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The impact of cell metabolic activity on biofilm formation and flux decline during cross-flow filtration of ultrafiltration membranes

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HIGHLIGHTS

- Physiological state of biofoulant impacted biofoulant activity.
- Biofoulant activity level did not correlate to biofilm activity level.
- Biofilm activity level was not a good indicator of flux decline.
- Stationary phase samples had more and unique EPS.
- Inactive cells resulted in the least amount of flux decline.

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ABSTRACT

One significant challenge to membrane filtration technologies is membrane fouling causing pressure drop, flux decline and eventually significant cost of membrane replacement. The objective of this research was to determine the impact of metabolic activity as measured in adenosine triphosphate (ATP) concentration of the pure culture of biofoulants on the membrane biofilm metabolic activity, biofilm formation rate, and operational flux decline. Our results showed that after 10–12 h of filtration, the biofilm ATP levels reach an equilibrium concentration (avg. 8 amol/cell) and do not appear to be related to biofoulant ATP levels from cells harvested in the late exponential growth phase regardless of initial ATP level. However, the bacterial growth phase affected the ATP activity of cells and membrane biofilms formed from biofoulants in the lag and stationary phases of growth contained similar levels of activity, and the exponential phase cells resulted in significant higher activity. Flux decline does not appear to be related to metabolic activity of the biofoulant or biofilm following 24 h of filtration but notably, there was much less flux decline when the biofoulant cells were inactive.

1. Introduction

Membrane separation is an attractive option for drinking water production since it produces biologically safe and consistently high quality drinking water and is more sustainable compared to conventional water treatment due to fewer additions of chemicals to raw water in the water treatment process and a smaller process footprint [1]. However, a serious impediment to more widespread implementation is membrane fouling, causing a significant decrease in flux with a corresponding increase in operational energy costs to maintain the design flow rate and, ultimately, membrane replacement. Among the different types of fouling (i.e. crystalline fouling, organic fouling, colloidal fouling, microbiological fouling), biofouling is considered a major problem in membrane separation systems [2] since other types of fouling can be controlled by reduction of foulant concentration in the feed water [3]. Biofouling is hard to control due to the ability of microorganisms present even at very small concentrations in nearly all water systems to colonize almost any surface and to survive under extreme conditions [4]. Furthermore, compared to planktonic cells, bacteria embedded in a biofilm are up to 1000-times more resistant to conventional approaches to mitigating biofouling including disinfection [5].

In order to control and develop effective anti-biofouling strategies in membrane filtration, it is essential to understand the mechanisms of biofouling. This work focuses on early stages of biofilm formation since the first step or immediate attachment of bacteria onto the membrane surface is a reversible process [6]. Thereafter, the biofilm growth process is dominated by the production of extracellular polymeric substances (EPS) which makes the attachment more firmly adhered and irreversible [7]. The hypothesis in this study is that the activity of planktonic cells in the feed water (biofoulant) will affect membrane biofilm cell activity, EPS formation, and consequently membrane performance (i.e. flux decline) in early stage biofilm formation. The activity of the biofoulant is likely to directly impact not only cellular activity in the biofilm but also the amount and composition of extracellular

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polymeric substances (EPS) formed [8,9], and consequently the membrane flux [10].

Adenosine triphosphate (ATP) has been used to determine the intracellular metabolic activity of biomass [11]. ATP is a fundamental biomolecule present in all viable cells whether their metabolism is aerobic or anaerobic and is degraded immediately after cell death [12]. Furthermore, ATP is stable in many studied organisms forming biomass [13]. Prediction of biofilm production can be achieved by analysis of biofilm formation rate [14], which is based on ATP analysis of the biofilm in monitoring biofilm and extraction and measurement of biomass ATP activity [15]. The quantity of ATP depends on the physiological state of the organisms and consequently on the biomass growth rate [16,17].

Therefore, the objective of this study was to investigate the effects of biofoulant activity in ultrafiltration membrane biofouling and the consequent effects on permeate flux. Short-term biofouling experiments with a mono-culture biofilm were carried out using a laboratory-scale ultrafiltration membrane filtration test unit and the activity of the pure culture of biofoulant, feed water, and biofilm accumulated on the membrane surface were investigated with ATP measurement. In addition, the knowledge of the metabolic activity of the membrane biofilm may assist in understanding the biofilm accumulation mechanisms to apply appropriate countermeasures and control of membrane biofouling.

2. Material and methods

2.1. Cross-flow filtration experiments

Cellulose acetate ultrafiltration (UF) membranes with a molecular weight cut-off of 20,000 Da (General Electric water and process technology, Minnetonka, MN) were used for the cross-flow filtration. Cross-flow experiments were carried out using membrane sheets mounted on the cell membrane (Osmonics Sepa CF, Minnetonka, MN) with an effective membrane area of 155 cm^2. A schematic diagram of the membrane filtration system has been shown in Fig. 1 [18].

