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Enhanced biological nitrogen and phosphorus removal using sequencing batch membrane-aerated biofilm reactor

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HIGHLIGHTS

- SBMABR adopted intermittent aeration and artificially enhanced biofilm shedding.
- The COD, nitrogen and phosphorus were removed simultaneously in a single SBMABR.
- Intermittent aeration and periodic backwashing enhanced phosphorus removal.

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ABSTRACT

A sequencing batch membrane-aerated biofilm reactor (SBMABR) combined with intermittent aeration and artificially enhanced biofilm shedding was employed to enhance biological nitrogen and phosphorus removal. A long-term running of 112 days was applied to evaluate the performances of SBMABR. The removal efficiencies of chemical oxygen demand (COD), NH_4^+–N, total nitrogen (TN) and total phosphorus (TP) in SBMABR were maintained above 90%, 96%, 91% and 85% respectively. The periodic backwashing could control the biomass effectively. The stable and abundant *amoA* and *nirS* genes were beneficial to maintain the high removal efficiency of SBMABR. Real-time PCR results revealed that SBMABR created the suitable survival environment for ammonia oxidizing bacteria (AOB) and denitrifying bacteria.

1. Introduction

The removal of nitrogen and phosphorus from municipal and industrial wastewater causes eutrophication of the surface waters (Tchobanoglous and Burton, 1991). It is urgent for modern wastewater treatment plants to remove nitrogen and phosphorus efficiently. Biological nitrogen and phosphorus removal is a promising method that protects water against eutrophication (Rittmann and McCarty, 2001). The nitrogen and phosphorus removals are usually implemented through the activities of different microbial communities. The biological nitrogen removal involves continuous processes of nitrification and denitrification. The rate-limiting step of nitrogen removal is oxidation of ammonium by ammonia oxidizing bacteria (AOB) (Wu et al., 2009). And the biological phosphorus removal is achieved through enhanced biological phosphorus removal process, in which phosphate accumulating organisms (PAOs) are exposed to alternate anaerobic and aerobic environments (Mino et al., 1998). The enrichment of AOB and PAOs simultaneously in a single bioreactor is complicated due to their different physiological characteristics. It provides a long biomass retention time for enrichment of slow-growing organisms such as AOB and alternate anaerobic/aerobic conditions for domestication of PAOs. Recently, some studies focused on biological nitrogen removal by the sequential batch biofilm reactor system (Gonzalez-Martinez and Wilderer, 1991; Goncalves and Rogalla, 1992; Morgenroth and Wilderer, 1998; Gieseke et al.,

Abbreviations: AOB, Ammonia Oxidizing Bacteria; CMABR, Conventional Membrane-aerated Biofilm Reactor; DO, Dissolved oxygen; HRT, Hydraulic Retention Time; MABR, Membrane-aerated Biofilm Reactor; PAOs, Phosphate Accumulating Organisms; SBMABR, Sequencing Batch Membrane-aerated Biofilm Reactor; TN, Total nitrogen; TP, Total phosphorus

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2002; Li et al., 2003; Terada et al., 2006). For phosphorus removal from biofilm system, the major limiting processes are the efficient removal of phosphorus-rich biomass from the reactor and the mass transfer of dissolved oxygen (DO) (Chiou and Yang, 2008).

The membrane-aerated biofilm reactor (MABR) is a novel technology for wastewater treatment, in which hydrophobic gas-permeable membranes were used as a carrier of biofilm and for bubbleless oxygen transfer (LaPara et al., 2006; Syron and Casey, 2008). The main difference between MABR and conventional biofilm reactor is the counter-diffusion, which induces a unique microbial stratification that varies from oxygen-rich to anoxic environment (Shanahan and Semmens, 2004a; Cole et al., 2004). In MABR, nitrifying bacteria are immobilized at the membrane surface so that oxygen can be directly supplied to them without the formation of bubbles. Therefore, the nitrification is promoted effectively (Brindle et al., 1998; Yamagiwa et al., 1998; Terada et al., 2004; Yamagiwa et al., 2004; Satoh et al., 2004). Several recent studies have reported successful applications of MABR technology for removing chemical oxygen demand (COD) and nitrogen simultaneously in a single bioreactor (Semmens et al., 2003; Hu and Yang, 2008; Wei et al., 2012; Hou et al., 2013). However, for enhancing biological phosphorus removal of MABR system, the backwashing of biofilm must be carried out at the maximum internal storage of phosphorus in PAOs (Falkentoft et al., 1999; Morgenroth and Wilderer, 1999). Furthermore, periodic backwashing controls the thickness of the biofilm and enhances mass transfer effectively.

In order to remove nitrogen and phosphorus efficiently and simultaneously in a single bioreactor, a sequencing batch membrane-aerated biofilm reactor (SBMABR) system was designed to obtain the functions of intermittent aeration, step-feed influent and backwashing. A contrast experiment between SBMABR and conventional membrane aerated biofilm reactor (CMABR) system was designed to compare the removal mechanisms of COD, nitrogen and phosphorus. Finally, real-time quantitative PCR were conducted at the end of each stage to determine the total bacterial concentrations and the proportion of AOB and denitrifying bacteria based on 16S rRNA, *amoA* gene and *nirS* gene respectively, in order to investigate the influence of different C/N ratio on the change of bacterial community inhabited in MABR biofilms.

