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A new method to measure and model dynamic oxygen microdistributions in moving biofilms☆

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A B S T R A C T

Biofilms in natural environments offer a superior solution to mitigate water pollution. Artificially intensified biofilm reactors represented by rotating biological contactors (RBCs) are widely applied and studied. Understanding the oxygen transfer process in biofilms is an important aspect of these studies, and describing this process in moving biofilms (such as biofilms in RBCs) is a particular challenge. Oxygen transfer in RBCs behaves differently than in other biological reactors due to the special oxygen supply mode that results from alternate exposure of the biofilm to wastewater and air. The study of oxygen transfer in biofilms is indispensable for understanding biodegradation in RBCs. However, the mechanisms are still not well known due to a lack of effective tools to dynamically analyze oxygen diffusion, reaction, and microdistribution in biofilms. A new experimental device, the Oxygen Transfer Modeling Device (OTMD), was designed and manufactured for this purpose, and a mathematical model was developed to model oxygen transfer in biofilm produced by an RBC. This device allowed the simulation of the local environment around the biofilm during normal RBC operation, and oxygen concentrations varying with time and depth in biofilm were measured using an oxygen microelectrode. The experimental data conformed well to the model description, indicating that the OTMD and the model were stable and reliable. Moreover, the OTMD offered a flexible approach to study the impact of a single-factor on oxygen transfer in moving biofilms.

In situ environment of biofilm in an RBC was simulated, and dynamic oxygen microdistributions in the biofilm were measured and well fitted to the built model description.

1. Introduction

Biofilms in natural environments offer a superior solution to mitigate water pollution. Artificially intensified biofilm reactors can be applied as an alternative wastewater treatment technology to the conventional activated sludge process (Patwardhan, 2003). Rotating biological contactors (RBCs), as representative biofilm reactors, have inherent advantages compared with the activated sludge process, including sufficiently long biomass detention time, smaller footprint, more microbial populations, stronger impact resistance, more stable ecological system, and lower energy cost coupled with a higher specific removal rate (Cortez et al., 2008; Han et al., 2012; Nicolella et al., 2000; Tonde et al., 2015). Oxygen plays a critical role in biological wastewater treatment (Ning et al., 2012), and strongly affects the biodegradation efficiency in RBCs. The oxygen transfer in RBCs behaves differently from other biological reactors due to the special oxygen supply mode that results from alternate exposure of the biofilm to wastewater and air, without additional oxygenating equipment. At the same time, biofilm, wastewater, and oxygen can be mixed adequately. Understanding

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the mechanisms of oxygen transfer and reaction in biofilm is essential to fully comprehend the mechanisms of the biodegradation process in RBCs. However, to the best of our knowledge, these mechanisms remain only partially described due to a lack of effective experimental tools to dynamically measure and analyze oxygen diffusion, reaction, and microdistribution in biofilms.

In recent years, microsensors have been widely used in biological fields (Bishop et al., 1995; Cantú-Lozano et al., 2015; Xiao et al., 2013). These devices have been applied to measure the microdistributions of substances in microbial aggregates such as biofilm and granular sludge. Oxygen microelectrodes are one of the most mature microsensors (Yu et al., 2004). *In situ* and *ex situ* measurements have been employed to determine oxygen microdistributions in biofilm using microelectrodes. In some biological reactors, such as the bioelectrochemical reactor, *in situ* measurement of biofilm is achievable because the biofilm can be fixed (Xiao et al., 2013). However, RBCs rotate continuously under normal operation, so the constantly moving biofilm provides a changing biofilm microenvironment. For this reason, it is difficult to measure oxygen microdistributions in biofilm *in situ* dynamically and accurately. Yu et al. (2004) used oxygen microelectrodes to measure oxygen concentration in biofilms *in situ* within an RBC treating domestic wastewater. However, to do this, the revolving discs had to be briefly stopped to measure the one-dimensional oxygen concentration profiles in biofilm (along the biofilm depth) on the RBC discs. Therefore, the experimental results did not represent the *in situ* oxygen microdistributions inside biofilm, because the external environment around biofilm was distinctly changed when the discs were stopped. Cantú-Lozano et al. (2015) immobilized a micromanipulator directly on the disc in an RBC, and used an oxygen microelectrode attached to the micromanipulator to measure the oxygen concentration in the biofilm on the disc. *In situ* measurement was possible with this method, but the micromanipulator and microelectrode were exposed to wastewater and air alternately along with the rotation of discs, potentially resulting in a change in determination accuracy and destruction to the microelectrode. Additionally, this approach is difficult to be used in RBCs without special design, especially in RBCs with additional moving components (e.g. moving fillers). Application of this approach limits the ability to study single-factor effects on oxygen transfer inside biofilm, while analyzing univariate impact is required to fully understand the bioprocess mechanisms in RBCs. An *ex situ* simulation was developed by De la Rosa and Yu (2005) to model the *in situ* biofilm environment in an RBC. They employed oxygen microelectrodes for three-dimensional mapping of the oxygen microdistribution in wastewater biofilms. In order to simulate the real conditions presented in the RBC, the experimental set-up exposed the biofilm sample to wastewater and air alternately by draining and filling the measuring chamber with wastewater. However, during the measurement, the chamber was kept filled, and there was no controllable relative movement between the biofilm and wastewater/air. During RBCs operation, the rotation rates of the discs influence oxygen and substrates transfer to biofilm, and the relative movement between the biofilm and the wastewater/air affects the internal mass transfer rate and the microbial activity inside the biofilm (Banerjee, 1996; Beyenal and Lewandowski, 2002; Guimerà et al., 2016). Therefore, the measurements without this relative movement likely do not represent the actual oxygen microdistributions in biofilm under normal operation. Thus, it is necessary to develop new methods to measure and analyze oxygen transfer in moving biofilm from RBCs.

