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Essential factors of an integrated moving bed biofilm reactor–membrane bioreactor: Adhesion characteristics and microbial community of the biofilm

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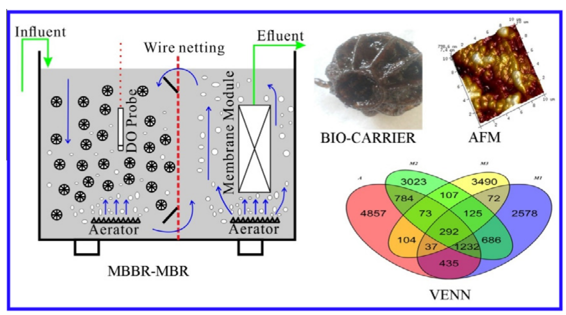
HIGHLIGHTS

- The roughness of biofilm is a convenient index to evaluate the maturity of biofilm.
- Tightly-bound protein and polysaccharide determines the stability of biofilm.
- The development of biofilm could be divided into three stages.
- *Gammaproteobacteria* are the most dominant microbial species in class level at the last stage.

GRAPHICAL ABSTRACT



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ABSTRACT

This work aims at revealing the adhesion characteristics and microbial community of the biofilm in an integrated moving bed biofilm reactor–membrane bioreactor, and further evaluating their variations over time. With multiple methods, the adhesion characteristics and microbial community of the biofilm on the carriers were comprehensively illuminated, which showed their dynamic variation along with the operational time. Results indicated that: (1) the roughness of biofilm on the carriers increased very quickly to a maximum value at the start-up stage, then, decreased to become a flat curve, which indicated a layer of smooth biofilm formed on the surface; (2) the tightly-bound protein and polysaccharide was the most important factor influencing the stability of biofilm; (3) the development of biofilm could be divided into three stages, and *Gammaproteobacteria* were the most dominant microbial species in class level at the last stage, which occupied the largest ratio (51.48%) among all microbes.

1. Introduction

Membrane bioreactor (MBR) process, combining a conventional activated sludge (CAS) process and membrane filtration, is an effective approach to purify both domestic and industrial wastewater (Huang and Lee, 2015; Mutamim et al., 2013). During the past decade, the merits of an MBR process, including higher removal of organic pollutants and nutrients, smaller space requirement, less excess sludge production, and reduced footprint, were gradually recognized and have aroused lots of academic interests in the fields of both applied and theoretic research. However, membrane fouling is still a great obstacle to limit the successful application of MBR in many fields (Lee and Kim, 2013; Poorasgari et al., 2014).

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So far, a general consensus has been reached on the inducement of membrane fouling, that is, the occurrence state and the concentration of biomass in MBRs, especially the suspended sludge (Lousada-Ferreira et al., 2015), has a very dominant influence on membrane fouling. Actually, the sludge in an MBR has two contradictory effects on its performance. On one hand, higher concentration of sludge means more biomass in the bioreactor, which is beneficial to decompose organic pollutants and convert nutrients; on the other hand, higher concentration of sludge also means more possibilities of suspended sludge particles and other substances depositing on the surface of membrane modules, which is an essential reason causing serious membrane fouling. For keeping more active biomass in an MBR, and at the same time retarding the negative effect of membrane fouling caused by the suspended sludge, a novel approach, adding carriers into an MBR to form an adhesive biofilm on the surface, has been invented, which is so called a moving bed biofilm reactor–membrane bioreactor (MBBR–MBR). Recently reported literature demonstrated that the novel configuration of MBBR–MBR has dominant advantages in the removal of micro pollutants and nutrients with less membrane fouling (Luo et al., 2015; Zhu et al., 2015).

Regarding of a bio-process for wastewater treatment, microorganisms are undoubtedly the main factor to be responsible for the degradation of organic pollutants. Nevertheless, so far, a common knowledge has been recognized, that is, not an individual microorganism species, but a whole microbial ecosystem, plays an actual role in degradation of organic pollutants. In a bioreactor, countless microorganisms may form one or several microbial communities, which compose a microbial ecosystem with a clear structure and explicit function. In this meaning, each bioreactor can be regarded as an artificial microbial ecosystem (Xia et al., 2010), and fully understanding of the microbial communities within a bioreactor is very useful to optimize the performance and management of a bio-process.

