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The influence of controlling factors on the start-up and operation for partial nitrification in membrane bioreactor

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ABSTRACT

In this study, the partial nitrification process was started-up successfully in a membrane bioreactor (MBR). The influence of temperature and DO was investigated by sequencing operation of membrane bioreactor. The preferred values were proved as 35 °C and 0.3–0.5 mg/L, respectively, and were indicated as indispensable controlling factors. In order to increase the sludge concentration, new seed sludge was added into the reactor, which caused the absolute destruction of the reactor performance. The results of reactor experiments showed that the free ammonia (FA) concentration of 74 mg NH_3/L, as the influent ammonium concentration of 600 mg N/L, was a useful and effective factor to recover the partial nitrification performance. Fluorescence in situ hybridization analysis indicated that nitrifiers hybridizing with NIT3 and NSR1156 were present and active in MBR, which were then eliminated under high FA concentration. The microbiological community analysis further provided the necessary biological information for the realization of partial nitrification.

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1. Introduction

It is an important issue to remove nitrogen components from wastewater nowadays since these components can be toxic to aquatic life, and cause oxygen depletion and eutrophication in receiving water (Brauer and Eitzer, 1997). Comparing with physicochemical processes, biological nitrogen removal is more effective and relatively inexpensive, so it has been widely developed.

In the last few years, several novel biological technologies have been developed, including partial nitrification, nitrifier denitrification, anaerobic ammonium oxidation (anammox) (Cervantes et al., 2001; Dong and Tollner, 2003; Chamchoi et al., 2008), and its combined system (completely autotrophic nitrogen removal over nitrite, Canon) (Gong et al., 2008). A combined system for nitrogen removal, based on partial nitrification in a first aerobic reactor with anaerobic ammonium oxidation in a second tank to ensure total nitrogen removal throughout an autotrophic process (Jetten et al., 1997), has various advantages, such as no need for external carbon addition, negligible sludge production and less energy and oxygen requirement than the conventional processes (Jetten et al., 2002). Partial nitrification, consisting in stopping the oxidation of ammonium at the stage of nitrite, is presented as a possible way to achieve the desired feed for anammox process (van de Graaf et al., 1995). The partial nitrification process is ideally suited to remove nitrogen from wastewater streams with high ammonium concentration (Hellinga et al., 1998), which has been recognized very promising for improved sustainability of wastewater treatment.

Influences of several factors on nitrite accumulation have been performed, such as free ammonia (FA) concentration by adjusting pH or temperature, dissolved oxygen (DO) concentration, and heterotrophic nitrification (Rhee et al., 1997). Controlling the DO at low concentration can create a favorable condition which is suitable for nitrite accumulation since the oxygen saturation coefficients of Monod kinetics for nitritation and nitratation are known to be 0.3 and 1.1 mg/L, respectively (Wiesmann, 1994). Only at temperature above 25 °C, it is possible for the ammonium oxidizers to effectively outcompete the nitrite oxidizers (Hellinga et al., 1998). Establishment and maintenance of a partial nitrification reactor successive performance require that nitrite-oxidizing bacteria can be washed out from the reactor by controlling the sludge retention time (SRT) or be out competed under execrable conditions compared with ammonium-oxidizing bacteria. Unfortunately, it seems to be difficult to achieve because ammonium- and nitrite-oxidizing bacteria can be found almost everywhere (Sinha and Annachhatre, 2007). Not all the process conditions which suppress nitrite oxidation are currently understood.

In recent years, membrane bioreactors (MBRs) have been widely used in wastewater treatment to achieve higher effluent quality, which is often difficult to be effectively met by conventional activated sludge process. The advantages of MBR are a high

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mixed liquid suspended solids (MLSS) concentration, a lower excess sludge production and the production of treated water can be reused (Meng et al., 2008). In addition, the space occupied by MBR systems is greatly reduced due to the absence of settling tanks and the reduction in bioreactor volume made possible by the higher biomass concentration. Unlike the conventional activated sludge system, the MBRs are characterized by a complete retention of the biomass inside the bioreactor for the use of membrane filtration, which controls and increases the SRT independently from the hydraulic retention time (HRT) (Meng et al., 2008). High SRTs enable one to increase the sludge concentration and the applied organic load, thereby increasing the pollutant degradation. This allows the development of slow-growing microorganisms able to remove pollutants contained in wastewater, resulting in improved removal rate.

In this study, our experiments were carried out in a completely stirred tank reactor (CSTR) with a submerged hollow fiber membrane module for a period of over 255 days (37 weeks). Primary experiments were carried out and the influences of DO and temperature on ammonium oxidation and nitrite accumulation were investigated after the seed sludge inoculation. In order to increase sludge concentration in the reactor, new seed sludge was added. Unfortunately, the performance of the reactor was destroyed absolutely. Hence, in order to recover partial nitrification process, experiments with the optimized parameters summarized from the previous investigations were implemented, and the concentration of ammonium in influent was increased to 600 mg N/L directly to utilize the inhibition effect of FA on nitrifiers. The change of microbiological community was investigated by the 16S rRNA-based molecular technique and fluorescence in situ hybridization (FISH) during the experiments.

