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Use of electrical resistance tomography (ERT) for the detection of biofilm disruption mediated by biosurfactants

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ABSTRACT

Inline measurement of biofilms could provide a valuable technology for water, food and bioprocessing industries to improve quality control and avoid contamination. This study presents the first use of electrical resistance tomography (ERT) to detect the removal of biofilms in a pipe. It also tests the effectiveness of sophorolipids and rhamnolipids for the disruption of *Bacillus subtilis* BBK006 biofilms in an industrial setting. Biofilms were grown on the inner side of a section of 1.5″ test pipe for 5 days using nutrient broth as the culture medium. After the medium was removed the test pipe was incorporated into a cleaning test circuit for the biofilm disruption experiment, where water, sophorolipids (0.4 g L^{-1}) or rhamnolipids (0.4 g L^{-1}) solutions were pumped through respectively for 30 min. ERT was used as an indirect evaluation of the film disruption progression. A cleaning parameter was identified based on zonal boundary averages which successfully measured the extent of biofilm removal.

1. Introduction

Biosurfactants are amphiphilic compounds produced on living surfaces, mostly on microbial cells or excreted extracellular hydrophobic and hydrophilic moieties. Bio-based surfactant global production was about 3.5 million tons in 2012, with a market value worth USD 6,588 million (Silva et al., 2014). Several industrial applications including detergents, agricultural chemicals and textiles, have been reported. More recently, biosurfactants have been considered as promising candidates for the inhibition of microbial biofilms (De Rienzo and Martin, 2016).

Microbial biofilms can be found on almost all natural and artificial surfaces that are submerged in or exposed to an aqueous solution. Given sufficient resources for growth, biofilms may grow rapidly into macroscopic scales even in very hostile environments. Although studies are made to exploit some of the beneficial side of the biofilm growth, such as fermentation, effluent processing, and microbial fuel cell development etc., in most cases, biofilms are shown to be detrimental. They can result in clogging, equipment failure, corrosion or contamination in an industrial production line (e.g. Cunault et al., 2015). There has been continuous and focused research recently into sourcing new generations of bacterial dispersal agents to develop more effective bacterial inhibition strategies as complete mechanical removal by hydrodynamic forces alone can be challenging (e.g. Paul et al., 2012; Lemos et al., 2015). Novel inline and non-destructive characterization techniques are in demand for insitu measurement of biofilms (Janknecht and Melo, 2003; Valladares Linares et al., 2016; Wang et al., 2018).

The electrical properties of biofilms have been reported on before and most have been found to be insulating. L'Hostis et al. (1997), proposed that a biofilm should be considered as a (nonconducting) porous material where overall conductivity is governed by the biofilm layer thickness, the electrolyte diffusion coefficient and the biofilm porosity. As such, for a given biofilm and media the conductivity is inversely proportional to layer thickness. This is consistent with most published work, for example, Muñoz-Berbel et al. (2006), used microchips as electrical transducers to measure *Pseudomonas aeruginosa* biofilm electrical impedance and found the impedance magnitude to decrease as the biofilm grew. Likewise, Dheilly et al. (2008), reported that the electrical conductance of biofilms of *P. aeruginosa* and *Bacillus subtilis* decreased with culture time as the film grew thicker and then increased with subsequent biofilm detachment. A small number of biofilms, such as

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Geobacter sulfurreducens, are conductive or produce current which is attributed to them having conductive phili (Malvankar et al., 2012).

We have reported before that pre-formed biofilms by different microorganisms can be disrupted by biosurfactants (De Rienzo et al., 2015; De Rienzo and Martin, 2016). The investigations also demonstrated the effect of mono and di-rhamnolipids against biofilms formed by mixed cultures under dynamic flow conditions. The current work uses two techniques to evaluate the effect of rhamnolipids and sophorolipids on the attachment and disruption of biofilm cells produced by a mixed culture: offline direct SEM analysis of an inner pipe surface combined with inline electrical resistance tomography (ERT).

ERT is an industrially proven non-intrusive imaging technique, which is commonly used to monitor processes requiring both temporal and special resolutions. The system typically consists of a ring of electrodes evenly spaced around the circumference of a tank or pipe (i.e., domain). Alternating electrical currents are injected into the fluid inside the domain. A cross sectional map of the electrical conductivity, which relates to the material composition inside the domain, can then be reconstructed based on the voltages measured on the boundary electrodes. ERT has been successfully used to monitor and control a wide range of engineering processes, including mixing (Rodgers and Kowalski, 2010), separations (Kowalski et al., 2010) and more recently inline monitoring of cleaning in place (CIP) (Henningsson et al., 2007; Sharifi and Young, 2013; Hou et al., 2016). In contrast to the impedance based biofilm detection methods previously reported (e.g. L'Hostis et al., 1997; Muñoz-Berbel et al., 2006; Dheilly et al., 2008) ERT is commercially available and proven in industrial process environments. In addition, as a tomographic technique it provides detection over a complete plane or volume so detection is less dependent on where the biofilm grows during operation or remains during cleaning. However, to the authors' best knowledge, the current investigation represents the first attempt to use the ERT method for monitoring the biofilm disruption process.

