Bioresource Technology 247 (2018) 259–266

Contents lists available at ScienceDirect

Bioresource Technology

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

Microbial dynamics of biofilm and suspended flocs in anammox membrane bioreactor: The effect of non-woven fabric membrane

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ARTICLE INFO

Keywords:
Anammox
Non-woven fabric
Substrate removal model
Microbial community dynamics
High-throughput sequence

ABSTRACT

Membrane bioreactor with non-woven fabric membranes (NWMBR) is developing into a suitable method for anaerobic ammonium oxidation (anammox). As a carrier, non-woven fabric membrane divided total biomass into biofilm and suspended flocs gradually. Total nitrogen removal efficiency was maintained around 82.6% under nitrogen loading rate of 567.4 mg N/L/d after 260 days operation. Second-order substrate removal and Stover-Kincannon models were successfully used to simulate the nitrogen removal performance in NWMBR. High-throughput sequence was employed to elucidate the underlying microbial community dynamics. *Candidatus Brocadia*, *Kuenenia*, *Jettenia* were detected to affirm the dominant status of anammox microorganisms and 98.2% of anammox microorganisms distributed in biofilm. In addition, abundances of functional genes (*hzs*, *nirK*) in biofilm and suspended flocs were assessed by quantitative PCR to further investigate the coexistence of anammox and other microorganisms. Potential nitrogen removal pathways were established according to relevant nitrogen removal performance and microbial community.

1. Introduction

Excessive discharge of anthropogenic nitrogen compounds in aquatic ecosystem has been a global concern causing a new wave of specific treatment technology innovation (Kong et al., 2016). Anaerobic ammonium oxidation (anammox) is a novel biotechnology method to minimize negative environmental impacts of nitrogen-rich wastewater. In this process, ammonium is converted to nitrogen gas under anaerobic conditions using nitrite as electron acceptor (Mulder et al., 1995), which extends the virgin nitrogen transformation pathways. Therefore, there is no need to convert all ammonium to nitrate under aerobic condition in nitrification process, or convert nitrate to nitrogen gas with carbon source under anoxic condition in denitrification process. As a result, anammox process could save 100% external carbon source, at least 50% oxygen and 90% operation cost, when compared to nitrification and denitrification process (Jetten et al., 2001; Tal et al., 2006). However, the growth rates (μ_{max}) of anammox microorganisms (such as *Candidatus Kuenenia*, *Candidatus Brocadia*, *Candidatus Jettenia*) are only 0.000083–0.00017 d^{-1}, which lead to the time-consuming start-up of anammox process (Ali et al., 2015; Dapena-Mora and Campos, 2006; Oshiki et al., 2011). It is nearly impossible to increase the microorganisms' growth rate significantly. Therefore, it is important to select reasonable reactor for anammox, which could reduce the wash-out of anammox biomass and shorten the start-up of anammox process.

Membrane bioreactor (MBR) is regarded as the most reasonable reactor and has been widely applied in lab-scale anammox researches. It is found that MBR can be used for the purification enrichment of anammox microorganisms with an unprecedented purity of 97.6% (van der Star et al., 2008). Tao et al. compared the anammox performance in MBR with that in SBR, the results of effluent suspended solids (SS, < 3 mg/L) and nitrogen removal (78% ammonium and 94% nitrite) indicated MBR was a more appropriate reactor for anammox process (Tao et al., 2012). The start-up time of anammox process in MBR (59 days) was also significantly shorter than that in SBR (101 days) (Wang et al., 2012). However, MBR with hollow fiber membrane module usually leads to expensive production/maintenance cost, which hinders its applications in anammox process. Therefore, the research emphasis is gradually gathered around the membrane bioreactor with non-woven fabric membrane modules (NWMBR), which is a hybrid of remarkable biomass-retaining ability and considerable low production/maintenance cost (Liu et al., 2008; Meng et al., 2014; Ni et al., 2010; Ren et al., 2015a). In NWMBR, non-woven fabric sheet is formulated into membrane module to replace traditional hollow fiber membrane

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http://dx.doi.org/10.1016/j.biortech.2017.09.070
Received 5 June 2017; Received in revised form 6 September 2017; Accepted 9 September 2017
Available online 19 September 2017
0960-8524/

module, not only acting as separation module for biomass retention but also as microbial carrier for biofilm formation. Shortened anammox start-up, increased the nitrogen removal, decreased membrane cost and alleviated membrane fouling could be achieved in NWMBR, when compared with those of traditional hollow fiber module MBR (Ren et al., 2015a).

Previous study confirmed that main biomass in anammox-NWMBR would attach on non-woven fabric membrane forming biofilm, while little biomass was maintained in suspended flocs (Ren et al., 2015a). Cultivated anammox microorganisms usually coexist with nitrifiers (Liu et al., 2008) and denitrifiers (Chamchoi et al., 2008) in anammox reactors. Different microorganisms generally distribute on different layers of biofilm based on different oxygen diffusion and gradients in the biological flocs (biofilm or suspended flocs). Coexisted nitrifiers could oxidize ammonium into nitrite on outer biofilm under aerobic condition ($0.2\% < O_2$), denitrifiers could reduce nitrite to nitrogen gas on middle biofilm under anoxic condition ($0\% < O_2 < 0.2\%$), and dominated anammox microorganisms could convert ammonium and nitrite simultaneously to nitrogen gas under anaerobic condition ($O_2 \approx 0\%$) which mainly concentrated on inner biofilm. Produced microenvironment on non-woven fabric membrane is suitable for coexistence of these microorganisms to realize the effective removal of nitrogen. However, literature about the microbial community dynamics in NWMBR is scarce.

