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Research article

Microbial community composition of a multi-stage moving bed biofilm reactor and its interaction with kinetic model parameters estimation

Xiaodong Wang [a, *], Xuejun Bi [b], Lars John Hem [a, c], Harsha Ratnaweera [a, b]

[a] Faculty of Science and Technology, Norwegian University of Life Sciences, P.O. Box 5003-IMT, 1432 Aas, Norway
[b] Qingdao University of Technology, State and Local Joint Engineering Research Center of Urban Wastewater Treatment and Reclamation in China, Fushun Road 11, 266033 Qingdao, China
[c] Oslo Water and Sewage Works, Herslebs Gate 5, 0561 Oslo, Norway

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ABSTRACT

Microbial community diversity determines the function of each chamber of multi-stage moving bed biofilm reactor (MBBR) systems. How the microbial community data can be further used to serve wastewater treatment process modelling and optimization has been rarely studied. In this study, a MBBR system was set up to investigate the microbial community diversity of biofilm in each functional chamber. The compositions of microbial community of biofilm from different chambers of MBBR were quantified by high-throughput sequencing. Significantly higher proportion of autotrophs were found in the second aerobic chamber (15.4%), while 4.3% autotrophs were found in the first aerobic chamber. Autotrophs in anoxic chamber were negligible. Moreover, ratios of active heterotrophic biomass and autotrophic biomass (X_H/X_A) were obtained by performing respiration tests. By setting heterotroph/autotroph ratios obtained from sequencing analysis equal to X_H/X_A, a novel approach for kinetic model parameters estimation was developed. This work not only investigated microbial community of MBBR system, but also it provided an approach to make further use of molecular microbiology analysis results.

1. Introduction

Moving bed biofilm reactors (MBBRs) have been widely applied in wastewater treatment plants since last decades (Di Trapani et al., 2014). MBBRs have proved to be successful in both domestic and industrial wastewater treatment, with respect to stable removal efficiency (Delnavaz et al., 2010), compact and high specific biomass concentration (Guo et al., 2010), and high cold-resistant ability (Hoang et al., 2014).

The performance of biological wastewater treatment systems relies on microbial community structure of the biomass (Flowers et al., 2013). With rapid development of molecular biological technology, microbial community diversity of wastewater treatment biomass has been increasingly investigated in recent years (Guo and Zhang, 2012; Jo et al., 2016). Moreover, the relationship of microbial community composition and environmental variables of activated sludge system has been investigated recently using high-throughput sequencing (Xu et al., 2017). Although microbial community of suspended growing activated sludge has been well studied, studies about community differences of fixed growing biofilm in different chambers of MBBR systems were rather limited.

The functional fractions in activated sludge and biofilm were usually classified into two groups: heterotrophic biomass and autotrophic biomass based on metabolic function. The quantity and activity of heterotrophic biomass and autotrophic biomass play central role in organic matter biodegradation and ammonia nitrification. Assessment of heterotrophic biomass and kinetic parameters of activated sludge model No. 1 - ASM1 (Henze et al., 2000) by respiration tests was initially introduced by Kappeler and Gujer (1992). Ochoa et al. (2002) applied the default values of kinetic and stoichiometric parameters to determine active heterotrophic and autotrophic biomass distribution via respiration tests. Hence, respiration tests were increasingly used coupled with kinetic models for biomass distribution quantification (Fernandes et al., 2013; Tsai and Wu, 2005).

* Corresponding author.
E-mail addresses: xiaodong.wang@nmbu.no, xiaodong.wang01@outlook.com (X. Wang).

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Simulation results may be biased if kinetic model parameters were adopted without calibration. Since the kinetic model parameters (e.g. heterotrophic growth rate and autotrophic growth rate) are difficult to be measured directly, they were usually estimated by performing batch test in earlier years (Kappeler and Gujer, 1992; Vanrolleghem et al., 1999). With the development of computer and modelling software, these parameters were more often being estimated via numerical approach, such as sensitivity analysis (Mannina et al., 2011b). However, it is quite often that model outputs were not sensitive to some kinetic model parameters, because biological process models was generally over-parameterized with dozens of variables and model parameters (Cosenza et al., 2014). Therefore, if the inner relationship of kinetic parameters could be established, computational power and time can be saved, and more insights of biochemical reaction of biological wastewater treatment may be obtained.

To the best of our knowledge, microbial diversity of biofilms in multi-stage MBBR system is underexplored. In this study, we investigated microbial community composition of different functional chambers of MBBR system. Moreover, the interaction between microbial composition and kinetic modelling provides insight for modelling and optimization of biological wastewater treatment process.

