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Real-time Raman based approach for identification of biofouling

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ABSTRACT

This study describes a proof-of-concept for a compact real-time surface-enhanced Raman spectroscopy (SERS)-online sensing approach for detection of biofouling in drinking water membrane filtration. In this study we created a custom-designed flow-cell that mimics a cross-flow membrane filtration system. This enables one to measure changes in surface-foulants, such as *Brevundimonas dimiuta* (BD) bacteria and adenine, under conditions that are similar to conventional membrane filtration systems. For measurements we used a common portable Raman-spectrometer with a laboratory Raman-probe in combination with a specially developed gold nanoparticle (Au NP) SERS-sensing area on filter-membranes. This allowed real-time detection of low concentrations of surface-foulants immediately after inoculation into an ultra-pure water reservoir under pressure-driven filtration conditions. We compared these online results with static measurements from an offline, sample-taking approach, using a confocal Raman-laboratory-microscope. The developed Au NP SERS-sensing-area on the membranes proved to be stable over a long period of surface fouling investigations and to suppress the strong interfering Raman-signal originating from the composition layer of most filtration membranes.

1. Introduction

Membrane technology is globally a key technology for water management and it is rapidly gaining industrial popularity. A major challenge in using membrane technology is membrane fouling [1], especially biofouling. It causes a decrease in filtration capacity, increases filtration time and reduces the lifetime of membranes [2–5]. Only a few bacteria can initiate the formation of a mature biofilm, which goes on to develop further layer by layer, into bacterial communities [6–10]. Therefore, once an undesired adhesion of bacteria has occurred, it is difficult to completely remove the biofilm formed. Because biofouling occurs just on the surface of membranes, a real-time monitoring solution that does not disturb the performance of membrane filtration systems would be desirable [11,12]. The flux-decline and increase of trans-membrane pressure can be used as an initial indication of membrane fouling [14,17]. Usually, the membranes are cleaned regularly to remove potential foulants and when their performance is deteriorated they need to be replaced [13,14]. In order to avoid excessive chemical cleaning and replacement of membranes it would be necessary to identify biomolecules and differentiate fouling types [9,15–17]. An optimum solution would be real-time monitoring of the membrane surface which can reduce the environmental impact and may be economically more feasible compared to the membrane replacement strategy [18,19].

There are many approaches to measure fouling formation in real-time. Promising optical methods are e.g. attenuated total reflection (ATR), confocal scanning laser microscopy (CSLM), Fourier transform infrared spectroscopy (FTIR), photo-sensors and bioluminescence [17,20]. When online observation is of importance, real-time Raman spectroscopy has been found particularly useful, though some challenges remain in its applicability [21–25]. Furthermore, Raman with SERS-enhancement allows real-time observation due to its ability to identify the chemical information, especially the differentiation of fouling types and their changes over time. Real-time observation of early-stage biofouling development and its mechanisms using e.g. standard

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process Raman-detection without SERS-enhancement is challenging. The main challenges are the interfering Raman scattering and the background fluorescence originating from the layer-composition of the membranes used for membrane-filtration which obfuscate the weak signal from the surface-foulants [26–28]. Surface-enhanced Raman spectroscopy (SERS) is a promising technique that allows non-invasive and in-situ studies of the growth of the bacteria fouling layers on membrane surfaces. This has for the first time been reported in our previous study [8] and has since then been recognized as a characterization technique for biofouling investigations [18]. One of the advantages of SERS is that it can easily be applied on surfaces. SERS also overcomes the limit of detection (LOD) especially in aqueous solutions, and it gives an enhancement of about 10^6 in scattering efficiency compared to normal Raman scattering [29]. Another key benefit of applying SERS compared to normal Raman spectroscopy is its ability to suppress the interfering Raman scattering originating from the layer-composition of most membranes, as demonstrated in this study. There are, however, several challenges related to real-time biofouling detection in cross-flow filtration systems, particularly in those that mimic industrial conditions. Firstly, the cross-flow velocity over the observed membrane makes it difficult to immobilize the SERS nanoparticles – required to enhance the weak Raman scattering – reliably to the membrane surface. Secondly, the pressure of water which is pumped through the system is high and vibrations caused by the water circulation pump can disturb the focusing of the probing system. Nevertheless, studies on biofouling behavior need to address these challenges for the applications to be suitable for industrial settings.

