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Tannic acid treatment to deter microbial biofouling in flow cell system and on RO membrane in drip flow reactor

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ABSTRACT

Membrane biofouling is a major obstacle, which considerably affects membrane performance and shortens membrane life span. In the present study, the biofouling prevention potential of the tannic acid (TA) was tested on single and mixed species culture under flow conditions in flow cell and on reverse osmosis (RO) membrane in drip flow reactor (DFR). Confocal laser scanning microscopy was used to acquire three dimensional (3D) images of biofilm samples and biofilm biovolume (μm^3) was calculated via IMARIS software. The continuous dosing of TA at 20 mg/L to a flow cell led to a 98.2% ($P < 0.05$) biofilm reduction by PAO1 and 84.6% ($P < 0.05$) by a multispecies culture isolated from an industrial RO membrane. Furthermore, the continuous addition of TA to DFR led to 96.6% ($P < 0.05$) biofilm reduction by PAO1 and 98.9% ($P < 0.05$) by multispecies species, which further indicated the anti-biofouling effect of TA on RO membrane. These results suggest that TA can be a potential agent for the control of RO biofouling.

Keywords: Biofouling; Tannic acid; Biofilm; *Pseudomonas aeruginosa*; RO multispecies

1. Introduction

Membrane filtration is important for water and wastewater treatment. Filtration process is based on membrane technology for seawater and wastewater purification into usable water. However, membrane biofouling is a major impediment, which considerably affects membrane performance and shortens membrane life span. Though, biofouling by microbial cells is a key challenge and it must be controlled for sustainable membrane bioreactors and membrane systems [1]. Microbial biofouling is a sequential development in which microbial cells adhere to the membrane surface and grow on the membrane. Due to membrane biofouling, membrane filtration decreases and it leads to financial loss and technical issues [2].

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Biofilm formation on different surfaces is a survival tactic for microbial cells [3]. It is not easy to control biofilm formation due to the fact that as long as even if trace amount of nutrients are available, bacterial cells will utilize them for their growth and as a result, they will cause membrane biofouling [2]. There is considerable number of bacterial cells present in the wastewater and seawater. Using disinfection or pretreatment methods of water and wastewater cannot keep sterility for longer time. Thus, the available bacterial cells can grow and they can cause membrane biofouling, even limited nutrients are available. Hence, membrane biofouling is a challenging problem, as it cannot be effectively mitigated by the available treatment methods [2].

Existing sterilization methods depends on the use of toxic chemicals, which are not only toxic for bacteria but also for environment. Also, most of these toxic chemicals are not effective in mitigating membrane biofouling. A promising and greener way to control membrane biofouling is the use of biological control approach [4–7]. This approach is centred on revealing the biofilm controlling mechanisms. Also, it is of immense need to identify natural products, with the ability to control membrane biofilm formation via targeting regulatory processes.

Some findings exhibit that some plant compounds and naturals extracts have the ability to control biofilm formation [8–11]. In our present study, Tannic Acid (TA) was chosen to evaluate its anti-biofilm activity against membrane biofouling. TA can be extracted from different plants including black tea, green tea, grape and cranberry etc. [12].

The focus of present study was to test the suitability of TA as an agent to control biofouling under flow conditions in flow cell and drip flow biofilm reactor on reverse osmosis (RO) membrane surface. The effect of TA was tested against single bacteria and RO multispecies community isolated from a wastewater treatment plant of Singapore. The potential of TA was also tested to disperse the pre-formed biofilm under flow conditions.

2. Materials and methods

2.1. Materials, medium and bacteria

For this study, TA (Sigma, USA) was used as a biofouling control agent. Nutrient broth (NB) (Difco, BD) medium was used, which is commonly used as a nutrient source of synthetic feed in laboratory scale RO [13]. NB medium was prepared in sterilized distilled water and medium was autoclaved at 121°C for 15 min.

For the initial part of this work, a single representative bacterium, *P. aeruginosa* PAO1 (green fluorescent protein (GFP) producing strain) was provided by Tim Tolker-Nielsen. RO mix culture was also employed in this study. This mix culture was isolated from a wastewater treatment RO plant of Singapore [14].

