Journal of Membrane Science 473 (2015) 36–44

Contents lists available at ScienceDirect

Journal of Membrane Science

journal homepage: www.elsevier.com/locate/memsci

Surface-enhanced Raman spectroscopy monitoring the development of dual-species biofouling on membrane surfaces

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ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form
5 September 2014
Accepted 7 September 2014
Available online 16 September 2014

Keywords:
Biofouling
Surface-enhanced Raman spectroscopy
Biofilm
Dual-species
Membrane fouling

ABSTRACT

Surface-enhanced Raman spectroscopy (SERS) was used to monitor the development of a dual-species biofilm formed by two model bacteria (*Brevundimonas diminuta, BD and Staphylococcus aureus, SA*) on a mixed cellulose ester membrane surface. The highly distinguishable SERS features of BD and SA as well as a semi-quantitative analysis of SERS were used to characterize dynamic changes in dominant species within the biofilm with culture time. SA dominated for the first 8 h but detached from the membrane after 24 h and were outcompeted by BD. SA also displayed differing behaviors in single and dual cultures, with no detachment in the former case but extensive detachment in the latter after 24 h. SERS results were in good agreement with that from scanning electron microscopy. Cell concentrations in solution and competition for limited nutrients accounted for changes in bacterial abundance in dual-species biofilms. Furthermore, bacterial attachment on the membrane as early as 1 h was detected by SERS, demonstrating its high sensitivity and capability for early diagnosis of biofouling. The extent of membrane biofouling was also monitored by plotting SERS peak intensity against culture time. This study suggests that SERS will provide insights into interspecies interactions in biofouling development and help the development of antifouling strategies.

1. Introduction

Membrane technology has been widely applied in the water industry because of its excellent performance in improving water quality. However, a major problem during long-term operation is membrane fouling, which can cause flux decline, more frequent membrane cleaning, high energy demand and reduced lifetime of membranes [1]. Amongst the various types of fouling such as biofouling [2–5], organic fouling [6,7], scaling [8], and colloidal fouling [9,10], biofouling is the most prevalent and problematic one. Biofouling is a biofilm problem caused by undesired adhesion of bacteria and subsequent biofilm development onto membrane surfaces. It occurs widely in various membrane technologies, such as membrane bioreactors [11–13], ultrafiltration [14], nanofiltration [3], reverse osmosis [2,4,5,15], forward osmosis filtration [8], and proton exchange membranes [16]. More seriously, once formed, it is difficult to be completely eradicated because only a few initial colonies of biofilms on the membrane surface can form a mature biofilm. Improved knowledge of biofilm development on membrane surfaces will facilitate the development of antifouling strategies.

Biofilms in both natural and engineered environments such as membrane bioreactors are typically composed of multiple species of bacteria [17,18]. Given the diversity of biofilms, interspecies interactions are very likely to play important roles in determining their development, structure and function. It has been reported that multi-species biofilms exhibit different behaviors from single-species biofilms, such as delayed growth, higher resistance to antibiotics and metabolic cooperation [19–21]. However, most of the recent work in biofilm formation and control studies on membrane surfaces focused on single-species biofilms, such as assessing the effect of membrane surface properties and permeate drag forces on bacterial adhesion and biofilm development [3,4,22,23]; evaluating the effect of nutrition levels on biofouling of a reverse osmosis membrane [2]; investigating the influence of active cells and a conditioning layer on early-stage biofilm formation on ultrafiltration membranes [24]; elucidating the effect of membrane biofouling on permeate flux, salt rejection, and boron removal [15,25]; and controlling membrane biofouling using Ag nanoparticles (NPs) or D-tyrosine [26,27]. In contrast, investigations on interspecies interactions and development of multi-species biofilms are rare and most of them do not focus on membrane surfaces [19–21,28]. To fully characterize a biofilm

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http://dx.doi.org/10.1016/j.memsci.2014.09.007
0376-7388/

and understand its development, it is necessary to develop reliable techniques that are capable of discriminating between bacterial species and monitoring their competitive or collaborative behaviors during biofilm development.

Amongst current techniques, confocal laser scanning microscopy (CLSM) has been very widely used. Various fluorescence probes have been applied to stain cells and extracellular polymer substances (EPS) to observe the 3D structure of biofilms. After that the development of cell biovolume and EPS in a biofilm layer can be determined using image-processing software [14–16]. Commonly used probes, such as live/dead SYTO9/propidium iodide fluorochrome, 4′6-diamidino-2-phenylindole (DAPI), crystal violet and Concanavalin A (ConA) lectin-dye conjugate, cannot clearly differentiate bacterial species because they are non-specific. To overcome this problem, green fluorescent proteins (GFP)-expressing bacteria and fluorescent in-situ hybridization (FISH) have been used to determine the abundance and the spatial distribution of various species within multi-species biofilms [21,28,29]. Additionally, scanning electron microscopy (SEM) can provide high-magnification and high-resolution visualization of the morphology and the distribution of adhered bacteria and mature biofilms on various substrate surfaces [30]. Identification of bacterial species and investigation of the development of multi-species biofilms can also be carried out by SEM for cells with discernable morphologies.