The feed water was circulated from the reservoir to the cell membrane and both the retentate (concentrate) and permeate outlets were recycled to the reservoir. The cross-flow experiment was performed for different filtration durations (4, 11, and 24 h) to understand changes in the activity of the formed biofilm (biofilm activity) during the first day of filtration. Biofoulant activity, defined as the cellular activity of model organisms present in the feed solution, was determined by collecting samples of the feed water following inoculation with bacteria at the beginning of the experiment. In addition, in order to represent a variety of possible growth conditions and physiological states of biofoulants that could be present in a real membrane-based water treatment system, the cross-flow filtration was carried out in 24 h with biofoulant in different growth phases (i.e. lag, late exponential and stationary phases).

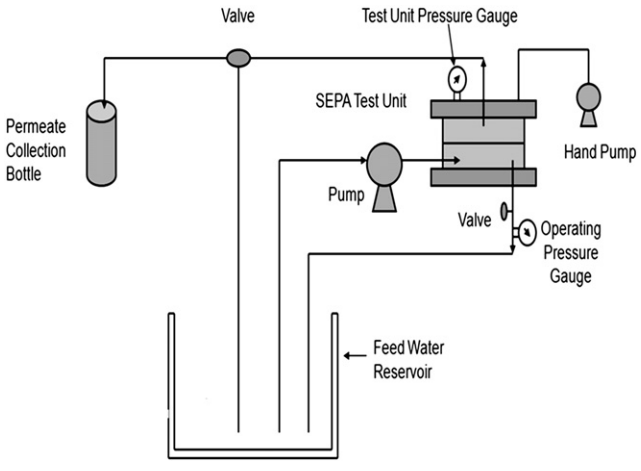

Fig. 1. Diagram of cross-flow membrane filtration system. Modified from Good et al. [18].

Cross-flow filtration was operated at a constant pressure of 172.36 kPa (25 psi) and a constant water temperature of 27 °C using a cooling fan. All membranes were pre-compacted for 12 h to reach a constant flux before starting the filtration period. The permeate flux (J) was measured during the filtration experiment and the final permeate flux was evaluated as normalized flux with respect to initial flux (J_0). The initial flux for all of the experiments was adjusted to 26 L/m^2-h. All of the cross-flow filtration experiments were carried out using synthetic feed water composed of deionized (DI) water and buffering chemicals as described in Zaky et al. [19]. During the filtration experiments using inactive bacteria, 0.2 mM sodium azide (NaN_3) was added instead of sodium chloride to prevent bacterial cell growth [10]. In this study, biofouling was investigated under consistent biological (i.e. dissolved organics, type of carbon and energy source); physical (i.e. cross-flow velocity, applied pressure, temperature, initial permeate flux); and chemical (i.e. membrane type, pH, ionic strength) conditions and the effects of cell deposition and biofilm growth on the membrane were characterized with different levels of biofoulant activity. Cell concentrations in the feed water and in the biofilm following 24 h of filtration as a function of biofoulant ATP activity were compared using spectrophotometery (OD_{600}). It should be noted that all of the experiments including the biofilm activity in different filtration durations, growth curve and ATP standard curve (data not shown) were carried out in triplicate set of tests to have consistent results.

2.2. Bacterial strain and growth conditions

A Gram-negative aerobic bacterium, *Pseudomonas fluorescens* Migula (ATCC # 12842) was used as the microorganism to produce the membrane biofouling. This rod-shaped bacterium has the optimal growth temperature of 27 °C, pH 7.0, and use glucose as its carbon source. Members of *Pseudomonas* genus are one of the most ubiquitous bacterial species in the environment and water systems and *P. fluorescens* is well known to be good biofilm producer due to its short generation time and resistance to temperature fluctuation [20,21]. The bacterial growth curve (cell counts vs. incubation time) was established at 27 °C in Luria–Bertani (LB) broth to determine the lag, exponential and stationary growth phases of the organism.

2.3. Cellular ATP determination

The intracellular adenosine triphosphate (ATP) concentration was measured using an ATP colorimetric/fluorometric assay kit (Biovision, Mountain View, CA) as the kit was previously reported [22,23]. To measure the amount of ATP, cell solutions were lysed in the assay buffer based on the cell concentration (1×10^6 cells in 100 μL of ATP assay buffer). ATP measurements were performed by extracting 50 μL of the sample and adding 50 μL of the ATP extract mixture, incubating at room temperature in the dark for 30 min and the amount of light produced was measured by the spectrophotometer in relative fluorescence units (RFU) which is proportional to the amount of ATP [24].

The ATP activity and cell number were measured as a function of incubation time. Growth phases were determined as follows: in the beginning of lag phase (t = 2 h), beginning of exponential phase (t = 9 h), mid-late of exponential phase which is the time that the cells were used in the membrane filtration experiments (t = 21 h), the transition between the exponential phase and the start of stationary phase (t = 27 h), and in the middle of the stationary phase (t = 36 h). ATP concentrations ranged from 4 amol/cell in the lag phase, up to 12 amol/cell in the late exponential growth phase, and then reducing to approximately 6 amol/cell in the stationary phase (data not shown). Dead (fixed) cells had an ATP concentration of approximately 2 amol/cell. In order to investigate the influences of bacterial cell activity on the membrane biofilm activity and membrane