Water Research 87 (2015) 282–291

Contents lists available at ScienceDirect

Water Research

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

Interrogating chemical variation *via* layer-by-layer SERS during biofouling and cleaning of nanofiltration membranes with further investigations into cleaning efficiency

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ARTICLE INFO

Article history:
Received 7 July 2015
Received in revised form
18 September 2015
Accepted 21 September 2015
Available online 25 September 2015

Keywords:
Biofouling
Biofilm
Chemical composition
Cleaning efficiency
Nanofiltration membrane
Surface-enhanced Raman spectroscopy

ABSTRACT

Periodic chemical cleaning is an essential step to maintain nanofiltration (NF) membrane performance and mitigate biofouling, a major impediment in high-quality water reclamation from wastewater effluent. To target the important issue of how to clean and control biofouling more efficiently, this study developed surface-enhanced Raman spectroscopy (SERS) as a layer-by-layer tool to interrogate the chemical variations during both biofouling and cleaning processes. The fact that SERS only reveals information on the surface composition of biofouling directly exposed to cleaning reagents makes it ideal for evaluating cleaning processes and efficiency. SERS features were highly distinct and consistent with different biofouling stages (bacterial adhesion, rapid growth, mature and aged biofilm). Cleaning was performed on two levels of biofouling after 18 h (rapid growth of biofilm) and 48 h (aged biofilm) development. An opposing profile of SERS bands between biofouling and cleaning was observed and this suggests a layer-by-layer cleaning mode. In addition, further dynamic biochemical and infrastructural changes were demonstrated to occur in the more severe 48-h biofouling, resulting in the easier removal of sessile cells from the NF membrane. Biofouling substance-dependent cleaning efficiency was also evaluated using the surfactant sodium dodecyl sulfate (SDS). SDS appeared more efficient in cleaning lipid than polysaccharide and DNA. Protein and DNA were the predominant residual substances (irreversible fouling) on NF membrane leading to permanent flux loss. The chemical information revealed by layer-by-layer SERS will lend new insights into the optimization of cleaning reagents and protocols for practical membrane processes.

1. Introduction

Water scarcity and strict regulations for the disposal of secondary wastewater effluent generates an urgent need for reclamation of this resource, especially in industrial processes requiring high consumption and wastewater generation (Bes-Pia et al., 2010). Nanofiltration (NF) has been widely utilized as a technology for high-quality water reclamation from wastewater effluent in industries, such as textiles, pulp and paper mill (Bes-Pia et al., 2010; Gonder et al., 2011; Judd and Jefferson, 2003). However, fouling is a major impediment in wastewater reclamation. Fouling causes a decline in permeate flux, requires frequent chemical cleaning, shortens membrane lifespan, and therefore significantly increases the operational cost of NF membrane plants (Guo et al., 2012).

The major types of membrane fouling include scaling (Zhang et al., 2012a), organic matter (Liu et al., 2012; Yao et al., 2010), colloidal (Tung et al., 2012) and biofouling (Al-Juboori and Yusaf, 2012; Baek et al., 2011; Chen et al., 2015; Sim et al., 2013; Zhang et al., 2012b). Amongst these, biofouling, caused by undesired microbial attachment and subsequent biofilm development onto the membrane surface, is the most prevalent and problematic fouling type (Flemming, 2002). Biofouling easily occurs, but is hard to eradicate, because bacteria are ubiquitous in wastewater effluent and tiny amounts of initial bacterial contamination on membranes can form a mature biofilm. Additionally, relative abundance of

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http://dx.doi.org/10.1016/j.watres.2015.09.037
0043-1354/

nutrients on membrane surfaces rather than bulk feed and the convective permeate flow also facilitates biofilm growth (Flemming et al., 1997). The typical biofouling (biofilm) structure is a microbial community with bacteria embedded into the self-produced matrix of extracellular polymeric substances (EPS). EPS are biopolymer mixtures mainly composed of protein, nucleic acids, polysaccharide and lipid, and account for 90% of the dry mass in most biofilms (Flemming and Wingender, 2010). EPS not only induce declines in permeate flux by increasing hydraulic resistance to permeate flow (Herzberg and Elimelech, 2007; Herzberg et al., 2009). They also act as a protective barrier to diffusion by retarding the adequate delivery of disinfection and cleaning agents to the embedded microorganisms (Nguyen et al., 2012), thus rendering such membrane cleaning less effective.

Periodic chemical cleaning and disinfection are essential steps in maintaining membrane performance (Li and Elimelech, 2004). Various chemical cleaning reagents, biocides and disinfectants, have been developed to control and reduce biofilm growth (Huang et al., 2009a; Liikanen et al., 2002; Mendret et al., 2009; Nguyen et al., 2012). The large consumption of cleaning reagents and accompanying energy costs impose a large economic burden on the operation of membrane plants. However, biofilm matrix has a complex chemical composition which varies dynamically with many factors such as development stages, nutrient supply, hydraulics and bacterial species (Chao and Zhang, 2012; Flemming and Wingender, 2010; Houari et al., 2010; Ivleva et al., 2010). For instance, more dead cells were observed in a mature and aged reverse osmosis biofouling compared to early stage (Herzberg and Elimelech, 2007). Storage time of biofouled NF membranes was found to affect both the membrane permeability and cleaning efficiency (Houari et al., 2009). Degradation of EPS by extracellular enzymes was reported to occur extensively in biofilms in order to self-supply carbon and energy sources (Flemming and Wingender, 2010). These observations suggest dramatic chemical variations in biofouling during formation and cleaning processes. A deeper understanding of biofouling composition and dynamic changes can greatly benefit the adoption of appropriate cleaning reagents and effective cleaning protocols. Given the fact that cleaning reagents mainly interact with the surface chemicals of biofouling, a tool capable of layer-by-layer interrogation of chemical composition is therefore required to investigate the cleaning mechanism and biofouling substance-dependent cleaning efficiency.