In this study we demonstrate a proof-of-concept solution for the use of online SERS-measurements in conditions mimicking cross-flow membrane filtration. We have developed a novel solution for preparing pressure and water-flux resistant SERS-sensing areas consisting of colloidal gold nanoparticles on filter-membranes and integrated them into filtration processes for online monitoring. The Raman-signal enhancement and stability of the set-up were tested over an extended period. The fabrication technique of SERS-substrates plays an important role in the performance of SERS [30–32]. The shape and size of the used Au NPs should be optimized in order to fit the pore size of specific membranes. Here we use a similar kind of approach for the mechanical attachment of Au NPs to the membrane surfaces as described in our earlier study for silver nanoparticles (Ag NPs) [8]. The porous and heterogeneous structure of many common membrane types (cp. Table 1) is beneficial for this type of SERS-sensing area immobilization and makes it stable for high cross-flow velocities and throughputs. Even though Ag NPs provide a stronger SERS-signal enhancement [33], we used Au NPs in this study because they are more stable compared to Ag NPs and they are non-toxic for bacteria, which is important especially in the early-stage of monitoring biofilm development [34,35].

Real-time SERS-investigations in this study focused on finding a robust method to sense biofilm fouling. This method could help improving cleaning and pre-treatment schemes [36]. Within this study we use the terms real-time and online as synonyms and the results are compared to a static offline, sample-taking approach.

2. Material and methods

2.1. Synthesis of gold nanoparticles

Colloidal Au NPs with an average dimension of about 120 nm at the longest side of the cross-section in an oval shape were synthesized by following the Frens-method [37]. Concisely, 100 mL of 0.01% (wt/vol) $HAuCl_4$ aqueous solution was heated to boil under vigorous and continuous stirring, followed by the immediate addition of 0.6 mL of 1% (wt/vol) trisodium citrate solution. The solution was kept boiling for about 1 h until the color changed to light ruby red. The final Au NP-solution was prepared by centrifugation at 3500 rpm for 5 min (Eppendorf 5430R centrifuge) and subsequently followed by the removal of the supernatant. The final dark ruby red Au NP-solution with a concentration of about 5400 mg/L was used for the SERS-substrate development. The biocompatibility and size-distribution have been described in our previous research [34].

2.2. Membrane and substrate preparation

The highly concentrated Au NP-solution (10 μL) was filtrated directly onto membranes and dried quickly under an applied mechanical pressure consequently immobilizing the Au NPs within the top-part of the membrane layer making them stable against elution [8]. An even circular visible area was formed right on the top-layer of the membrane as a SERS-sensing area.

The Au NP SERS-sensing-area was prepared on various commercial and home-made membranes with different pore sizes and compositions (Table 1). The immobilization of the Au NPs on membranes used for cross-flow filtration was done just before the membranes were placed into the flow-cell. The Au NP SERS-sensing-area was visible as an evenly ruby red colored circular detection area as shown in Fig. 1A.

2.3. Foulant samples

Biofilm model-bacteria *Brevundimonas dimiuta* (BD) were extracted from a drinking water pipeline as described earlier [9,38]. Pure adenine (Sinopharm Chemical Reagents Co., Ltd., China), a readily available small water soluble molecule (135.13 g/mol) was used since adenine and adenosine have been identified to contribute to SERS-spectra of biofilms.

2.4. Bacteria adhesion process on membranes with and without SERS-substrate

Commercial NF90 polyamide nanofiltration (NF90), mixed cellulose ester (MCE) microfiltration and home-made polysulfone (PS) ultrafiltration-membranes with and without the Au NP SERS-sensing-area were prepared for monitoring bacteria adhesion. The home-made PS ultrafiltration-membranes were used in the online flow-cell and long-term substrate-performance-measurements (Sections 3.3 and 3.4). The applicability of the developed Au NP SERS-sensing-area on membranes was tested offline on commercial NF90 and MCE-membranes.

Table 1
Comparison of the level of the interfering Raman-signal of different pure non-fouled filter-membranes determined with a confocal Raman-scanning-microscope.

Membrane type	Filtration type	Selective layer	Dominating composition	Level of interfering Raman-signal
1. NF90 (Dow,USA)	Nanofiltration	UF-layer	Polyamide	Strong
2. NF270 (Dow, USA)	Nanofiltration	UF-layer	Polyamide	Strong
3.PS (home-made)	Ultrafiltration	UF-layer	Polysulfone	Strong
4. PVDF (0.1 μm, Millipore, USA)	Microfiltration	MF-layer	Polyvinylidene fluoride	Weak
5. MCE (0.1 μm Millipore, USA)	Microfiltration	MF-layer	Mixed cellulose ester	Weak
6. PC (0.22 μm Millipore, USA)	Microfiltration	MF-layer	Polycarbonate	Moderate