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Towards physicochemical and biological effects on detachment and activity recovery of aging biofilm by enzyme and surfactant treatments

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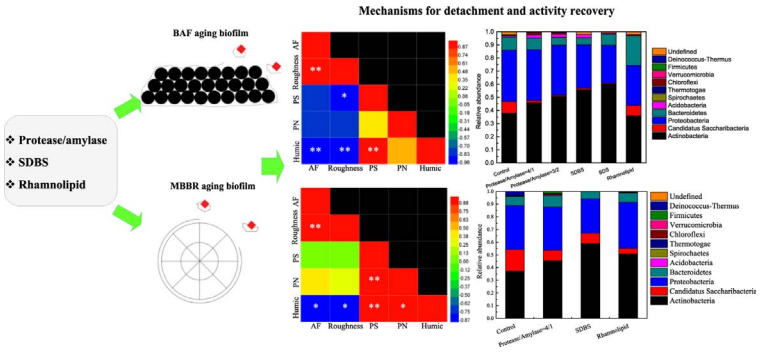
GRAPHICAL ABSTRACT



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ABSTRACT

In order to explore physicochemical and biological effects on detachment and activity recovery of aging biofilm by enzyme and surfactant treatments, two kinds of biofilm processes, i.e. biological aeration filter (BAF) and moving bed biofilm reactor (MBBR), and multiple indicators including water quality, biofilm morphology, activity and microbial community structure, were employed. Results showed that detachment of aging biofilm was mainly attributed by extracellular polymeric substance (EPS) solubilization and dispersion, and activity recovery of aging biofilm mainly depended on biological effects of dominant bacteria. Phosphorus metabolism related bacteria, such as *Microbacterium* and *Micropruina*, were responsible for BAF biofilm regeneration. More abundant microbial community structure of MBBR regenerated biofilm was found, and biofilm activity was not only related to phosphorus metabolism related bacteria, but also to denitrifying bacteria. Rhamnolipid performed best on aging biofilm detachment and regeneration, giving a clue for effective activation of aging biofilm in wastewater treatment systems.

1. Introduction

During the operation of biofilm-based wastewater treatment system, biofilm will get thicker gradually and may affect the mass transfer efficiency of nutrients, leading to the decrease of biofilm activity (Lazarova and Manem, 1995) and the aging state of biofilm. Aging biofilm may reduce wastewater treatment efficiency and even result in the collapse of the system (Bassin et al., 2012). Therefore, the evaluation of biofilm activity and timely detachment and regeneration of aging biofilm has always been an important issue in biofilm-based wastewater treatment.

Evaluation methods of biofilm activity can be divided into two

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categories: indirect evaluation based on effluent quality (usually by COD and NH_4^+-N) and direct evaluation based on biofilm, respectively. The indirect method is reliable, however, biofilm activity is not linearly related to the effluent quality (Lazarova and Manem, 1995), moreover, effluent quality always shows a significant change after biofilm activity reduced to a certain extent, thus the indicators including specific oxygen uptake rate (SOUR), bacterial live cell ratio, and adenosine triphosphate (ATP) content are commonly used to evaluate biofilm activity directly (Hao et al., 2009; Sotirova et al., 2012).

Aging biofilm needs to be detached or removed from carrier surfaces by artificial intervention and then regenerated to recover its activity, and there are a few methods reported for biofilm detachment in the fields of food, paper industry, biomedicine and environment (Simões et al., 2010). It is known that surfactants are amphiphilic molecules with both hydrophilic and hydrophobic groups and have a good solubilization effect towards extracellular polymeric substance (EPS) of biofilm (Splendiani et al., 2006). Chen and Stewart reported that sodium dodecyl sulfate (SDS) removed more biofilm and killed less cells compared with acid (Chen and Stewart, 2000), indicating an environment-friendly agent against biofilm removal. A combination use of sodium dodecyl benzene sulfonate (SDBS) and NaOH also achieved good results on membrane anti-biofouling (Chen et al., 2015). Particularly, biosurfactants, secreted through microbial metabolism (Rodrigues et al., 2006), have significant advantages including high specificity, biodegradability and low toxicity, among which rhamnolipid is a kind of commercially viable biosurfactant and has been widely used in biofilm removal (Dusane et al., 2010). Kim et al. found that membrane flux increased by 20% after treated with 300 mg/L of rhamnolipid for 6 h (Kim et al., 2015a). In another study, it was also found that removal rates of polysaccharide and protein of biofilm EPS on membrane were 31.6% and 79.6%, respectively, after treated by 300 mg/L of rhamnolipid (Kim et al., 2015b). However, there was only few information available for the direct application of rhamnolipid in activity recovery of aging biofilm in wastewater treatment systems.

Enzymes can catalyze the decomposition of specific components in biofilm EPS and promote biofilm detachment and desorption of microbial cells. Si and Quan reported that the combination of vanillin with protease or deoxyribonuclease could inhibit biofilm formation in wastewater treatment system (Si and Quan, 2017). Similar results were also found in our previous study that the biofilm pellets could be effectively decomposed by protease and amylase (Huang et al., 2014). Due to the complexity of biofilm composition and high specificity of enzymes, a mixed formula using multiple enzymes may be favorable for effective control of wastewater treatment biofilm.

