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Kinetic characteristics and bacterial structures in biofilm reactors with pre-cultured biofilm for source water pretreatment

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ABSTRACT

The growth and accumulation of functional bacteria were limited due to the deficiency of substrates in biofilm reactors for source water pretreatment. In this study, pre-cultured biofilm (PCB) formed on different carriers was used to treat oligotrophic source water, and the kinetic characteristics and bacterial structures was evaluated under realistic conditions. Experimental results showed that PCB formed under substrate-enhanced conditions decreased substrate affinity of biofilm, but effectively improved the operation performance of biofilm reactors. In particularly, biofilm formed on elastic stereo media had the lowest apparent half-saturation coefficient (*Ks*) for substrates, which was more easily to accommodate oligotrophic niche. Interestingly, biodiversity of biofilm was obviously increased after long-term exposure to oligotrophic niche, and decreased functional bacteria were replaced by others with similar function. Basically, *Proteobacteria* and *Planctomycetes* were dominant in biofilm samples under both substrate-enhanced and oligotrophic conditions.

1. Introduction

The increasing discharge of wastewater with nitrogen and organics has seriously polluted source water in the world, especially in developing countries (Benner et al., 2013; Zhang et al., 2014; Yang et al., 2015). Biological treatment process is generally considered as a promising technology for the treatment of polluted source water due to its advantages of lower operation cost and less secondary pollution (Yang et al., 2015). Up to date, biofilm reactors have been widely used as pretreatment processes for the treatment of polluted source water (Feng et al., 2012a; Zhang et al., 2014; Yang et al., 2015).

However, bacteria growth and reproduction are limited due to the deficiency of substrates in source water treatment systems (Egli, 2010; Yang et al., 2015). Especially, the initial formation of biofilm in source water pretreatment systems usually lasted for an extremely long operation period of even several months (Seredynska-Sobecka et al., 2006; Yang et al., 2014). Extra substrates addition was considered as one useful strategy to enhance the formation of biofilm and increase the pollutants removal performance. However, operation risks including increasing precursors of disinfection by-products (DBPs), nitrite accumulation and decreasing substrates affinity may be introduced (Yang et al., 2015; Wang et al., 2017).

Inoculation of pre-cultured biofilm (PCB) could enhance the startup performance and reduce the operation risks of substrates addition in biofilm systems treating polluted source water (Yang et al., 2014). However, few studies focus on the kinetic characteristics and bacterial structures in biofilm systems with PCB for source water treatment. In this study, PCB of different biocarriers formed under substrate-enhanced conditions were inoculated into three lab-scale biofilm reactors for the treatment of polluted source water. The objectives are to (1) investigate the responses of operation performance and kinetic characteristics of PCB for source water pretreatment, and (2) reveal the changes in bacterial structures and potential functional bacteria in source water treatment systems.

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2. Materials and methods

2.1. Experimental setup

Three lab-scale biofilm reactors (R_1, R_2 and R_3) with identical working volume of 15 000 mL were built up by polyvinyl plastic. Biofilm carriers including suspended ball carrier I (SB1), suspended ball II (SB2) and elastic stereo media (ESM) were filled into reactors R_1, R_2 and R_3, respectively. R_1, R_2 and R_3 had identical carrier filling ratio of 5.44% (v/v) with the surface area of 3.23, 2.05 and 7.35 m^2, respectively. Three reactors were placed outdoor and operated under temperature of 17.0–31.5 °C. Aeration pump was employed to provide dissolved oxygen (DO) for biofilm reactors with an air-fluid flow ratio of 1:1.

2.2. Characteristics of polluted source water

Polluted source water was obtained from a river located in Zhejiang Province of China. The source water has been seriously polluted by ammonia, organics and turbidity. The major water quality is presented as follows: NH_4^+-N 0.37–2.48 mg L^{-1}, NO_2^--N 0.10–0.37 mg L^{-1}, NO_3^--N 1.27–4.43 mg L^{-1}, COD_{Mn} 3.57–7.05 mg L^{-1}, UV_{254} 0.0645–0.1882 cm^{-1}, turbidity 19.2–95.6 NTU, DO 2.63–6.98 mg L^{-1} and pH 6.38–7.49.

2.3. Operation procedures

2.3.1. Formation of PCB

To stimulate the growth of microorganisms, an independent operation stage (P_1, Days 1–27) was operated under substrate-enhanced conditions for the formation of PCB. Ammonium sulfate and ethanol were added into influent and used as nitrogen source and organic carbon source, respectively. Thus, NH_4^+-N concentration in influent of reactors was increased from 1.47 ± 0.57 mg L^{-1} to 6.49 ± 0.19 mg L^{-1} with COD_{Mn} level increased from 4.48 ± 0.31 mg L^{-1} to 7.16 ± 0.54 mg L^{-1}. In P_1, three reactors were operated under batch operation mode, and effluent was recycled as influent by a recirculation peristaltic pump at a hydraulic retention time (HRT) of 4 h. The treated water was replaced by fresh influent in every 4–8 d. Each replacement of feeding water was defined as an operation round, and five operation rounds named round 1, 2, 3, 4 and 5 were operated.