2. Materials and methods

2.1. Bacteria strains and culture conditions

Candida bombicola ATCC 22214 was stored in nutrient broth with 20% glycerol at −80 °C. The standard medium for the production of sophorolipids was glucose/yeast extract/urea (GYU) medium (10% w v^{-1} glucose, 1% w v^{-1} yeast extract, 0.1% w v^{-1} urea). The fermentation medium contained the same growth medium, with rapeseed oil, as a second carbon source, being fed at regular intervals to induce sophorolipid production.

Burkholderia thailandensis E264 was maintained on nutrient agar slants at 4 °C, each slant was used to obtain a bacterial suspension, with the optical density (570 nm) adjusted to give 10^7 CFU mL^{-1} for each of the strains used. The standard medium for the production of rhamnolipids by *B. thailandensis* E264 was nutrient broth (NB) (8 g L^{-1}), with glycerol (20 g L^{-1}). For the biofilm experiments *B. subtilis* BBK006 and *Staphylococcus* sp. were stored in nutrient broth plus 20% glycerol at −80 °C, and used when needed.

2.2. Production of rhamnolipids (RLs)

An Electrolab FerMac 360 fermentation unit was used to perform batch cultivation of *B. thailandensis* E264. The microorganisms used in this study was aerobically (0.5 vvm) incubated in nutrient broth, at 30 °C, during 120 h at 400 rpm. Rhamnolipids were extracted using a foam fractionation system (De Rienzo et al., 2016).

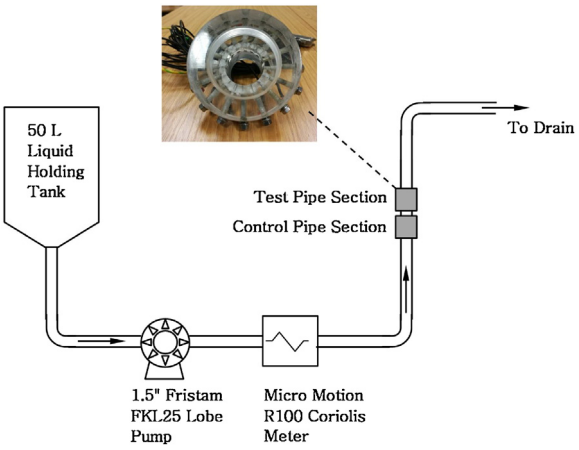

Fig. 1 – Schematic diagram of the experimental setup (inset photo showing the test pipe section with ERT electrodes).

2.3. Production of sophorolipids (SLs)

A crude sophorolipid mixture was obtained as the settled product from fed batch cultivation of *C. bombicola* ATCC 22214, operated without the use of antifoam (Shah et al., 2005) feeding glucose and rapeseed oil rather than waste frying oil. The dry matter content of the crude mixture sophorolipid was adjusted to 45% v v^{-1}.

2.4. The test pipe section, ERT electrodes, and growth of biofilm

Fig. 1 shows a schematic diagram of the experimental setup and an image of the ERT test section. The biofilm growth and disruption experiments were all conducted on a section of 1.5″ nominal sized transparent acrylic pipe. The test pipe section was also fitted with a single plane of 16 electrodes for the ERT measurements. The electrodes were all made of M5 threaded stainless steel rods. They were installed at equal interval around the pipe circumference, with one end flush with the inner surface of the test pipe. Such a configuration ensured that the ERT electrodes were in good electrical contact all the time with the process fluid flowing inside the test pipe section. Note that the electrodes also provided a source of active surfaces for the micro-organisms and biofilms to grow.

For the biofilm growth, the inner side of the circular test pipe section was first inoculated with 50 mL of nutrient broth containing 10^7 colony forming units (CFU) from an overnight culture of each microorganism. It was then incubated at 30 °C for 5 days to allow cells to adhere, with a daily change of the medium to maintain the bacteria viability. Afterwards, the medium was removed, and the test pipe section was ready to be inserted into the cleaning test circuit for the disruption experiments.

2.5. Cleaning test circuit, disruption experiments, and ERT measurements

The test circuit consisted of a 50 L stainless steel cleaning liquid holding tank, a 1.5″ lobe pump (Fristam FKL 25, USA) to pump the cleaning liquid, a Coriolis meter (Micro Motion R100, USA) to monitor and regulate the flowrate, and 1.5″ pipework including the test pipe section. In particular, the test pipe section was mounted upright, with the cleaning liquid flowing upwards. This was to ensure that the test pipe was fully filled