Surface-enhanced Raman spectroscopy (SERS) can provide fingerprint information on molecules, bacteria and biofilms without the need for staining [7,31–36]. The strong electromagnetic enhancement typically provided by Ag or Au NPs can provide SERS with extremely high detection sensitivity, down to single-bacteria and even single-molecule levels [37]. SERS, combined with multivariate analysis, has been demonstrated to be a powerful and reliable tool to discriminate bacteria down to species and strain levels because of its fingerprinting information, high sensitivity, high speed, and minimal requirements for sample preparation [35,38,39]. Therefore, SERS has great potential in studying the behavior of different bacterial species in multi-species biofilms. In addition, SERS analysis is non-destructive because there is no need to remove the biofilm from substrates. Its ability for semi-quantitative analysis also allows the detection of fouling extent on membranes. Chao and Zhang recently applied SERS to study chemical variation in single-species biofilms in different growth phases, from initial attachment to mature biofilms [36]. Ivleva et al. used SERS to analyze chemical compositions and their distribution in a mature multi-species biofilm (22 and 82 days old) inoculated from the activated sludge of a wastewater treatment plant [33]. However, both works focused on chemical variations, including polysaccharides, proteins and DNA, but did not include discussions on bacterial behaviors and interspecies interactions during the development of biofilms. We recently used SERS to monitor the fouling process of proteins on polyvinylidene fluoride (PVDF) membranes and compared the fouling propensities of three kinds of proteins [7]. Recently, Lamsal et al. used SERS and normal Raman spectroscopy to examine fouling caused by natural organic matter on a nanofiltration membrane [40]. However, to our knowledge, SERS has been rarely applied to characterize collaborative or competitive behaviors of different bacterial species during the development of multi-species biofilms on membrane surfaces.

Therefore, SERS was employed as a new and non-invasive technique to study the development of dual-species biofilms from the initial bacterial attachment to biofilm formation on a mixed cellulose ester membrane. Two model bacteria, *Brevundimonas diminuta* (BD) and *Staphylococcus aureus* (SA) were used in this investigation. The distinguishable SERS features of BD and SA and the semi-quantitative analytical ability of SERS were used to monitor the dynamic evolution of the dominant species in dual-species biofilms and biomass changes (extent of biofouling) with culture time. Different behaviors of bacteria in single- and dual-cultured biofilms were also investigated and compared. SERS judgments were confirmed by acquiring SEM images of dual- and single-species biofilms. In addition, the ability of SERS in early diagnosis of bacterial attachment and nutrition adsorption as early as 1 h after exposure of the membrane surface to the bacterial suspensions was also demonstrated.

2. Experimental

2.1. Bacterial species

Brevundimonas diminuta (BD) extracted from drinking water distribution pipeline is a gram-negative rod-shaped bacterium belonging to the phyla *Proteobacteria. Staphylococcus aureus* (SA) (ATCC 25923) from Guangdong Culture Collection Center of Microbiology, China, is a gram-positive coccus bacterium belonging to the phyla *Firmicutes*. There are three reasons for the selection of these two bacteria: 1) *Proteobacteria* have been found in abundance within biofilms in membrane bioreactors or microfiltration systems [17,41,42], and *Firmicutes* have been identified as pioneer bacteria responsible for membrane surface colonization [41,43]; 2) BD and SA display highly distinguishable SERS features, which can be clearly observed in Fig. 1. 3) BD and SA have different shapes, which facilitates the observation of individual species by SEM to validate SERS judgments.

2.2. Micro-filtration membrane preparation

Mixed cellulose ester membranes (MCE) with pore size of 0.1 μm were purchased from Millipore (Cat no. VCWP02500) and used as substrates for biofilm formation. MCE membranes with

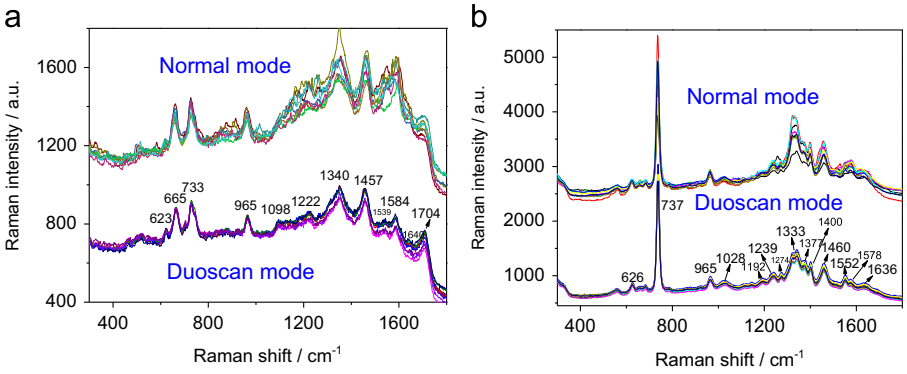

Fig. 1. SERS spectra acquired from different spots on BD (a) and SA (b) biofilms grown for 48 h using the Duoscan mode ($30 \times 30\ \mu m^2$) and normal mode (about 3 μm in diameter).