2.3.2. Long-term operation of PCB for source water treatment

Three reactors with PCB were changed to continuous-flow operation mode, and real polluted source water was treated in this period (P_2, Days 28–178). HRT of three reactors was set at 4 h during Days 28–98, and the NH_4^+-N loading rate (ALR) and COD_{MN} loading rate (CLR) were varied of 2.3–14.9 mg L^{-1} d^{-1} and 24.1–40.1 mg L^{-1} d^{-1}, respectively. In the following operation days (Days 99–178), the HRT was shortened to 1.91 h. Correspondingly, ALR and CLR were varied of 4.6–25.1 mg L^{-1} d^{-1} and 44.9–88.7 mg L^{-1} d^{-1}, respectively (Table S1).

2.4. Analysis of bacterial communities

Biofilm carriers obtained from reactors R_1, R_2 and R_3 on Day 27, 52 and 137 were used for analysis of bacterial communities, and the detail information of each biofilm sample is shown in Table 1. Total DNA of biofilm samples was extracted using a soilDNA kit (OMEGA) according to the method provided by producer. The V4 region of 16S rDNA genes were amplified using primers of 520F and 802R with the sequences of 5' - barcode + GCACCTAAYTGGGYDTAAAGNG - 3′ and 5' - TACNVGGGTATCTAATCC - 3', respectively. Polymerase chain reaction (PCR) amplification was conducted using 25 μL reaction system including 0.25 μL NEB Q5 DNA high-fidelity polymerase, 0.5 μL dNTPs (10 mM), 5 μL 5 × PCR reaction buffer, 5 μL 5 × high GC buffer, 1 μL DNA template (20 ng), 1 μL forward primer, 1 μL reverse primer and 11.25 μL sterilizing ultrapure water. The PCR conditions with the methods for the detection of PCR were identical to that published by Feng et al. (2017).

A mixture of purified clone library was used for Miseq sequencing analysis using a MiSeq machine in Personal biotech Co. Ltd (Shanghai, China). After trimming barcodes and primers, defective reads with incognizable reverse primer, shorter than 150 bp or containing ambiguous bases were removed from library. Sequences with similarity of higher than 97% were clustered into one operational taxonomic unit (OTU), and the subsequent analysis of bacterial information was based on OTU.

2.5. Routine analysis of water samples

Influent and effluent of biofilm reactors were routinely sampled for the analysis of water quality. Unfiltered samples were used for analysis of COD_{Mn} and turbidity. NH_4^+-N, NO_2^--N, NO_3^--N and UV_{254}-detectable substances (UV_{254}) of water samples were determined after filtration through 0.45-mm glass membrane according to the Standard Methods issued by Chinese SEPA (2002). DO meter (YSI Model52, USA) was used for DO and temperature determination.

2.6. Calculations

2.6.1. Modified S-K model

Modified Stover-Kincannon (S-K) model (Eq. (1)) was useful to predict the pollutants removal potential in biofilm reactors treating polluted source water (Yang et al., 2015).

$$\left(\frac{dS}{dt}\right)^{-1} = \frac{K_B}{U_{max}}\frac{V}{QS_0} + \frac{1}{U_{max}} \quad (1)$$

Where, dS/dt is substrate removal rate (mg L^{-1} d^{-1}), U_{max} is maximum substrate removal rate (mg L^{-1} d^{-1}), K_B is saturation constant (mg L^{-1} d^{-1}), Q is inflow rate (L d^{-1}), V is the effective volume of reactors (L, 15 L in this study), S_0 and S are influent and effluent substrate concentration (mg L^{-1}), respectively.

2.6.2. Modified Monod model

Modified Monod model (Eq. (2)) could be used to quantify the kinetic characteristics of NH_4^+-N or organics removal (Lee et al., 2014; Yang et al., 2015).

$$\mu = \mu_{max}\frac{S}{K_S + S} \quad (2)$$

Where, μ is substrate removal rate (mg L^{-1} d^{-1}), μ_{max} is maximum substrate removal rate (mg L^{-1} d^{-1}), S is substrate removal concentration (mg L^{-1}), and K_s is Monod coefficient or apparent half-saturation coefficient (mg L^{-1}).

2.6.3. Modified kinetic model for analysis of temperature influence

In biofilm reactors, reaction rate (μ) could be expressed by equation (3) due to the diffusive limitation of substrates (Salvetti et al., 2006).

$$\mu = kc^a \quad (3)$$

Where, μ is substrate removal rate (mg L^{-1} d^{-1}), k is rate constant that related to biofilm thickness, diffusion coefficient and temperature, c is limiting substrate concentration (mg L^{-1}), and a is a reaction coefficient (dimensionless).