Process modeling is a widely accepted approach to describe and predict the substrate removal performance in biological systems. Anammox process has been widely simulated by different models such as first-order, second-order substrate removal and Stover–Kincannon models (Ni et al., 2010). These data are beneficial in the operation optimization for better nitrogen removal performance. However, no relevant model has been applied in NWMBR to depict or predict the nitrogen removal performance.

The aims of this study are twofold. The first is to evaluate the nitrogen removal performance of NWMBR and predict the removal capacity using substrate removal models. The second is to investigate the microbial community dynamics and relevant extracellular polymeric substance (EPS) release in NWMBR during nitrogen-rich wastewater treatment. To the best of our knowledge, all these attempts are firstly applied in a NWMBR for anammox process.

2. Materials and methods

2.1. Experimental set-up

A novel NWMBR occupying two non-woven fabric membrane modules was developed in this study. Configuration and operation strategy of this NWMBR had been described in the previous study (Ren et al., 2015a). Nitrogen characteristics of influent for entire experiment were 126 mg/L of ammonium and 151.2 mg/L of nitrite. Hydraulic retention time (HRT) was shortened from 96 h to 12 h by adjusting the pump injection rate. Sludge retention time (SRT) was increased from 114 d to 560 d due to the gradually formed biofilm. The non-woven fabric membrane module was fabricated from polyethylene terephthalate (PET) non-woven fabric.

2.2. Characterization of non-woven fabric

Morphology of non-woven fabric sample (1 × 1 cm) was characterized by the field emission scanning electron microscope (SEM, NanoSEM-450, FEI) after gold coating. A contact angle system (A-200, MAIST) was employed to detect the hydrophobicity or hydrophilicity of the non-woven fabric surface. Water droplet was set to 7 μL and calculation method was Young-Laplace (Circle) algorithm for hydrophobic (hydrophilic) contact angle.

2.3. Chemical analysis

Concentrations of nitrogen compounds and mixed liquor suspended solids (MLSS) were measured according to the APHA (Gilcreas, 1998). Water samples from influent and effluent were collected every 4 days for the nitrogen compounds concentration calculation, and every 10 days for the effluent SS calculation, while the reactor MLSS (biofilm and suspended flocs) was only measured on day 260. Nitrogen loading rate (NLR) and nitrogen removal rate (NRR) were calculated according to the concentrations of nitrogen compounds in influent and effluent. Trans membrane pressure (TMP) was monitored by a vacuum-table.

2.4. Kinetics models for nitrogen removal

Different substrate removal models were employed to depict and predict the nitrogen removal performance and capacity.

2.4.1. First-order substrate removal model

The equation of first-order substrate removal model could be expressed as:

$$-\frac{dS}{dt} = \frac{QS_i}{V} - \frac{QS_e}{V} - k_1 S_e \tag{1}$$

where $(-dS/dt)$ is the substrate degradation rate in reactor (g/L/d), Q is the influent flow rate (L/d), V is the reactor volume (L), S_i is the substrate concentration in influent (g/L), S_e is the substrate concentration in effluent (g/L) and k_1 is the first-order substrate removal rate constant (1/d).

The substrate degradation rate is nearly negligible under pseudo-steady-state conditions. Therefore, the virgin equation could be simplified as:

$$\frac{QS_i}{V} - \frac{QS_e}{V} = \frac{S_i - S_e}{HRT} = k_1 S_e \tag{2}$$

where HRT is the hydraulic retention time (d).

2.4.2. Second-order substrate removal model

Second-order substrate removal model is described as (Grau et al., 1975):

$$-\frac{dS}{dt} = k_{2(S)} X \left(\frac{S_e}{S_i}\right)^2 \tag{3}$$

where $K_{2(S)}$ is the second-order substrate removal rate constant and X is the mean biomass concentration in the reactor.

After integration and linearization, the following equation is obtained:

$$\frac{S_i\ HRT}{S_i - S_e} = HRT + \frac{S_i}{k_{2(S)} X} \tag{4}$$

The parameter of $S_i/K_{2(S)}X$ is usually considered as a constant and the modified second-order model is given as below:

$$\frac{HRT}{E} = a + b\ HRT \tag{5}$$

where E is the total substrate removal efficiency (%), a replaces the virgin $S_i/K_{2(S)}X$ and b is a constant.

2.4.3. Stover-Kincannon model

Original Stover–Kincannon model is expressed as (Stover and Kincannon, 1982):

$$\frac{dS}{dt} = \frac{U_{max} \frac{QS_i}{V}}{k_b + \frac{QS_i}{V}} \tag{6}$$

where U_{max} is the maximum substrate utilization rate constant (g/L/d) and K_b is the substrate saturation constant (g/L/d).