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Positive impact of biofilm on reducing the permeation of ampicillin through membrane for membrane bioreactor

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HIGHLIGHTS

- Positive effect of biofilm in MBR on retention of antibiotic was explored.
- A novel method was used to monitor the permeation process of antibiotics.
- Biofilm increased 3–28% resistance which in turn improved rejection of antibiotics.
- Permeation of antibiotic follows first-order with diffusion as the main mechanism.
- Compact structure of biofilm with EPS could explain the additional resistance.

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ABSTRACT

The membrane bioreactor (MBR) has recently been the focus of research for the treatment of emerging contaminants such as antibiotics in wastewater. Although the biofilm on membrane in an MBR has been considered a cause of "membrane biofouling", its positive impact on removing pollutants has not been well-studied. This study was designed to investigate the retention effect on the permeation of ampicillin (AMP) by the biofilm coated on cellulose acetate (CA) membrane (commonly used for MBRs) utilizing a novel method based on microbial sensitivity test. The bioflim layer (thickness of 12–16 μm) increased the resistance of the membrane for AMP permeation by 3–28%. Diffusion appeared to be the main driving force for the mass transfer of AMP across the membrane. Besides, the biofilm increased the retention of AMP by 23% but exhibited similar adsorption capacity with comparison of the suspended activated sludge, which indicates that the compact structure of the biofilm was the major contributor for the added retention effect on AMP by the biofilm-coated CA membrane. This study suggests that biofilm (biofouling) in MBRs increases the retention of small-molecule constituents such as antibiotics. A delicate trade-off between reduced wastewater throughput and increased retention of contaminants should be obtained when an MBR is designed and operated.

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1. Introduction

Tons of antibiotics are produced and consumed globally each day. As a result, residual antibiotics are continuously discharged into wastewater treatment plants (WWTPs) (Brown et al., 2006; Larsson et al., 2007). Antibiotics must be treated effectively in WWTPs in order to avoid the development of the antibiotic resistance in the ecosystem (Kümmerer, 2003). Due to the antimicrobial nature of antibiotics, conventional biological treatment technology such as the activated sludge (AS) system needs to be improved to handle this type of constituents in wastewater. In this context, the membrane bioreactor (MBR), which combines the advantages of both biodegradation and membrane filtration (Urbain et al., 1996), has emerged as an applicable technology to treat antibiotics in wastewater.

Several studies have reported the use of MBRs to remove antibiotics in wastewater. For example, Radjenovic et al. (2007) and Kim et al. (2007) reported the performance of MBRs for the removal of antibiotics in wastewater in pilot and full scale, respectively. They both found that the MBR exhibits higher removal efficiency for antibiotics than the conventional AS technology. But the treatment efficacy in each of the above two studies was

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below 70%, which was much less effective than the reverse osmosis (RO) process (treatment efficacy >95%). The contrast of the treatment efficacies by the MBR and the RO could be attributed to the difference in pore size of the membranes for the MBR and the RO. Generally, the membrane process in an MBR could be classified as the microfiltration (MF) or the ultrafiltration (UF) according to the operating range (Metcalf and Eddy, 2004), which means that the membrane alone in the MBR could not retain small molecules like antibiotics as the RO did. It was thought that membrane in an MBR could be helpful for the retention and enrichment of functional bacteria capable of more efficient degradation of antibiotics (Clara et al., 2004; Schroder et al., 2012).

However, recent studies have shown that the membrane in MBRs is more than a barrier for bacteria that are responsible for the biodegradation of organic constituents in wastewater. Sahar et al. (2011) investigated the removal of several antibiotics by a conventional AS system, by an AS system coupled with a subsequent UF step and by an MBR, respectively. It was found that the MBR demonstrated 15–42% higher removal efficiency over the AS system for all tested antibiotics. Meanwhile, the AS system with a subsequent UF had a similar removal efficiency of antibiotics as the MBR did. Since biodegradation was excluded from the UF step, and the UF membrane pores were obviously larger than the antibiotic molecule, the author inferred that it should be due to the enmeshment effect of the target antibiotic by the biofilm attached on the UF membrane. Generally, the biofilm developed on membranes has been regarded as the major reason for diminishing the flux of permeate ("membrane biofouling") in MBRs (Baker and Dudley, 1998; Chang et al., 2002). However, Sahar et al. (2011) suggest that the biofilm may contribute to prevent constituents such as antibiotics in the bulk fluid from releasing across the membrane. In this sense, the positive impact of the biofilm attached to the membrane in an MBR on the removal of antibiotics is yet to be confirmed.

This study was designed to evaluate the positive role of the biofilm in enhancing the retention of antibiotics in wastewater through the membrane for the MBR. Ampillin (AMP), one of the most widely used antibiotics, was chosen as the model compound and the cellulose acetate (CA) membrane, commonly used for MBRs, was used as the model membrane. Microbial sensitivity test was adopted to measure the resistance to AMP permeation caused by the biofilm coated on the CA membrane. This novel methodology for evaluating the performance of the membrane in an MBR for the removal of antibiotics has the benefits of (1) reducing analytical cost for antibiotics, and (2) reducing the amount of experimental wastewater.