Biological aeration filters (BAF) using fixed carriers and moving bed biofilm reactors (MBBR) using suspended carriers (Barwal and Chaudhary, 2014; Biswas et al., 2014; Calderón et al., 2012), are commonly used biofilm processes in wastewater treatment, respectively. View from the current study, research on BAF were mainly focused on performance improvement of the carriers (Wu et al., 2015a,b; Han et al., 2009; Liu et al., 2010), combination of BAF and other processes such as advanced oxidation or adsorption (Zou, 2015; Wu et al., 2015a,b), and microbial community structures of BAF biofilm (Shi et al., 2015). On the other hand, research on MBBR mainly concentrated on the start-up of MBBR (Zhu et al., 2015; Wu et al., 2015a,b), influences of different operation conditions (Barwal and Chaudhary, 2014; Bassin et al., 2012), and also on the microbial community structures (Zhu et al., 2015; Biswas et al., 2014; Fu et al., 2010). Nevertheless, there has been very little coverage in the detachment and activity recovery of BAF and MBBR aging biofilm. Therefore, it is of great necessity to carry out the further study of physicochemical and biological effects on detachment and activity recovery of aging biofilm by enzyme and surfactant treatments, based on the foregoing background and our previous work (Huang et al., 2014; Yu et al., 2016). This study offered a systematic insight into the involved mechanisms and has important guiding significance for efficient regulation of biofilm in biofilm-based wastewater treatment.

2. Materials and methods

2.1. Experimental set-up

Lab-scale BAF and MBBR reactors were employed. BAF reactors were made of polymethyl methacrylate and filled with clay ceramic particles (CCP). The cylindrical reactors were with a diameter of 6.5 cm and a height of 50 cm, respectively. Each reactor was filled with glass drops (3–5 mm in diameter) on a platform as the supporting layer (6 cm in height), then filled with CCP as the filler layer (30 cm in height), leaving a headspace of 10 cm to save wastewater and be good for aeration. Aerating devices were installed at the bottom of reactors to supply oxygen, with one diffuser in each reactor. The BAF system was inoculated with activated sludge obtained from the anoxic/oxic (A/O) process at a sewage plant for 48 h. The reactors were then fed with synthetic wastewater including glucose of 500 mg/L, NH_4Cl of 190 mg/L, and KH_2PO_4 of 44 mg/L to make sure that influent chemical oxygen demand (COD), ammonium nitrogen (NH_4^+-N) and N/P were 500 mg/L, 50 mg/L and 5:1, respectively. What's more, the synthetic wastewater also contained following compositions: $MgSO_4{\cdot}7H_2O$ 0.51 mg/L, $CaCl_2{\cdot}2H_2O$ 0.26 mg/L, NaCl 1 mg/L, $FeSO_4{\cdot}7H_2O$ 0.2 mg/L, $MnSO_4{\cdot}H_2O$ 0.12 mg/L, $ZnSO_4{\cdot}7H_2O$ 0.18 mg/L, $CuSO_4{\cdot}5H_2O$ 0.16 mg/L, and H_3BO_3 0.1 mg/L. Na_2CO_3 of 110 mg/L was added to adjust pH to about 7–8. Hydraulic retention time (HRT) and air/liquid ratio (A/L) were 12 h and 10:1, respectively.

MBBR reactors were cubic, with a height of 30 cm and a bottom length of 10 cm, respectively. Aeration devices were arranged at the bottom of the reactors to provide dissolved oxygen. Plastic suspended carriers in the reactors were with an outer diameter of 25 mm and a height of 10 mm, respectively. The reactors were inoculated with activated sludge for 48 h, and then fed with synthetic wastewater including glucose of 500 mg/L, NH_4Cl of 95 mg/L, and KH_2PO_4 of 22 mg/L. Na_2CO_3 of 60 mg/L was added to adjust pH to about 7–8, and hydraulic retention time (HRT) was 12 h. The BAF and MBBR systems were both operated at room temperature ranging from 25 to 30 °C.

2.2. Enzyme and surfactant treatments and biofilm regeneration

A whole running process containing start-up, biofilm maturation, biofilm aging and in situ activity recovery, was carried out in BAF reactors. In our previous study (Yu et al., 2016), two kinds of enzyme preparations (i.e. protease/amylase = 4/1 and protease/amylase = 3/2) and three kinds of surfactants (SDBS, SDS and rhamnolipid) were screened for treatment of aging biofilm. Then, BAF aging biofilm were treated by preferred enzymes and surfactants for 13 days, and the reactors kept running for another 12 days for biofilm regeneration. Variations of effluent qualities, biofilm morphology and activity, and microbial community structures were monitored, and mechanisms for aging biofilm detachment and activity recovery were investigated.

The sampling and preservation of carriers with aging biofilm of MBBR was according to our previous work (Huang et al., 2014), after that the carriers with aging biofilm were treated by enzymes and surfactants in stirring devices for 8 h. And then, the carriers were taken out, rinsed three times with tap water, and put into the empty MBBR reactors to start biofilm regeneration test as introduced in Section 2.1. The reactors were operated for 30 days. EPS and morphology of MBBR detached aging biofilm, effluent qualities, biofilm activity and microbial community structures, were systematically investigated.

2.3. Characterization of the effluent and biofilm

Concentrations of effluent qualities containing chemical oxygen demand (COD), ammonia nitrogen (NH_4^+-N), protein, polysaccharide and humic acid, were measured in triplicate according to standard