Confocal laser scanning microscopy (CLSM) is widely used in biofilm studies. CLSM can characterize the three-dimensional structure of biofilm and quantify the biovolume of bacteria and EPS by staining biofilm with different fluorescent probes (Yuan et al., 2015). Nevertheless, because of the complexity of EPS compositions and a broad fluorescence spectrum, CLSM cannot achieve high specificity for structural analysis. Raman spectroscopy provides whole-organism fingerprint information with all related biomolecules (nucleic acids, protein, lipid, polysaccharides and their metabolites) displaying distinct spectral features, and thus has been utilized to study the chemical heterogeneity of biofilms (Andrews et al., 2010; Ivleva et al., 2009; Sandt et al., 2007; Wagner et al., 2009). However, application of Raman spectroscopy for biofouling on NF membrane has two challenges: 1) very weak signal (one Raman photon out of 10^8 incident photons) and thus low detection sensitivity; and, 2) interference of Raman bands from membrane materials. Surface-enhanced Raman spectroscopy (SERS) has been developed for biofilms studies due to its ultrasensitivity, even down to single molecule levels (Kneipp et al., 2008), relying on significant electromagnetic enhancement of up to 10^6–10^{14} over normal Raman scattering provided by silver and gold nanoparticles (Ag NPs and Au NPs). Raman signals of molecules adsorbed or in close proximity to these NPs can be greatly enhanced. This short-distance enhancement effect of SERS can thus exclude membrane interferences, and more importantly, ensure revelation of chemicals only on the surface layer of biofouling. SERS has been successfully applied to study chemical composition and distribution on mature biofilms (Ivleva et al., 2009, 2010), chemical variation during biofilm formation from initial attachment to mature biofilm (Chao and Zhang, 2012), dynamic evolution of microbial structure during biofilm development on cellulose membranes (Chen et al., 2015), and protein fouling on PVDF membranes (Cui et al., 2011). However, the biofilm cultivation conditions in previous investigations (*i.e.*, under static or flow-cell cultivation and/or on glass slides) were far removed from that of real membrane biofouling on commercial membranes under pressure-driven crossflow filtration. Moreover, only biofilm formation processes were addressed, largely ignoring the key cleaning process. Considering the differences of biofouling chemical composition between formation and cleaning processes, the biofouling variation during the cleaning process requires more attention. Outcomes will contribute to the selection of cost-effective cleaning reagents and protocols.

To target chemical variation of membrane biofouling and the important issue of how to clean biofouling more efficiently, this work developed a crossflow membrane filtration system to simulate wastewater reclamation using commercial DOW NF90 membranes, synthetic wastewater effluent and a model bacterial strain. A layer-by-layer SERS tool was employed to interrogate the chemical variations during both the biofouling and cleaning processes. Biocompatible Au NPs were used for SERS acquisition to eliminate possible artifacts caused by microbial toxicity of Ag NPs (Chao and Zhang, 2012; Cui et al., 2013, 2015; Ivleva et al., 2010). Dynamic chemical and infrastructural changes within biofouled membranes and their effects on cleaning processes were studied. Biofouling substance-dependent cleaning efficiency and the persistent chemicals contributing to the permanent flux loss were also evaluated. These studies are important towards our understanding of the cleaning process and factors affecting cleaning efficiency.

2. Materials and methods

2.1. Synthetic wastewater effluent and model bacterial strain

An enriched synthetic wastewater effluent was used for NF membrane biofouling development in a crossflow filtration system, based on the secondary effluent quality of a wastewater treatment plant with high-rate biological processes (Herzberg and Elimelech, 2007). Its composition included: 341 mg/L sodium citrate, 61 mg/L KH_2PO_4, 42 mg/L $NaHCO_3$, 117 mg/L NaCl, 148 mg/L $MgSO_4{\cdot}7H_2O$, 50 mg/L NH_4Cl and 1:1000 diluted Luria Bertani (LB) broth in deionized water (Herzberg and Elimelech, 2007). LB broth was prepared by adding 10 g tryptone (Oxoid Ltd., England), 5 g yeast extract (Oxoid Ltd., England) and 10 g NaCl into 1 L deionized water. Unless otherwise stated, all chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd, China. LB broth and synthetic wastewater effluent were sterilized at 121 °C for 30 min before use.

Brevundimonas diminuta is a gram-negative rod-shaped bacterium belonging to phyla Proteobacteria; it is found in abundance within biofilms in membrane filtration systems (Kwon et al., 2011). After overnight cultivation in LB broth at 37 °C and 180 rpm, *B. diminuta* reached the later exponential phase with a final optical density (OD_{600}) of 1.0 and concentration of 10^9 CFU (colony forming unit)/mL. After centrifugation at 7000 rpm for 5 min, *B. diminuta* pellets were re-suspended in synthetic wastewater effluent and used as inoculum in the crossflow filtration system.