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Nitrifier characteristics in submerged membrane bioreactors under different sludge retention times

*Tao Yu, Rong Qi, Dong Li, Yu Zhang, Min Yang**

State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Haidian District, Beijing 100085, China

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ABSTRACT

Three submerged membrane bioreactors (MBRs) were operated continuously for 230 days by feeding with synthetic inorganic wastewater (NH_4^+-N, 100 mg L^{-1}) under different solids retention times (SRTs. M_{30d}, 30 days; M_{90d}, 90 days; $M_{infinite}$, no sludge purge) to examine the influence of SRT on nitrification performance and microbial characteristics. All the reactors could oxidize NH_4^+-N to NO_3^--N effectively without accumulation of NO_2^--N. M_{30d} with the shortest SRT showed significantly higher specific ammonium oxidizing rate (SAOR, 0.22 kg NH_4^+-N kg^{-1} MLSS day^{-1}) and specific nitrate forming rate (SNFR, 0.13 kg NO_3^--N kg^{-1} MLSS day^{-1}) than the other two MBRs (0.12–0.14 kg NO_3^--N kg^{-1} MLSS day^{-1} and 0.042–0.068 kg NO_3^--N kg^{-1} MLSS day^{-1}, respectively). Short SRT led to low extracellular polymeric substances (EPS) concentration and long operating cycle. The nitrite oxidizing bacteria (NOB) ratios by both the fluorescence in situ hybridization (FISH) (3.6% for M_{30d} and 2.1–2.2% for M_{90d} and $M_{infinite}$) and MPN (1.4×10^7 cells g^{-1} MLSS for M_{30d} and 6.2×10^5 and 2.7×10^4 cells g^{-1} MLSS for M_{90d} and $M_{infinite}$) analyses showed that M_{30d} favored the accumulation of NOB, which was in accordance with the SNFR result. However, the ammonia oxidizing bacteria (AOB) ratios (3.5%, 3.2% and 4.9% for M_{30d}, M_{90d} and $M_{infinite}$) were not in accordance with the SAOR result. PCR-DGGE, clone library and FISH results showed that the fast-growing *Nitrosomonas* and *Nitrobacter* sp. were the dominant AOB and NOB, respectively for M_{30d}, while considerable slow-growing *Nitrosospira* and *Nitrospira* sp. existed in $M_{infinite}$, which might be an important reason why $M_{infinite}$ had a low SAOR and SNFR.

1. Introduction

The most important point of membrane bioreactors (MBRs) is that this technology permits biological wastewater treatment under desirable solids residence times (SRTs), which allows the systems to keep a sufficient amount of slow-growing microbes, such as ammonia oxidizing bacteria (AOB) and those specializing in degrading refractory compounds, by choosing a relatively long SRT (Stamper et al., 2003; Xing et al., 2000). A long SRT, at the same time, will permit a stable treatment and low excess sludge production (Fan et al., 2005; Howell et al., 2003). The higher biomass concentration also increases shock tolerance, which is particularly important where feed is highly variable (Bin et al., 2009). However, too long an SRT may result in membrane fouling due to the accumulation of large amount of EPS in the MBR systems (Djamila et al., 2008). How to choose a proper SRT to achieve high water quality while prevent serious membrane fouling is therefore an important issue for the operation of MBRs.

* *Corresponding author*. Tel.: +86 10 62923475; fax: +86 10 62923541.
E-mail address: yangmin@rcees.ac.cn (M. Yang).
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On the other hand, activated sludge is a complicated ecological system where different types of microbes cooperate or compete with each other. Our previous study has shown that a large amount of heterotrophic bacteria coexisted with AOB and nitrite oxidizing bacteria (NOB) even in an MBR fed with inorganic ammonia-bearing wastewater (Hongyan et al., 2005b). It was considered that these heterotrophs live mainly on EPS and dead AOB and NOB. Different microbes have different growth rates. Therefore, the selection of SRT may affect microbial community structures as well as bioactivities in the biological systems.

It is considered that almost no excess sludge will be generated if an MBR is operated without sludge purge. However, it is not known if such an operation mode will adversely affect the bioactivity and performance of the MBR system. So in the present study, three reactors (M_{30d}, M_{90d} and $M_{infinite}$) fed with inorganic ammonia-bearing wastewater were established, and the reactor performance, bioactivity and microbial community structures of the MBRs were investigated under different SRTs (M_{30d}, 30 days; M_{90d}, 90 days; $M_{infinite}$, no sludge purge) for 230 days.