2.2. Flow cell setup and operation

Biofilm mitigation experiments were carried out in flow cells (Fig. 1) comprising of three separate channels (1 mm^3 × 4 mm^3 × 40 mm^3) [15]. Each channel was injected by 800 μL of PAO1-GFP or RO multispecies diluted to an OD_{600} of 0.1. After injecting bacterial culture in flow cells, the flow channels were incubated for 1 h, after which biofilms were grown in medium (0.3% of NB (24 mg/L) with 0.2% glucose) pumped through at flow rate of 9 mL/channel/hr. TA was added to the feed tank at desired concentrations. Microbial biofilm dispersal potential of TA (20 mg/L) was also evaluated via treating pre-formed (24 h) PAO1-GFP biofilms in flow cells. Biofilms were developed in the medium (0.3% of NB with 0.2% glucose) in flow cells for 24 h. Then, TA (20 mg/L) with nutrient medium was passed through the flow chambers for 24 h. Controls without TA was run concurrently.

2.3. Drip flow biofilm reactor system and operation

The drip flow biofilm reactor consists of a rectangular base held at a 10° angle by adjustable legs. Four separate channels are bored into the base resulting in four independent sampling opportunities for each run performed. Each channel has two small pegs to hold the 18.75 cm^2 (25 mm × 75 mm × 1 mm) coupon in place. The covers contain rubber o-rings to form an air tight seal, bacterial air vent gas exchange ports, and a mini-nert valve used for the inlet. The mini-nert valve consists of a rubber septum, into which a needle is placed to deliver the media.

The drip flow biofilm reactor is a flexible reactor system; it can be easily adapted to model a variety of conditions in the laboratory. Each channel in the drip flow reactor (DFR) was injected by 5 ml of bacterial culture (PAO1-GFP or RO multispecies) diluted to an OD_{600} of 0.1. After injection of bacterial

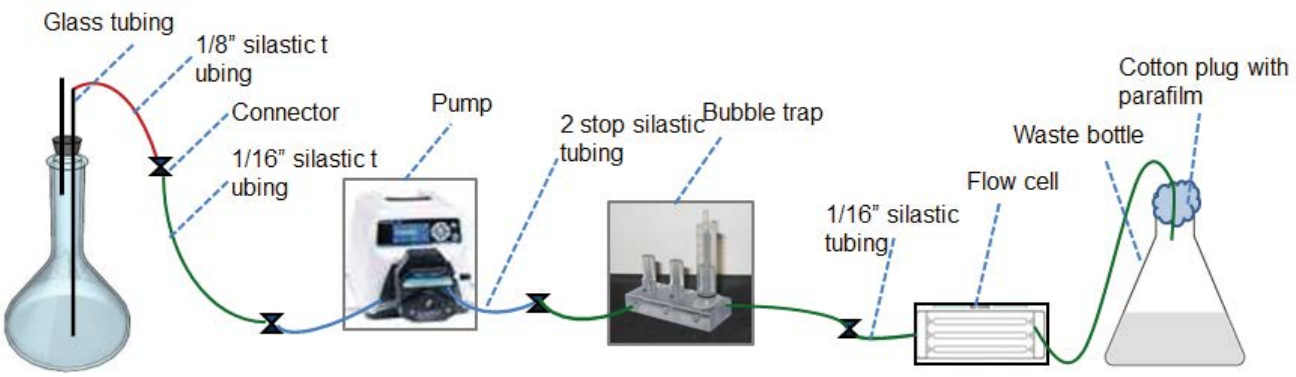

Fig. 1. Flow cell system.

cells, DFR was incubated for 3 h, after which biofilms were grown in medium (0.3% of NB (24 mg/L) with 0.2% glucose (2 g/L)) pumped through at flow rate of 0.7 ml/channel/min. TA was injected to the feed tank at desired concentrations.

2.4. Confocal laser scanning microscopy (CLSM) of biofilm samples

After each run of flow cell and drip flow biofilm reactor, the flow channels of flow cells were gently rinsed by injecting 800 μL of phosphate buffered saline (PBS), while RO membrane samples were rinsed with 1 ml of PBS. Then, RO multispecies biofilm samples were stained with SYTO-9 (Invitrogen) by adding 1 mL working solution (3 μL of the stock solution diluted in 1 mL of 0.85% NaCl) on RO coupons and 800 μL in flow cells and samples were incubated in dark for 40 min. After incubation time, excess stain was washed with PBS. Both GFP strain containing biofilm in flow cells and RO multispecies biofilm on RO membrane were viewed using confocal laser scanning microscope (CLSM) (Nikon Eclipse 90i, part of the A1R hybrid confocal spectral imaging system, with an argon laser (488 nm). Also, for every sample, three dimensional (3D) images were acquired and IMARIS software (7.31) was used to calculate the average biovolume (μm^3) for every sample.

