. The concentration of the suspended biomass added to the ACBER was 4000 mg/L as a mixed liquor suspended solid (MLSS) and the ratio of Volatile Suspended Solids (VSS) to Total Suspended Solids (TSS) was 82% [23]. In the proposed system, the first step studied nitrate removal in the presence of Ibuprofen (Ibu) representing a difficult biodegradable organic carbon source to achieve both nitrate and Ibu removal simultaneously. The efficiency for removal of pollutants against operating parameters has been tested and discussed elsewhere, indicating a high level of efficiency for the system. Properties of the granules generated during this system operation will be discussed in the following sections.

2.2. Heterotroph and autotroph nitrate reducer's population determination in granules

The most probable number (MPN) method was used to identify the autotroph and heterotroph population density of the denitrifying bacteria [24]. This method has been used in other studies to identify the denitrifiers in soil and aqueous samples. For calculating the MPN, the formed granules in the ACBER were sampled. The granule samples are gently vortexed for 15 min, then the suspended granules of bacteria were divided into two aliquots for simultaneous determination of the HDB and ADB population. As described by Tang et al., the 10-fold dilution series was used to calculate the MPN with the following protocol: 1) Preparing HDB[a] and ADB[b] sterilized growth medium; 2) Adding 10 mL of each prepared dilution by deionized water into 100 mL of each for replication per dilution of sterilized growth medium, 3) Incubation of HDB and ADB tubes in 30 °C for 14 days, and 4) Estimation of MPN using positive tubes of each dilution of HDB and ADB, respectively.

Note: [a] HDB growth medium constitutions were: 2.0 g KNO_3, 5.0 g $C_6H_5Na_3O_7$, 0.2 g $MgSO_4{\cdot}7H_2O$, 1.0 g K_2HPO_4, 1.0 g KH_2PO_4 per 1 L of deionized water.

[b] ADB growth medium constitutions were: 2.0 g KNO_3, 9.8 g $NaHCO_3$ (the same C/N with HDB medium), 0.2 g $MgSO_4{\cdot}7H_2O$, 1.0 g K_2HPO_4, 1.0 g KH_2PO_4 per 1 L of deionized water.

Note: before inoculating the pH of both growth medium was adjusted to 7.2 using 1 M HCL and NaOH.

Please cite this article in press as: E. Hoseinzadeh, et al., Low frequency-low voltage alternating electric current-induced anoxic granulation in biofilm-electrode reactor: A study of granule properties, Process Biochem (2017), http://dx.doi.org/10.1016/j.procbio.2017.02.019