2. Materials and methods

2.1. Experimental system

Fig. 1 shows the schematic diagram of the experimental system consisting of three identical MBRs (M_{30d}–$M_{infinite}$). A submerged hollow fiber membrane module (Mitsubishi Rayon Co. Ltd., Tokyo) with an average pore size of 0.4 μm and surface area of 0.2 m^2 was used for each MBR. The working volume of each reactor was 12 L and the HRT was controlled at 16 h. Air was supplied at a rate of 1 L min^{-1} to maintain dissolved oxygen (DO) at 3–5 mg L^{-1}. The pH in the three reactors was 7.5–8.0 and the temperature was kept at 20 ± 1 °C. The permeate flux was suctioned by a metric pump controlled with a timer (Hongyan et al., 2005a). The operational cycle included 4 min of suction and 1 min break. M_{30d} and M_{90d} were operated at an SRT of 30 days and 90 days, respectively, and $M_{infinite}$ was operated without sludge purging. The three systems were operated in parallel for 230 days. The membranes were rinsed with water when the operating pressure suddenly increased.

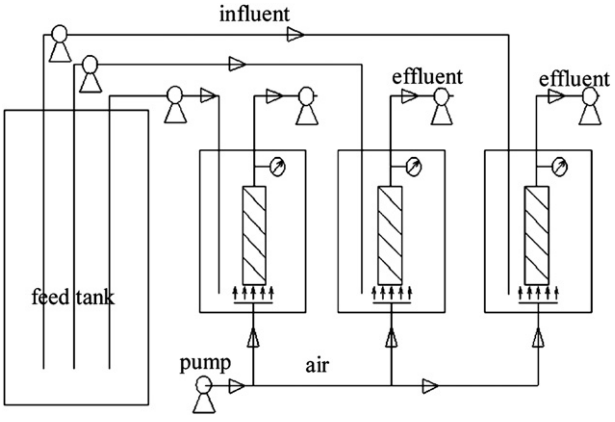

Fig. 1 – Schematic diagram of membrane reactors.

Seed sludge was taken from one sewage treatment plant in Beijing, and inoculated to MBRs with an average MLSS of 4300 mg L^{-1}. Synthetic inorganic wastewater (Hongyan et al., 2005a) with an influent NH_4^+-N of approximately 100 mg L^{-1} was fed to the MBRs soon after the inoculation of seed sludge to give a volumetric loading rate of 0.15 kg NH_4^+-N m^{-3} day^{-1}. Influent and effluent samples were take from each reactor to analyze NH_4^+-N, NO_2^--N, NO_3^--N and EPS, and sludge samples were taken to analyze MLSS, specific ammonium oxidizing rate (SAOR), specific nitrate forming rate (SNFR) and microbial community structures. SAOR and SNFR were used to determine the activities of activated sludge in oxidizing ammonia and nitrite, respectively. Detailed methods have been described previously (Hongyan et al., 2005a). EPS was extracted according to the methods proposed by Frolund et al. (1996). The content of polysaccharides was measured according to a phenol–sulphuric acid method and that of protein was determined with the Lowry method.

2.2. Most probable number (MPN) enumeration and plate counting

The MPN was used for the enumeration of AOB and NOB, respectively, together with the FISH method (Hongyan et al., 2005a). 1 mL sample taken from a reactor was dispersed by sonication. Serial 10-fold dilutions of the inoculum sources were prepared in sterile distilled water and 1 mL portions were transferred to MPN tubes containing 9 mL of the enumeration medium. Five replicate tubes were prepared per dilution for enumeration. The MPN tubes were incubated for 30 days at 28 °C. Production and oxidization of nitrite in each tube were examined by colour determination after adding zinc powder and Griess reagent, respectively. The colony-forming unit (CFU) was used for the heterotrophs by depositing 0.1 mL serial dilutions on LB agar plates as inoculum. After incubating for 24 h under 37 °C, the numbers of the colonies on the plates were counted.

2.3. FISH analysis

Following sonication on ice for about 3 min, activated sludge samples were fixed for 3 h with 4% paraformaldehyde at 4 °C and stored in a 1:1 mixture of phosphate-buffered saline (PBS, pH 7.4) and ethanol at −20 °C (Amann et al., 1995). 3 μL samples were placed in wells of the slides and immobilized for 3 h. Then the slides were dehydrated for 5 min using 50%, 80%, 98% ethanol, respectively. The hybridization and washing procedures was the same with the protocol previously described by Amann et al. Fluorescent hybridized cells were analyzed with an epifluorescence microscope (Axioskop2 mot plus, Zeiss, Germany) equipped with a cooled CCD camera (AxioCam MRm, Zeiss Corp., Germany). The proportion of the nitrifiers was calculated with the software provided by Zeiss (Axio Vision 4.1). At least 40 views were obtained for each sample to give an average result.

Probes NSO190 (5′-CGA TCC CCT GCT TTT CTC C-3′), NIT3 (5′-CCT GTG CTC CAT GCT CCG-3′) and EUB338 (5′-GCT GCC TCC CGT AGG AGT-3′) were used for AOB, NOB and active bacteria, respectively (Mobarry et al., 1996).