2.5. Statistical analysis

Statistical analysis was also carried out where indicated. For this purpose, a two-tailed Student's *t*-test ($P < 0.05$) was used for evaluating the significance of results. Statistics was carried out by Microsoft Excel 2010 (Microsoft Corporation, USA). All experiments were carried in triplicate.

3. Results and discussion

3.1. Biofilm control in flow cell

TA was evaluated for its anti-biofilm potential under flow conditions in flow cells. Knowing the biofilm control efficacy over time is an important consideration when using any strategy to control biofouling in industrial settings. To test that TA has any anti-biofilm control potential over time under flow conditions, biofilm experiments were performed for different time periods (24, 48 and 72 h). A range of concentrations (20, 50 and 100 mg/L) of TA was evaluated to control the biofilm formation via PAO1-GFP. It was found that 100 mg/L of TA exhibited 99.98% reduction in biovolume of biofilm after 72 h compared to control (Fig. 2) and 50 mg/L of TA also showed >97% of biofilm reduction (97.6%, 99.6% and 99.9% of biofilm reduction after 24, 48, and 72 h, respectively) (Fig. 3). While, TA at 20 mg/L showed slightly lower biofilm reduction (75.9%, 96.5%, and 98.2% after 24, 48, and 72 h, respectively) compared to 50 and 100 mg/L of TA (Fig. 4). These results exhibited that the concentrations tested have the ability to mitigate (>70%) biofilm. On the ground of these results for TA, further tests employed 20 mg/L of TA for biofilm dispersal experiments.

The potential of TA (20 mg/L) to disperse a biofilm was also assessed by treating pre-formed (24 h old) biofilms of PAO1-GFP. It was found that at 20 mg/L of TA, biofilm

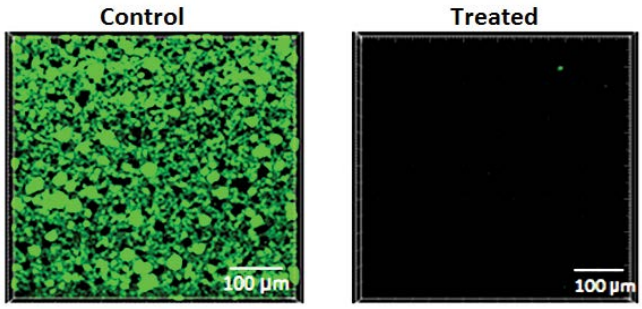

Fig. 2. Treated (TA of 100 mg/L) vs. untreated (control) PAO1-GFP biofilm after 72 h, confocal images showing bacterial biofilm, scale bars = 100 μm.

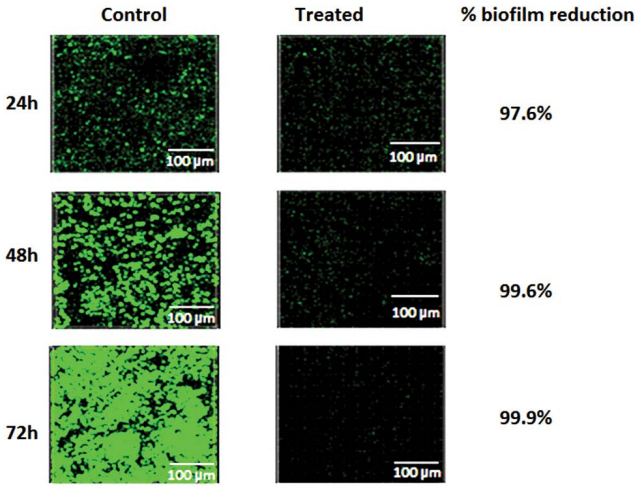

Fig. 3. Treated (TA of 50 mg/L) vs. untreated (control) PAO1-GFP biofilm, confocal images showing bacterial biofilm, scale bars = 100 μm.

