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Start-up of the anammox process and membrane fouling analysis in a novel rotating membrane bioreactor

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HIGHLIGHTS

- A rotating flat-sheet membrane bioreactor was employed to start up anammox process.
- The anammox process was successfully started up within around 16 days.
- The hydrodynamic conditions were investigated by particle image velocimetry.
- After 60 days of operation, the membrane fouling in the novel MBR was very slight.

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ABSTRACT

A rotating flat-sheet membrane bioreactor (RFMBR) was employed to start up anammox process, in comparison with a conventional membrane bioreactor (CMBR). The anammox process was successfully started up within around 16 days in both bioreactors. The particle image velocimetry (PIV) analysis showed a larger velocity gradient and a stronger shear stress on membrane surface in RFMBR than in CMBR. At the end of the experiment, the mean particle size of anammox granules achieved 899 μm in RFMBR, while the value reached 809 μm in CMBR, and the trans-membrane pressure (TMP) reached 4 and 16 kPa in RFMBR and CMBR, respectively. Furthermore, the scanning electron microscope (SEM) observation of the biofilm formed on membranes illustrated that a much thinner biofilm with the thickness of 35 μm was formed in RFMBR, compared to the value of 120 μm in CMBR.

1. Introduction

Discharge of nitrogen from wastewater into surface water bodies may result in eutrophication, toxicity to aquatic species, as well as emissions of nitrous oxide to atmosphere during the denitrification [1]. The traditional biological nitrogen removal process mainly consists of two sub-steps, i.e. autotrophic nitrification and heterotrophic denitrification. However, this traditional process is costly due to needing supplementation of oxygen for nitrification and external carbon sources for denitrification [2], and is complicated when treating highly concentrated nitrogen wastewaters with low C/N ratio [3]. Anaerobic ammonium oxidation (anammox) process, a newly discovered biochemical pathway that allows coupling between ammonium oxidation with nitrite reduction to nitrogen gas (N_2) as the terminal product under anoxic conditions [4,5], provides an attractive alternative for nitrogen removal. In fact, the anammox process has since been successfully employed to treat various ammonium-rich wastewaters [6,7]. Nevertheless, start-up of the anammox process is always a challenge for practical applications [8], due to anammox bacteria, the bacteria responsible for anammox process, growing at a very slow rate with a doubling time of approximately 11 days [9].

Reactor configuration is one of the factors influencing the anammox start-up process. Various reactors were developed and optimized to enrich anammox bacteria and start up anammox process, such as fixed bed biofilm reactor (FBBR) [7,10–12], sequencing batch reactor (SBR) [9,13–15], rotating biological contactor (RBC) [16–18], fluidized bed reactor [4,7], gas-lift reactor [19], granular sludge bed reactor (GSBR) [20], trickling filter [21], upflow anaerobic sludge blanket (UASB) [22], as well as upflow sludge bed filter (UBF) [23]. It is widely acknowledged that the enrichment of slow-growing microorganisms requires efficient retention of biomass [24]. Nevertheless, in the above-mentioned reactors, start-up of the anammox process was inevitably impeded by a continuous loss of anammox biomass via the effluent, leading to more difficult cultivation of the biomass [9,24].

In recent years, the membrane bioreactor (MBR) becomes a new hotspot for start-up of the anammox process due to its full biomass retention, either immersed MBR (iMBR) [8,24,25] or sidestream MBR (sMBR) [2,26]. For the more compact and energy efficient immersed iMBR [27], a significantly shorter start-up period and higher biomass

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purity was obtained in previous study compared to other reactor configurations. But membrane fouling was still an issue in these investigations. For example, in the experiment of Wang et al. [8], membrane pressure increased rapidly in the first 2 weeks and the module was chemically cleaned on day 43. Van de Star et al. [24] replaced membrane module every 10 days to prevent biofilm growth on the membrane surface. In the research of Trigo et al. [25] that employed a membrane sequencing batch reactor (MSBR), a backwashing period of 3 min was set in a 6 h cycle to minimize fouling and the permeation time was only 18 min in a cycle.

Recently, shear-enhanced membrane filtration using a moving membrane module to mitigate membrane fouling has attracted much attention. Zuo et al. [28] introduced a new bioreactor named submerged rotating membrane bioreactor (SRMBR), the membrane module of which comprised several rotatable round flat-sheets and a hollow rotating axis. The flat-sheet plates are parallel to each other, and rotate around the hollow rotating axis. The equilibrium permeate flux rose with the increase in rotary speed of membrane plates, proving that rotation of membrane module could enhance shear forces on membrane surface and mitigate membrane fouling. But the SRMBR also had some drawbacks, such as weak turbulence created by the membrane module, as well as difficult washing and replacement of the membrane material.

In this research, a rotating flat-sheet membrane bioreactor (RFMBR) was proposed and used to start up anammox process, in comparison with a conventional membrane bioreactor (CMBR). The membrane fouling was analyzed through the trans-membrane pressure (TMP) rise and biofilm formation on membranes. The particle image velocimetry (PIV), a widely used technique that can provide velocity field in fluids [29], was employed to investigate the hydrodynamic conditions in the reactors. In addition, the morphology of anammox granules and relative abundance of anammox bacteria in total bacteria was also analyzed by scanning electron microscope (SEM) and florescence in situ hybridization (FISH), respectively.