2. Materials and methods

2.1. AS inoculum and biofilm-coated membrane

AS inoculum was collected from a local WWTP and acclimated (mixed liquor suspended solids of 2 mg L^{-1}, aeration rate of 0.12 m^3 h^{-1}) in a 5-L tank reactor (the acclimation tank) at 25 ± 2 °C for a month. 2 L AS was fed with 2 L synthetic wastewater in a fill-and-draw mode with 23-h aeration, 40-min sedimentation, 5-min withdraw of the supernatant in the upper half reactor, and 5-min feeding to the original volume of 4 L. The synthetic wastewater contained (in mg L^{-1}) sodium acetate 2700, NH_4Cl 175, K_2HPO_4 45, $FeSO_4{\cdot}7H_2O$ 10, $CaCl_2{\cdot}2H_2O$ 30, and $MgSO_4{\cdot}7H_2O$ 12.5 in water, with pH of 7.0–7.2 (Tijhuis et al., 1994).

After AS acclimation, flakes of the CA membrane (850 pieces, diameter of 25 mm and pore size of 0.45 μm; Jingju Technical, Xiamen, China) were incubated in the acclimation tank for 3 d. Then, the biomass-attached membranes were transferred into another 5-L tank reactor (the biofilm development tank) for the development of biofilm without suspended AS. The biofilm development tank contained the same synthetic wastewater and was operated with the same operational parameters as the AS acclimation tank, except for a mechanical stirrer of 160 rpm supplying the dispersing force of these membrane carriers.

Biomass and biofilm dry weights were measured according to Standard Methods (APHA, 1998). Biofilm was detached from the membrane through ultrasonification (15 min) prior to biomass dry weight analysis. The biofilm thickness was monitored by a microscope (Olympus CX41RF, Tokyo, Japan) with a clamp holding the membrane support in the vertical direction to the view plane. The average thickness of the biofilm was determined to be 13.5 μm. When the biofilm mass accumulated on the membrane varied within a range of 10%, biofilm-coated membranes were removed from the biofilm development tank and used in the AMP permeation study.

2.2. Permeation of AMP through membrane

Microbial sensitivity plate assay as described by Anderl et al. (2000) and Singh et al. (2010) was adopted to measure the permeation of AMP through clean CA membrane or biofilm-coated CA membrane and quantify the resistance of the biofilm to the permeation of AMP. A schematic of the experimental setup for the permeation of AMP through membrane is shown in Fig. 1. The AMP in the solution in the steel cylinder permeated through the membrane and reached the surface of the Luria-Bertani (LB) plate solid medium which was pre-loaded with an indicator microbe on the surface. The amount of AMP permeated through the membrane was proportional to the area on the LB plate that showed inhibition to microbial growth as a result of the antimicrobial effect of AMP. *Staphyloccocus aureus* ATCC25923 (in-house culture) was used as the indicator microbe. Minimum inhibitory concentration (MIC) of AMP (Sangon Biotech, Shanghai, China) to this bacterial strain was determined to be 0.25 mg L^{-1} according to the conventional disk diffusion test (Bauer et al., 1966).

The permeation experiment was conducted according to the following procedures. (1) Diluted culture of *S. aureus* (100 μL, approximately 10^6 CFU mL^{-1}) was evenly smeared on the surface of the sterilized LB solid medium. (2) A sterilized (by UV exposure for 15 min on each side) clean CA membrane with a diameter of 15 mm was placed on the surface of the LB plate to avoid direct contact between the biofilm and the agar culture on the LB plate. (3) A biofilm-coated CA membrane with a diameter of 10 mm (the membranes were trimmed from Φ 25 mm to Φ 10 mm using a puncture machine) was gently flushed with Mill-Q water, blotted dry with a tissue paper, and then placed on the top of the clean CA membrane. (4) A sterilized steel cylinder with a diameter of 6 mm and a length of 10 mm was placed on top of the biofilm-coated CA membrane and 0.2 mL of AMP solution at a concentration greater than the MIC to *S. aureus* (i.e., 10–60 mg L^{-1}) was added into the steel cylinder. (5) The AMP permeation assembly (Fig. 1) was covered with a lid and sealing tapes, and stored at 4 °C to allow AMP to

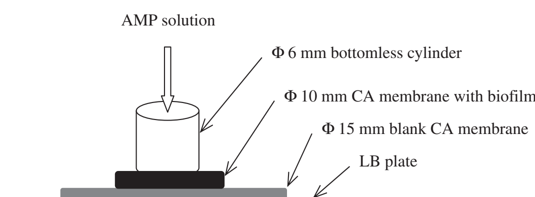

Fig. 1. Schematic illustration of the experimental assembly to monitor the AMP permeation process, based on the microbial sensitivity test.

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