2. Methods

2.1. Experimental set-up

The reactor had a working volume of 14 L, which was well equipped with a submerged hollow fiber membrane module (polypropylene, hydrophilic, pore size: 0.1 μm, filtration area: 0.2 m^2, Hangzhou Kaihong Membrane Technology Co. Ltd., China). Water-bath as used to control temperature of liquid inside reactor to be within 35 ± 1 °C (Kim et al., 2006). The air was supplied through the reactor liquid phase using an air sparger at the bottom. DO concentration and pH value in the reactor were measured by a DO (YSI 55/12 FT, USA) and pH (Sartorius PB-10, Germany) meter, respectively. The oxygen concentration was varied during the start-up and step-wise reduced by subsequent adjustment of oxygen loading by gas flowmeter. The influent pH was maintained around 8 by addition of $KHCO_3$ when required. Full mixing within the reactor was achieved with one set of mechanical stirrer, which is at the bottom of the liquid phase, comprising of two blades. The feeding solution of synthetic wastewater was added to the reactor using peristaltic pump. Constant flux was maintained by adjusting the rotation rate of the peristaltic pump.

2.2. Biomass and synthetic wastewater

The reactor was inoculated with 8 L sludge from an activated sludge treating domestic wastewater from a wastewater treatment plant, Dalian, China. The seed mixture contained initial biomass concentrations of 11.15 g MLSS/L and 6.25 g MLVSS/L. The new seed sludge which was added to increase the sludge concentration in the reactor, having initial biomass concentrations of 12.58 g MLSS/L and 10.07 g MLVSS/L.

The synthetic wastewater used in this experiment was a mineral medium supplemented with 70 mg NH_4^+–N/L in the form of $(NH_4)_2SO_4$ initially, which was increased to 300 mg N/L gradually along the course of the experiment. HRT was maintained at 16 h, so nitrogen loading rate was varied from 105 to 450 mg N/L d. On day 175, the concentration was increased to 600 mg N/L directly. HRT was changed to 12 h, relevant nitrogen loading rate was 1200 mg N/L d. The composition of the mineral medium was (g/L): $KHCO_3$ 1.25, KH_2PO_4 0.025, $CaCl_2 \cdot 2H_2O$ 0.3, $MgSO_4 \cdot 7H_2O$ 0.2, $FeSO_4$ 0.00625, EDTA 0.00625. The compositions were based on the previous studies (Third et al., 2001).

2.3. Partial nitrification reactor operation strategy

In order to obtain optimal effluent as the feed for anammox process (composition of equimolar amounts of ammonium and nitrite), investigations were carried out during primary experiments. The reactor was run for 159 days. The influent ammonium concentration was increased from 70 to 300 mg N/L step-wise in the start-up course. Afterwards, experiments in conditions of various DO and temperature were implemented. On day 170, the reactor performance was destroyed by adding new seed sludge. According to previously reported (Taichi et al., 2008), high influent ammonium concentration (>500 mg/L) was useful for the realization of partial nitrification. And so, in this case, influent ammonium concentration was raised to 600 mg/L to recover the partial nitrification performance, and then the reactor was run for over 80 days. The sludge samples were collected after regular intervals and microbial population shift was studied using molecular techniques.

2.4. Chemical analysis of water quality

The analyses of mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), NH_4^+–N, NO_2^-–N and NO_3^-–N, were performed in accordance with standard methods (APHA, 1998). NH_4^+–N and NO_2^-–N were measured by using the different colorimetric methods and NO_3^-–N was analyzed by using ultraviolet spectrophotometric method. The pH was determined potentiometrically with a digital, portable pH meter. The DO level was measured with a digital, portable DO meter (YSI, Model 55, USA).

2.5. FISH analysis

Biomass cultured on the supporting fabrics was used for FISH and 4′-6-diamidino-2-phenylindole (DAPI) staining. Biomass samples were obtained as described above. In situ hybridization was performed according to the standard hybridization protocol (Amann, 1995). The following 16S rRNA-targeted oligonucleotide probes, ordered from TaKaRa (Dalian, China) were used for in situ detection of AOB (aerobic ammonia-oxidizing bacteria): beta-proteobacterial AOB-specific NSO190 (40% formamide; length position: 190–208) (Biesterfeld et al., 2001) labeled with Cy3 (red). Nitrobacter-specific NIT3 (40% formamide; length position: 1035–1048) and Nitrospira-specific NTSPA662 (35% formamide; length position: 662–679) labeled with FITC (green) (Wagner et al., 1996). CNIT3 and CNTSPA662 were used as competitive probes to avoid any mismatch during the hybridization. The domain-specific EUB338, EUB338-II and EUB338-III (Daims et al., 1999) labeled with fluorescein isothiocyanate (FITC) was used for in situ detection of all bacteria. For image acquisitions, an epifluorescence microscope (OlympusBX51, Japan) was used together with the standard software package delivered with the instrument (version 4.0).

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