

**“The influence of natural organic matter on biofilm growth,
chlorine efficacy, and by-product formation in water
distribution systems”**

Basic Information

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Conference papers and presentations

Xue Zheng and Youngwoo Seo, 2011, Quantitative Analysis of Biofilm Susceptibility against Mode Disinfectants, in Water Environmental Federation (WEF) Disinfection 2011 conference, Cincinnati, OH, 235-242.

Wang, Zhikang, Zheng Xue, and Youngwoo Seo; 2010, Influence of Bacterial Extracellular Polymeric Substances (EPS) on Biosorption of Natural Organic Matter in Water Distribution System, 240th American chemical society national meeting, Boston, MA, USA.

Wang, Zhikang and Youngwoo Seo; 2011, Affect of Phenotypic Variation on Biosorption of Natural Organic Matter (NOM) Under Simulated Drinking Water

Distribution System Conditions, Borchardt Conference, University of Michigan, Ann Arbor, MI, USA.

Xue, Zheng, and Youngwoo Seo; 2011, Factors Modulating Biological Stability of Water in a Model Distribution System, Association of Environmental Engineering and Science Professors Education and Research Conference, Tampa, USA (Submitted).

Xue, Zheng, Cyndee Gruden, and Youngwoo Seo; 2011, Efficacy of model disinfectant on biofilm and biofilm detachment, American Water Works Association Water Quality Technology Conference, Phoenix, Arizona, USA. (Submitted)

Wang, Zhikang and Youngwoo Seo; 2011, Biosorption of Natural Organic Matter in Water Distribution System, American Water Works Association Water Quality Technology Conference, Phoenix, Arizona, USA. (Submitted)

1. PROBLEM AND RESEARCH OBJECTIVES

In water supply, treatment, and distribution systems natural organic matter (NOM), , creates various problems as it is highly reactive and is not fully remediated by conventional water treatment procedures. Consequently, NOM creates two major problems in water supply, yield, and quality. First, NOM can be utilized by bacterial biofilm (consortium of bacteria attached with surfaces) as a carbon and nutrient source, thus leading to subsequent deterioration of water system infrastructure (biocorrosion and biofouling), as well as deterioration of water quality in water treatment, distribution and storage systems (Lechevallier, et al., 1991). Second, NOM is highly reactive with chlorine-based disinfectants and creates unwanted disinfection by products (DBPs), which can potentially cause cancer in humans with long term exposure.

Biofilm formation and bacterial regrowth are dependent upon complex interactions between drinking water characteristics, as well as engineering and operational parameters (LeChevallier, et al., 1996). The efficiency of residual disinfectants is critical for the reduction of total bacterial cell counts in bulk water and incidences of biological contamination in drinking water. However, the role of natural organic matter on biofilm disinfection and viability of detached biofilm has not been well studied. Specifically, the impact of NOM interaction with both biofilm and detached biofilm clusters still remains elusive.

The objective of this study is to examine the role of dissolved NOM on biofilm growth and susceptibility to model disinfectants. Strains from an opportunistic pathogen, *Pseudomonas aeruginosa* (both wild type and mutant strains) with different alginate EPS secretion capabilities were used to investigate (i) NOM interaction (adsorption) with biofilm, (ii) the impact of the presence of natural organic matter on the viability of both attached and detached biofilm.

2. STATEMENT OF RESULTS OR BENEFITS

In water distribution systems, biofilm survival has been reported despite the regulatory presence of residual disinfectants. Natural organic matter (NOM), which is ubiquitous in drinking water systems, contributes to biofilm growth as a carbon source as well as increasing disinfectant demand. In this study, strains from an opportunistic pathogen, *Pseudomonas aeruginosa* (both wild type and mutant strains) with different extracellular polymeric substance (EPS) secretion capabilities were used to cultivate single species biofilms. Biofilms were grown in a continuous flow system under low nutrient condition simulating the drinking water distribution system. Post chlorine disinfection, biofilm was visualized using a confocal laser scanning microscope (CLSM) followed by image analysis software to quantify biofilm EPS content and spatial distribution of viability. The survival rate of detached cells from PAO1 biofilm was analyzed by flow cytometry to differentiate live, dead and membrane compromised cells considering the presence or absence of NOM- materials known to consume residual . Both biofilm and detached cluster viability were confirmed utilizing the plate count method.

Results

The following results were obtained from this study.