2. Materials and methods

2.1. Experimental set-up

The RFMBR and CMBR are the same as those in our previous study [30]. The membrane module of RFMBR is composed of 9 flat-sheets and 2 plates. The diameter of each plate is 160 mm, and the effective height and width of each flat-sheet is 153 and 39 mm, respectively. The two sides of each flat-sheet are covered with polyvinylidene difluoride (PVDF) membrane with an average pore size of 0.2 μm. The flat-sheets are vertically and symmetrically placed on the edge of the plates. The angle between plane of each flat-sheet and its corresponded radius is fixed at 30°. The hub of the bottom plate is connected with gears that are driven by an adjustable speed electromotor.

Two flat-sheet membrane modules are vertically and symmetrically installed in the two sides of the CMBR. The membrane material is the same PVDF used in RFMBR. An outlet connected with external outlet pipe is opened on the bottom of each module. The reactor is equipped with a mechanical stirrer driven by an electromotor in the middle. The total effective filtration of both reactors is 0.09 m^2.

2.2. Operational strategy

Each reactor was inoculated with anammox activated sludge from an upflow anaerobic sludge blanket (UASB) which had been operating for more than 2 years in our laboratory. After inoculation, the initial SS in each reactor was 2232 mg/L. The MBRs were continuously fed with the same synthetic wastewater (medium composition shown in Table 1) by peristaltic pumps and were operated in the mode of constant flux for 3 stages: the flux was 10, 6 and 8.5 L/(m^2h) for stage I (days 0–8), II (days 8–37) and III (days 37–61), corresponded to a HRT of 14.4, 24 and 17 h, respectively. The rotational speed of the membrane module in RFMBR was moderately set to 20 rpm according to our previous study [30], when the membrane fouling rate was relatively low; whereas the stirrer in CMBR worked at a speed of 60 rpm in order to provide enough forces to make the biomass suspended. The synthetic wastewater was replaced every day to avoid the changes in feed composition and was purged with pure nitrogen gas during the preparation process to remove the oxygen (influent DO<0.1 mg/L). The temperature in the MBRs was maintained at about 33 °C. The sludge retention time (SRT) was infinite since there was no sludge waste from the reactors during the whole experimental trial except for sampling. According to the previous reported ratio of nitrite consumption to ammonia consumption in anammox reaction (1.32) [9], the medium concentrations of $(NH_4)_2SO_4$ and $NaNO_2$ were initially set to about 150 and 200 mg N/L, respectively, to maintain the ratio of ammonia to nitrite at 1:1.33, and the N-loading rate was changed by varying the HRT.

Table 1
Medium composition.

Medium composition	Concentration (mg/L)	Medium composition	Concentration (mg/L)
$(NH_4)_2SO_4$	550	$NaNO_2$	300
KH_2PO_4	50	$KHCO_3$	500
Trace solution I	1 mL/L	Trace solution II	1 mL/L
Trace solution composition I	Concentration (g/L)	Trace solution composition I	Concentration (g/L)
EDTA	10	$FeSO_4 \cdot 7H_2O$	18
Trace solution composition II	Concentration (g/L)	Trace solution composition II	Concentration (g/L)
KCl	1.4	NaCl	1
$CaCl_2 \cdot 2H_2O$	1.4	$FeSO_4 \cdot 7H_2O$	1

2.3. Analytical methods

2.3.1. Chemical analysis

The concentrations of ammonium, nitrite, nitrate, suspended solids (SS) and volatile suspended solids (VSS) were determined according to standard methods for the examination of water and wastewater described in detail by American Public Health Association [31]. DO and pH were measured by a DO meter (YSI55/12FT, USA) and a pH meter (Sartorius PB-10, Germany), respectively. The particle size was obtained with a laser particle size analysis system (Mastersizer 2000, Malvern, UK).

The extraction of bound extracellular polymeric substances (EPS), normalized as the sum of protein (PN) and polysaccharide (PS), was performed based on a cation ion exchange resin (Dowex-Na form) method [32]. For quantitative analysis of proteins, the modification of Lowry method described by Frolund et al. [33] was used with bovine serum albumin as standard. The anthrone method modified by Raunkjer et al. [34] was employed for the quantification of polysaccharides with glucose as standard.

2.3.2. Fluorescence in situ hybridization (FISH) analysis

FISH analysis was used to investigate the proportion of anammox bacteria to background bacteria. Paraformaldehyde cell fixation and FISH analysis were performed according to the standard hybridization protocol [35,36], using oligonucleotide probes for eubacterium (EUB338 plus) and anammox bacteria (AMX820) [21,37]. The hybridization was performed on 4% (w/v) paraformaldehyde-fixed sludge samples. A Leica TCS-SP2 confocal scanning laser microscope (CSLM) (Leica, Germany) was employed to acquire images.

2.3.3. Morphological observation

The granule morphology was analyzed by a camera (Canon EOS 550D, Japan), a light microscope (Olympus CX21, Japan), as well as the scanning electron microscope (SEM, JEOL JSM-5600LV, Japan). The surface and section morphology of the biofilm on fouled membranes