The most common configuration of an MBBR–MBR includes two containers (Duan et al., 2015; Leyva-Díaz et al., 2013), in which membrane modules and carriers are usually installed or put in separately. In one of the containers, the membrane modules totally retain solid particles or macromolecular substances, which guarantee high quality of effluents, while, in the other container, carriers provide sufficient space for the growth of microbial community, which is responsible for degrading organic pollutants and converting nutrients, and between the two containers, a pump is used to recycle the mixed liquid suspended solids (MLSS). Unlike a traditional MBR, most of the biomasses in an MBBR–MBR adhere to the surface of carriers, and they form a dense layer of biofilm, which minimize the concentration of suspended sludge flocs/particles, and also reduce the potential of membrane fouling (Di Trapani et al., 2014). In this regard, the physico-chemical characteristics and microbial communities of the biofilm in an MBBR–MBR are very primary factors to influence the start-up and the operational performance of bioreactor.

In a bio-treatment process, including biofilm systems and activated sludge processes, the metabolism of microorganisms generally secrets lots of biopolymers. With these substances, microorganisms may aggregate together to form a complex microbial community with an explicit environmental function. In a CAS process, the biopolymer is the key component contained in the skeleton of sludge flocs, while, in a biofilm system, the biopolymer is the only factor to be responsible for forming a stable biofilm and adhering microorganisms on the carriers with enough physical stability. Generally, two technical terms are used to express the content of biopolymers in biofilm, one is extracellular polymeric substances (EPS), and the other is total organic carbon (TOC). EPS can be classified as bound EPS and soluble EPS, but only the bound EPS determines the adhesion characteristic of biofilm and influences the composition of microbial communities. The bound EPS can also be further classified as tightly-bound EPS (TB-EPS) and loosely-bound EPS (LB-EPS), which have different influence on the adhesion characteristics of biofilm. Among them, TB-EPS is found in cell wall, which is mainly responsible for keeping cells together in clusters; and the main role of LB-EPS is bonding different clusters to form stable micro-colonies (Pellicer-Nacher et al., 2013). The chemical components in EPS are very complex, but protein (PN) and polysaccharide (PS) are the major components, which generally account for more than 85% (wt.) (Baroutian et al., 2013), and accordingly, these components in different EPS are termed as tightly-bound PN (TB-PN), loosely-bound PN (LB-PN), tightly-bound PS (TB-PS), and loosely-bound PS (LB-PS). When the term TOC is used to describe the content of biopolymer in a biofilm, it includes all of the organics, therefore, tightly-bound and loose-bound biopolymer can be also expressed with TB-TOC and LB-TOC, respectively. In this meaning, different EPS and TOC may have various influences on the adhesion characteristic and strength of biofilm, and they are essential factors to affect the establishment and stability of the biofilm (Xue et al., 2012).

Combining an MBBR and an MBR into a single bioreactor would make up an integrated MBBR–MBR (IMBBR–MBR). This new configuration not only reduces the requirement of space and energy consumption, but also stimulates the mass transfer between the MBBR and MBR very quickly. Based on the above analysis, the adhesion characteristics and the microbial community are of primary importance to understand the essence of an IMBBR–MBR. However, in the reported literature, there is still very little information available about the adhesion characteristics and microbial community of the biofilm in this kind of bioreactor, such as roughness, morphology, adhesion force, microbial communities and their variation from the start-up to the stable operation. Therefore, the present investigation aims at revealing the essential factors that affect the successful start-up and performance of an IMBBR–MBR, thus, two closely related aspects were especially considered: (1) the variation of adhesion characteristics of biofilm within the bioreactor over operational time; (2) the composition of microbial communities and their varying trend over time in the biofilm.

2. Materials and methods

2.1. IMBBR–MBR configuration and experimental process

The experiment was conducted in a rectangle bioreactor ($35 \times 25 \times 25$ cm) with an effective volume of 20 L. The bioreactor was divided equally by a metal screen, one part acted as the moving bed bioreactor (MBBR) area, and the other part as the membrane bioreactor (MBR) area. Simulated wastewater was prepared in a water supply tank, and was transported to the MBBR area through an accurate peristaltic pump. In the MBBR area, round polythene carriers (Table 1) were filled with 35% ($V_{carrier}/V_{reactor}$) filling ratio. In the MBR area, a membrane module (hydrophilic PVDF hollow membrane with a pore size of 0.22 μm and surface area of 0.5 m^2) was mounted vertically in the middle of this part. Air was blown through two aerators mounted at the bottom of the MBBR and the MBR area after accurate gauging. The schematic diagram is shown in Fig. 1.

The inoculated sludge was collected from the secondary sedimentation tank of the Lijiao municipal wastewater treatment plant (located at Haizhu District, Guangzhou, China). The constantly moving state of the carriers in the bioreactor caused a great difficulty to form a stable biofilm on the surface of carrier. For stimulating fast attachment of biofilm on the carriers, a special strategy was adopted in the experiments, which included the following steps: (1) when the inoculated sludge was taken to the