- 1) All tested cultures showed lower biosorption capacities compared to the counterparts without divalent ions (Huh?). In the presence of divalent ions, biosorption of NOM is proportional to the amount of capsulated EPS on bacteria culture.
- 2) The amount of EPS produced is positively related to biofilm viability in both the presence and absence of NOM. Resistance to disinfection was significantly enhanced in EPS overproduction biofilm compared to EPS deficient strains. Due to chemical reactions between NOM and residual disinfectants in the bulk solution, the presence of NOM improved detached biofilm resistance to chlorine residuals.

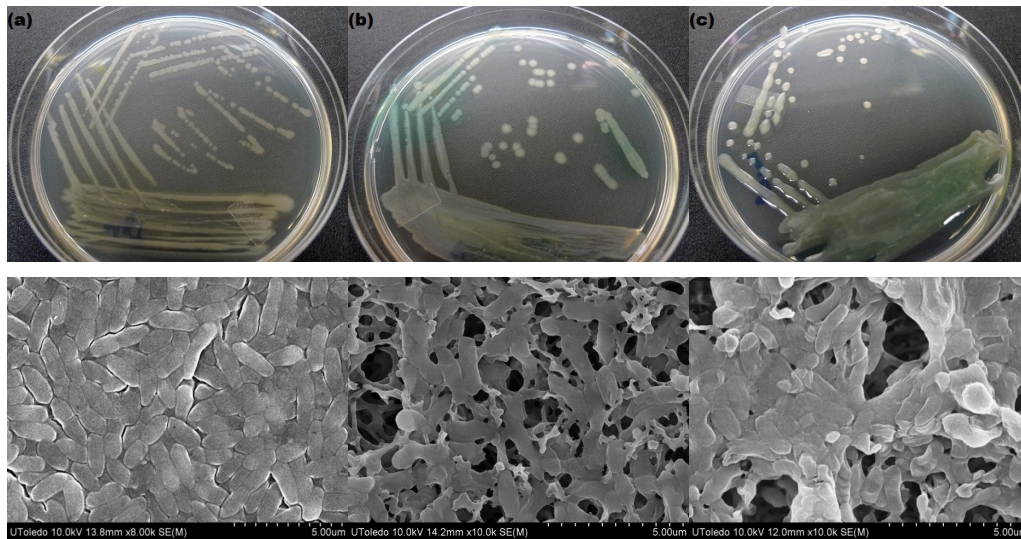
Benefit

Presence of NOM and biofilm formation greatly increases biological and chemical instability in the drinking water supply, which will decrease water yield and result in increased operational costs. Presence of NOM and biofilm also requires higher disinfectant doses to comply with Environmental Protection Agency regulations. From this study, the minimum required dose of chlorine to control both bacterial biofilm and detached biofilm clusters was elucidated. The results from the proposed study will enable local water utilities to incorporate biofilm control strategies by effectively utilizing disinfectant with consideration of biofilm / NOM prevalence.

3. MATERIALS/METHODOLOGY

Pseudomonas aeruginosa strain PAO1 and two mutant strains *algT* (inhibited alginate EPS production) and *mucA* (overproduction of alginate EPS) were used in this experiment (Figure 1). All strains were grown in one-tenth strength LB broth at 37°C with mixing and then harvested during the late-exponential phase. The bacterial suspensions were prepared by centrifugation at 2,000 × *g* for 15 min, allowing for minimal removal of cell-bound EPS. The cells were diluted in chlorine demand free (CDF) buffer as a bacterial suspension.

Figure 1 – *P. aeruginosa* strains on agar plates and under SEM. (a) *algT*; (b) PAO1; (c) *mucA*



Solution preparation

A 0.02 strength LB broth was used as a biofilm growth medium to create nutrient limited growth conditions mimicking low-carbon drinking water environment. For the biofilm cultivation experiments with NOM, filtered (0.45µm) Suwannee River NOM (SR-NOM) was added to the medium, resulting in a final NOM concentration of 2 ± 0.2 mg/L (Croue, et al., 1999). Chlorine solutions were prepared by adding Clorox bleach (The Clorox Co., Oakland, CA) to autoclaved dionized water. The free chlorine concentration was determined by the N, N-diethyl-*p*-phenylenediamine (DPD) method.