2. Materials and methods

2.1. MBBR system description

A multi-stage laboratory scale MBBR was set up for this study. As is shown in Fig. 1, domestic wastewater was transported from primary clarifier of the WWTP to a storage tank, which provided constant flow of influent to the MBBR system. The MBBR system was consisted by three functional chambers: anoxic chamber (AN), first aerobic chamber (AE1), and second aerobic chamber (AE2). Each chamber of the reactor was filled with suspended plastic biocarriers. The density of carriers was 0.95 $g \cdot cm^{-3}$ and the specific surface area of the carriers was 600 $m^2 \cdot m^{-3}$. The volume of each chamber was 5 liters. The quantity of carriers in each chamber was 0.32 m^2, in terms of surface area. Dissolved oxygen (DO) in AE1 and AE2 was always higher than 6 $mg \cdot L^{-1}$ to avoid mass transfer limitation among inner layers of biofilm (Ødegaard, 2006). Carriers in upper stream chambers cannot be transported to the downstream chambers, because the opening between each chamber was much smaller than the diameter of carriers. The hydraulic retention time in each chamber was 2 h, and the total hydraulic retention time was 6 h. Nitrified liquid was recycled back to AN for denitrification, with recycle ratio of 1:1.

To create steady-state condition for this study, the concentrations of chemical oxygen demand (COD) and ammonia nitrogen ($NH_4^+ - N$) of inlet wastewater were adjusted to 700 $mg \cdot L^{-1}$ and 50 $mg \cdot L^{-1}$ respectively, by adding sodium acetate and ammonium chloride. The laboratory MBBR system was setup inside the laboratory, where an air conditioner was used to maintain the temperature at 12 °C. Besides, to provide necessary references, a paralleled activated sludge (AS) system was running as a sequencing batch reactor (SBR) with the same daily loading rate. A SBR cycle was four hours (one hour of anoxic reaction, two hours of aerobic reaction, and one hour for sedimentation and refilling, 6 cycles per day). After each cycle, 50% of the supernatant was discharged to regain space for refilling influent. The sludge age was kept as 18 days during the entire test period. Both the MBBR and activated sludge systems have been operated for 40 days before this study was carried out.

2.2. Samples collection and wastewater analysis

After 40 days of steady-state operation (at 12 °C), biofilm samples were collected from every chamber of MBBR during a period of seven days. Five carriers were taken randomly from each chamber every other days, and in total 20 carriers were collected from each chamber. Biofilm was scratched from the carriers and stored at −80 °C immediately after the carriers were taken out from MBBR system. The AS samples were collected from the paralleled SBR system at the same time when biofilm samples were collected.

The influent and outlet water quality of each chamber was analyzed once per day. COD and soluble COD (SCOD) were analyzed using Hach-Lange test kits (LCK 314/514), following the recommended method on spectrophotometer DR 3900. Total suspended solids, ammonia nitrogen ($NH_4^+ - N$), nitrate ($NO_3^- - N$), nitrite ($NO_2^- - N$) and orthophosphate ($PO_4^{3-} - P$) were analyzed following Standard Methods (APHA et al., 2012). The seven-day average water quality of influent and outlet of each chamber was shown in Table 1.

2.3. DNA extraction, PCR and high-throughput sequencing

DNA was extracted from 1.5 mL of AN, AE1, AE2 and AS samples respectively using PowerSoil DNA Isolation Kits (Mo Bio Laboratories, USA) according to manufacturer's instruction. The extracted DNA was checked using 1% agarose gel electrophoresis. The V3-V4 region of the 16S rRNA gene was amplified from extracted DNA using universal primers 338F (ACTCCTACGGGAGGCAGCA) and 806R (GGACTACNNGGGTATCTAAT) (Du et al., 2017). The mixture of 4 μL of 5× FastPfu Buffer, 2 μL of dNTP (2.5 mM), 0.4 μL of each

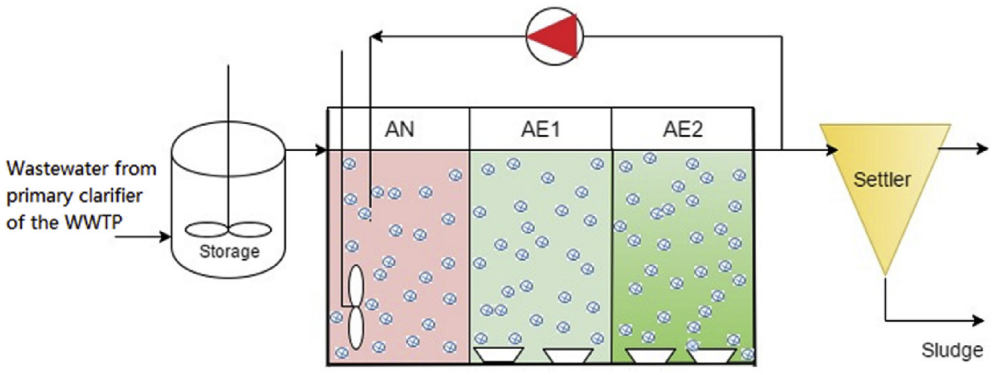

Fig. 1. The flow diagram of the laboratory scale wastewater treatment system. In the MBBR system, AN, AE1 and AE2 represents anoxic chamber, first aerobic chamber and second anoxic chamber, respectively.