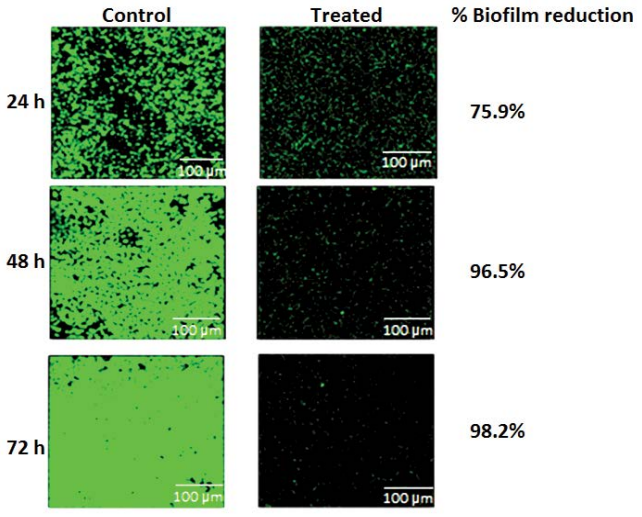

Fig. 4. Treated (TA of 20 mg/L) vs. untreated PAO1-GFP biofilm, confocal images showing bacterial biofilm, scale bars = 100 μm.

was increased (54.9%) after 24 h exposure. This result exhibited that TA cannot disperse pre-formed biofilm under flow conditions tested (Fig. 5). Based on few studies it is stated that microbial biofilms get resistance to chemicals or inhibitors under nutrient limited conditions compared to the conditions in which required concentration of nutrients are available [16]. These results demonstrated that biofilm dispersal is a different phenomenon from the biofilm prevention. Biofilms can be inactivated by the agents tested but bacterial cells in the biofilms can still stay remain attached to the surface. By inactivating metabolic activity and growth of bacteria in biofilm has never been expected to detach biofilm from the surface, as biofilm are made up of bacterial cells and EPS [17].

A range of concentrations (20 and 50 mg/L) of TA was tested to prevent the biofilm formation by RO multispecies. 50 mg/L of TA showed 96.85% of biofilm biovolume reduction after 72 h compared to control (Fig. 6a). While, TA at 20 mg/L showed slightly lower biofilm reduction (84.61%) compared to 50 mg/L of TA (Fig. 6b). Based on these results, subsequent experiments used 20 and 50 mg/L of TA for biofilm reduction in drip flow biofilm reactor on RO membrane coupons.

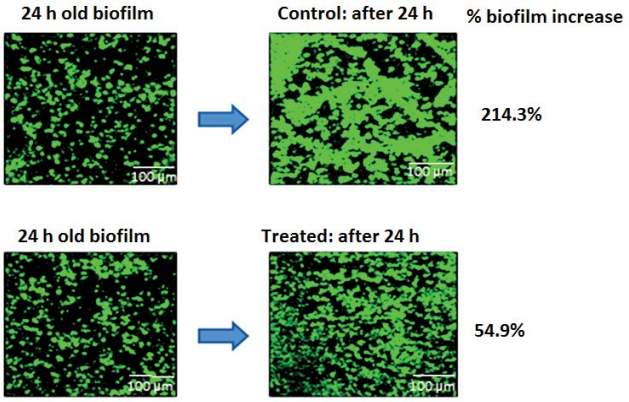

Fig. 5. Treated (TA of 50 mg/L) vs. untreated PAO1 biofilm, confocal images showing bacterial cells, scale bars = 100 µm.

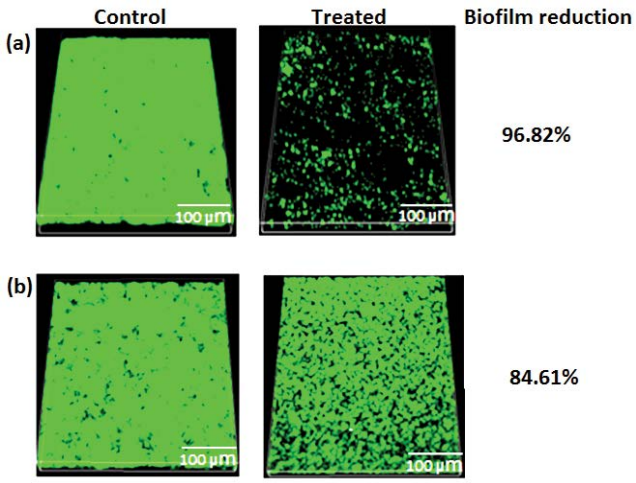

Fig. 6. Treated vs. untreated RO multispecies biofilm after 72 h of flow cell operation. (a) TA of 50 mg/L and (b) TA of 20 mg/L. Confocal images showing bacterial biofilm, scale bars = 100 µm.