Biofilm cultivation in flow cell system

Single-species biofilms were grown in continuous-culture flow cells (channel dimensions, 1.6 by 12.7 by 47.5 mm; flow rate, 0.2 ml/min) at room temperature. The flow cell contains a standard glass microscope slide on one side and a glass cover slip on the other side. Flow rate of the flow cells simulated the laminar flow with an average flow velocity of 0.16 mm/s throughout each experiment. Under this flow condition, a residence time that enhanced biofilm formation was achieved. Two carboys were used as medium feeding and chlorine supply reservoirs respectively. All

feeds to reactors were delivered using a multichannel peristaltic pump (ISMATEC, Glattbrugg, Switzerland) and silicone tubing (Masterflex, Vernon Hills, IL). Flow cells, tubing and solutions were sterile at the initiation of each experiment. Operation and sampling of the flow cells followed aseptic technique throughout the experiments. The flow cell channels were inoculated with bacterial suspension and incubated without flow for 2 hours at room temperature for initial bacterial attachment. After 2 hours, flow rate was gradually increased to 0.2 ml/min. For each experiment, the two channels in one flow cell were operated in parallel under identical conditions, although one channel received chlorine and the other served as a non-chlorinated control. Chlorine concentration was maintained at 0.5 mg/L at the flow cell inlet throughout the disinfection process. Flow cell effluent was collected every 30 minutes for 2 hours in total and quenched with 0.1 M sodium thiosulfate before further analysis.

Figure 2 – Flow cell system set up



Confocal Laser Scanning Microscopy and Image Analysis

The biofilm on the cover slides was visualized by fluorescent staining with *BacLight* LIVE/DEAD bacterial viability staining kit (Molecular Probes Inc.) to differentiate live and dead cells. Extracellular polysaccharide in the biofilm formed by *P. aeruginosa* was visualized with Alexa 633 conjugated concanavalin A (ConA-Alexa 633). Biofilms were visualized with a Leica confocal laser scanning microscope (CLSM) equipped with a 63X oil immersed objective and a 20X objective. The CLSM images were further processed with mathematical analysis to determine total biomass, EPS content, and surface characteristics using image analysis. For each culture strain, experiments were repeated at least three times. All image analysis was based on at least 5 images of one sample.

Flow cytometry analysis

The total cell amount and viability distribution from the detached biofilm was quantified as a function of fluorescence intensity measured with flow cytometry. Samples were run at high speed and approximately 10,000 events were taken for each measurement. Data were acquired in log mode by a FACScalibur flow cytometer (BD Biosciences, San Jose, CA) and analyzed using CELLQUEST software (BD Biosciences, San Jose, CA). The flow cytometer was equipped with an argon laser set at 15 mV and turned to an excitation wavelength of 488 nm. Propidium iodide (PI) and SYTO9 (InVitrogen, Carlsbad, CA) were used in combination as membrane compromised cells and intact cells marker respectively. Cell concentration was determined by adding microsphere standard (InVitrogen, Carlsbad, CA) and adjusted to a concentration of $\sim 10^6$ cells/ml. On the basis of negative and positive controls, the analysis using flow cytometry was performed by making a comparison plot between the PI and the SYTO9 fluorescence to quantify the cellular viability.

NOM adsorption tests

Seven concentrations (5 mg/L, 7.5 mg/L, 10 mg/L, 12.5 mg/L, 15 mg/L, 17.5 mg/L, 20 mg/L) of absorbate solutions were diluted from three NOM stock solutions (SR NOM, SR humic acid standard, SR fulvic acid standard) (100 mg/L). Adsorption isotherms were conducted in triplicate for each culture variant with methods mimicking kinetic tests. Tests were conducted with and without divalent ions at a fixed pH. The isotherm equilibrium time was selected as 5 h based upon the results from kinetic experiments.

3. PRINCIPLE FINDINGS AND SIGNIFICANCE

Principle Finding

1) Our results indicate that in the presence of divalent ions, all tested cultures exhibit higher biosorption capacities compared to the counterparts without divalent ions. The possible mechanisms are: i) the existence of divalent ions could compress the electrical double layers which reduce the repulsive force between cultures and NOM. ii) Functional groups in EPS, microbial cell membrane and NOM play a significant role, possibly bridging of biomolecules by divalent ions. In water distribution systems,

2) The biofilm CLSM image analysis reveals that alginate EPS production has an effect on biofilm viability. The biofilm viability is found to be positively related to its EPS content. The EPS overproducing *muca* biofilm shows comparatively high resistance to chlorine, when compared to the wild type and EPS deficient strains. The presence of NOM did not significantly affect the viability of biofilm due to insignificant interaction between NOM and biofilm in the absence of divalent ions. However, the presence of NOM enhances the detached cell viable rate, which would lead to bacteria regrowth or reattachment in distribution system.

Significance

- 1) Sorption mechanism of NOM on biofilm was elucidated under relevant conditions in water distribution system.
- 2) From this study, the required dose of chlorine to control both bacterial biofilm and detached biofilm clusters was elucidated. The results from the proposed study will enable local water utilities to incorporate biofilm control strategies by effectively utilizing disinfectant with consideration of biofilm / NOM prevalence.

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