2. Materials and methods

2.1. Reactor configuration and operational conditions

The laboratory-scale SBMABR and CMABR (Fig. 1a and b) are both consisted of a membrane module and a cylindrical plastic shell. The membrane module contains 120 hydrophobic polypropylene dense hollow fibers with the length of 1000 mm, which were purchased from Hydroking Sci & Tech. Ltd., (Tianjin, China) and filled in the cylindrical shell. Oxygen supply rates of the membrane module are measured as 7.08 g/m^2 d and 10.29 g/m^2 d at the intramembrane pressures of 0.10 MPa and 0.15 MPa, respectively. The thickness of hollow fiber membrane is as thin as about 30–40 μm. The total volume of the feed tank, bioreactor and pipeline is 2.6 L. Synthetic wastewaters were fed to the two MABR systems with a hydraulic retention time (HRT) of 12 h. The main components of the synthetic wastewaters were: NH_4^+–N 60–70 mg/L, total phosphorus (TP) 9–13 mg/L, COD 170–190 mg/L (Condition I), 290–330 mg/L (Condition II) and 400–440 mg/L (Condition III) respectively. The COD/total nitrogen (TN) ratios of three conditions were 3, 5 and 7 respectively. Glucose, ammonium sulfate and dipotassium phosphate were the main source of COD, NH_4^+–N and TP in the synthetic wastewaters. The nutrient solution was consisted of the following compounds: K_2SO_4 45 mg/L, $MgSO_4 \cdot 7H_2O$ 55 mg/L, $NaHCO_3$ 300 mg/L, Na_2CO_3 100 mg/L, $Fe_2(SO_4)_3$ 0.1 mg/L, $CuSO_4 \cdot 5H_2O$ 0.1 mg/L, $MnSO_4$ 0.1 mg/L, and $ZnSO_4$ 0.1 mg/L.

The step-feed influent, intermittent aeration and backwashing applied in SBMABR system were the main differences from CMABR. In SBMABR, synthetic wastewater was stored separately in two tanks. The influent 1 was consisted of NH_4^+–N, TP and the nutrient solution. The influent 2 only contained organics. The purposes of separate storage can be divided into two parts. The first one is to avoid the serious lack of energy source needed in anaerobic phosphorus release process and denitrification process, which was caused by the excessive intake and removal of COD by heterotrophic microorganisms. Secondly, the two kinds of wastewaters are the simulations of high organics (high C/N) and high ammonia nitrogen (low C/N) wastewaters. The separate storage is beneficial to remove the contaminants in two kinds of wastewaters simultaneously, which has a high value of practical application. All these would possess not only an important theoretical meaning but also the realistic significance in a certain degree. The alternate anaerobic/aerobic condition caused by intermittent aeration is beneficial to nitrification/denitrification process and domestication of PAOs. The backwashing process could remove the phosphorus-rich biomass from the reactor and control of biofilm thickness effectively. For achieving intermittent aeration and step-feed influent, automatic control system was installed to control aeration, feed pump and circulating pump as can be seen in Fig. 1a. The influent 1 tank and the influent 2 tank were connected to the bioreactor through the automatically-controlled three-way valve. The operation procedure of SBMABR is shown in Fig. 1c (the color areas represent the equipment operation stage). At the end of each cycle, a 3-min backwashing which was provided by the reversal of the peristaltic pump (circulating pump) was carried out. The reverse flow at high velocity (0.05 m/s) washed away part of biofilm which does not adhere firmly. In CMABR system, the influent contained all components of the synthetic wastewater and automatic control system was not installed because of the needless of intermittent aeration, step-feed influent and backwashing. Synthetic wastewater was just pumped into the bottom of bioreactor from a feed tank and cycled in the system. The feed flow velocity of the two MABR systems was 0.03 m/s which is controlled by circulation pump. The aeration times of SBMABR and CMABR were 8 h and 12 h respectively. In order to compare the performances between the two bioreactors at the same aeration strength, the aeration pressures of SBMABR and CMABR were fixed at 0.15 and 0.10 MPa respectively. The water temperature was maintained at 20 ± 2 °C and the influent pH was between 7.8 and 8.3.

2.2. DNA extraction and real-time PCR: Analysis of nitrifiers, denitrifiers and total bacteria

At the end of each stage, biomass from three random sites of the membrane was carefully sampled using a razor, following which the samples were immediately stored at −80 °C until further analysis. DNA was extracted from the activated sludge used for initial seeding and from the biofilm samples (0.5 g each) collected at each stage using the Power Soil DNA Isolation Kit (MOBIO), according to the manufacturer's protocols.

Real-time PCR analysis was employed to quantify the copy number of the *amoA*, *nirS*, and 16S rRNA genes. Quantification of *amoA* fragments (500 bp) was implemented using the primer set *amoA*-1F (5′-GGGGTTTCTACTGGTGGT-3′) and *amoA*-2R (5′-CCCCTCKGSAAAG-CCTTCTTC-3′). Quantification of *nirS* fragments (423 bp) was carried out using the primer set *nirS*-Cd3aF (5-AACGYSAAGGARACSGG-3′) and *nirS*-R3cd (5′-GASTTCGGRTGSGTCTTSAYGAA-3′). Quantification of 16S rRNA gene was conducted using the primer set (530 bp) Eu27F/Eu518R (5′-GTATTACCGCGGCTGCTGG-3′). The primer characteristics and conditions for real-time PCR are shown in Table 1. The PCR reaction mixture consisted of (1) 10 μL of 2 × Evagreen, 2.5 μL (*nirS*