In this study, a new method was developed to meet this demand. We reported a model for oxygen diffusion, reaction, and microdistribution in biofilm produced by RBCs, and the design and manufacture of an experimental device called the Oxygen Transfer Modeling Device (OTMD). The external environment around the biofilm under normal operation in RBCs was precisely simulated by this device as the biofilm was kept static. This allowed dynamic measurement of the oxygen concentration in biofilm varying with time and depth. The measurement results likely represent oxygen microdistributions in the biofilm during normal RBCs operation. Additionally, the device offers a flexible approach to study the impact of a single-factor, such as exposure time or immersion time, on oxygen concentration in biofilm by appropriately controlling the simulation parameters. Application of this method will facilitate research on the transfer and reaction mechanisms of oxygen or other substances in the inner layer of moving biofilms.

2. Materials and methods

2.1. Biofilm and the RBC

The biofilm measured in this study was taken from a Rotating Biological Cage, which is a modified conventional RBC. The reactor is composed of rotating cages and reaction tank, and a schematic of the reactor is shown in Fig. S1. The rotating cages were penetrated by a driving shaft and consisted of discs (polypropylene, diameter: 150 mm, thickness: 30 mm), fillers (polypropylene, diameter: 25 mm, thickness: 10 mm), and wrappage (polypropylene, pore size: 15 mm). Each rotating cage was divided into four equal-volume parts separated by plastic net to avoid all fillers clumping together during disc rotation. The specific surface area of the fillers was 500 m^2/m^3, providing increased surface for the attachment of microorganisms. The Rotating Biological Cage in this study was used for synthetic refractory wastewater treatment. The synthetic wastewater was composed of four parts: substrates (ammonium and Chemical Oxygen Demand (COD)), salt, trace elements, and mineral medium. Ammonium, salt, and COD were introduced as NH_4Cl, NaCl and glucose, respectively, and trace elements were supplied at 1.25 mL/L wastewater. The composition and content of the trace element solution (adapted from van de Graaf et al. (1996) and Ma et al. (2012)) are shown in Table S1. The composition and content of the mineral medium (adapted from Jin et al. (2008)) are shown in Table S2. The parameters of the synthetic wastewater used as influent and some data of the Rotating Biological Cage are listed in Table S3. COD, ammonium and the salinity were measured according to standard methods (Chinese, 2002).

The biofilm used in this study was produced by the Rotating Biological Cage, after 7 months of stable operation. The reactor was operated continuously with 50% submersion of cages and 3 rpm cage rotation rate. The effective volume was 22.8 L, the flow of influent was 10.0 L/d, and the hydraulic retention time (HRT) was 54.7 h. The dissolved oxygen (DO) levels in the biofilm were determined by an oxygen microsensor (Unisense, OX10, Denmark). Biofilm was taken out with the fillers and fixed on the carrier in the OTMD.

2.2. Oxygen Transfer Modeling Device (OTMD)

The OTMD was mainly composed of biofilm, wastewater, a power system, and a bracing system. The biofilm was fixed by the bracing system, and its external environment under normal operation was simulated by wastewater and the power system. The power system included three apparatuses to separately provide wind, wastewater rotation, and vertical movement. The wind and wastewater rotation movement were designed to model the relative movement between the biofilm and air/wastewater, and the wastewater vertical movement was used to simulate the alternate exposure of the biofilm to air and wastewater. The wastewater in