3.2. Biofilm control in DFR on RO membrane

PAO1 and RO multispecies biofilm development was also investigated in DFR on RO membrane coupons to observe TA's anti-biofilm activity on the biofilm formation on the RO membrane surface. After 48 h, 50 mg/L of TA showed 97.8% of PAO1 biofilm biovolume reduction compared to control (Fig. 7 (a)), while TA at 20 mg/L showed 96.6% of PAO1 biofilm reduction (Fig. 7b). These results exhibited that at 20 mg/L of TA still can reduce biofilm formation more than 90%. Based on these results, subsequent experiments used 20 mg/L of TA for biofilm reduction in drip flow biofilm reactor on RO membrane coupons against RO multispecies. For the biofilm formation of RO multispecies, TA at 20 mg/L also showed 98.9% reduction (Fig. 8). The overall results indicated that TA can reduce biofilm formation on RO membrane in DFR and flow cells against PAO1 and RO multispecies.

The clear mechanism for the biofilm control via TA is still not clearly understood, while few reports explained that biofilm control could be due to enzyme inhibition and precipitation of proteins [18,19]. Also, it is reported that TA can alter the surface charge and it can interfere in the attachment of

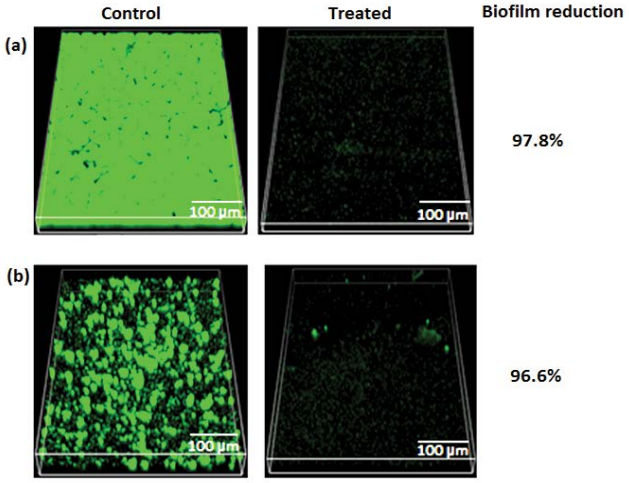

Fig. 7. Treated vs. untreated PAO1 biofilm after 48 h of drip flow reactor operation. (a) TA of 50 mg/L and (b) TA of 20 mg/L. Confocal images showing bacterial biofilm, scale bars = 100 µm.

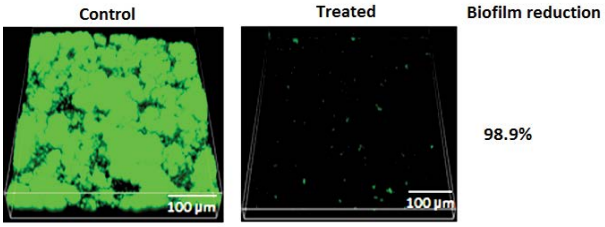

Fig. 8. Treated (TA of 20 mg/L) vs. untreated RO multispecies biofilm after 48 h of drip flow reactor operation, confocal images showing bacterial biofilm, scale bars = 100 µm.

cells on surface [20]. Furthermore, TA can control biofilm via inducing stringency [21]. Few studies exhibit that tannins can also mitigate biofilm via controlling quorum sensing [22,23]. As TA can control biofilm formation, when TA was supplied from 0 h till 72 h, it may be used in the continuous mode of RO system without flux. Also, TA exhibited biofilm control potential against multispecies isolated from wastewater, which indicates that a wide range of bacterial biofilm could be mitigated.

4. Conclusions

Here, TA was tested for its ability to control biofouling by a single species biofilm as well as multispecies biofilm under different flow conditions on RO membrane and on glass surface. CLSM (3D images) exhibited that TA reduced bacterial biofilm of PAO1 and successfully controlled RO multispecies biofilm formation. The continuous addition of TA to a flow cell led to biofilm reduction by PAO1 and by a multispecies culture isolated from an industrial RO membrane. Furthermore, the continuous addition of TA to DFR also led to biofilm reduction by PAO1 and by multispecies, which further indicating the anti-biofouling effect of TA on RO membrane against multispecies. These results suggest that TA may be a potential agent for the control of RO biofouling in RO system. However, the suitability of TA has to be further studied in RO simulators or lab scale RO systems.

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