Chlorinated Wastewater and its Potency to form Microbial Biofilm

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Background and Significance

- Biofilm – a community of microbial cells that cling together the in forms of chains attached to a surface (Sengupta, B. *et al.*, 2016; Chattopadhyay, I. *et al.* 2022; Scialla, S. et al. 2021) through excreting DNA, protein, and polysaccharides (EPS).
- The present study is designed to study chlorinated wastewater and its efficacy to form microbial biofilm from Nacogdoches (NWWTP) and San Augustine (SAWWTP) wastewater treatment plants (WWTP).
- To compare these efficacies to sterilized water (ST) and tap water(TW) which were collected by autoclaving distilled water and directly from a running tap respectively.
- Bacterial sample(*Bacillus thuringiensis* (Bt)), a commonly used gram positive, biofilm forming bacteria was used to grow biofilms in sterilized water, tap water, and chlorine treated wastewater from the WWTPs,.
- Increase in optical density (OD) at 600 nm indicated growth in all the cultures, while other techniques such as light microscopy, scanning electron microscopy (SEM) and ion exchange chromatography (IC) were used to study the extent of biofilm growth in the chlorine treated wastewater compared to sterilized water and tap water.

Hypothesis

- Chlorinating wastewater will negatively affect its ability to form microbial biofilm

Objectives

- To confirm the effectiveness of biofilm formation by *Bacillus thuringiensis* (Bt) in Nacogdoches chlorine (Cl_2) treated water(NC) and San Augustine chlorine (Cl_2) treated water (SAC).
- Compare the effect of chlorine (Cl_2) treated wastewater with sterilized and tap water.

Methods and Materials

Bacillus thuringiensis (Bt) ATCC 33679, obtained from the American Type Culture Collection) was grown on liquid Luria Broth (LB, obtained from RPI) culture in an incubator at 37ºC with shaking at a speed of 109 rpm overnight. The optical density (OD) was measured at 600 nm to determine the growth of the bacteria. A final OD_{600} of 0.03 was chosen to initiate biofilm formation. Bt was grown in five different environments: i. 100% LB media; ii. LB with 50% sterilized water (v/v); iii. LB with 50% tap water (v/v); iv. LB with 50% NWWTP Cl_2 water (v/v); v. LB with 50% SAWWTP Cl_2 water (v/v) in mica as well as sterile 24-multiwell culture plates (obtained from Costar) with volume 500 µl/well at 37°C for 24 hours in a static condition. After incubation period, OD_{600} of the cell samples was measured by the Berthold TriStar^2 Plate Reader and the supernatants was carefully removed for ion exchange analysis. Brightfield image of the plates were taken, and SEM images of the mica was taken for each water samples.

Imaging Studies

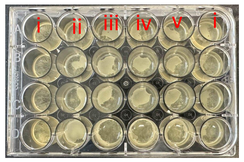

Figure 1. Bt grown in 24-wells plate for 24 hours.

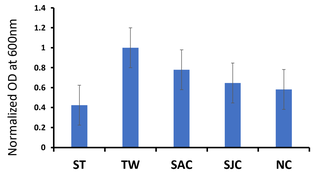

Figure 2. Optical density of supernatant of Bt solutions from plate.

Figure 3. Sputter coated mica for SEM imaging.

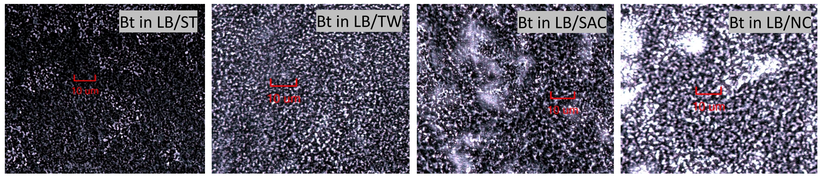

Figure 4 : Brightfield Images of Biofilms after 24 hrs at 40x Magnification

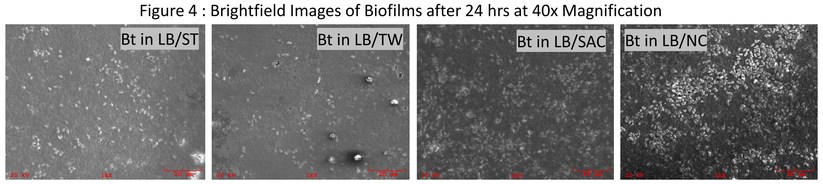

Figure 5: Scanning Electron Microscope (SEM) images of Biofilms after 24 hours at 1000x magnification

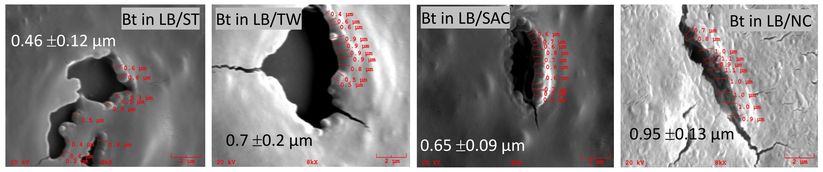

Figure 6: Thickness of the Biofilms measured at 8000x magnification at 45^0 tilted angle.

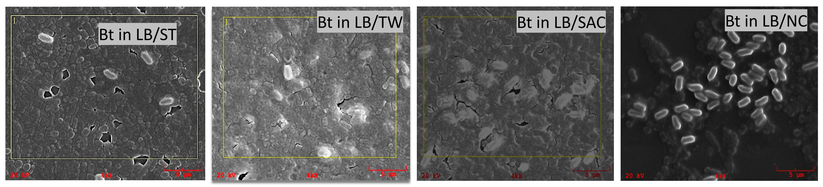

Figure 7: Scanning Electron Microscope (SEM) images of Biofilms after 24 hours at 4000x magnification

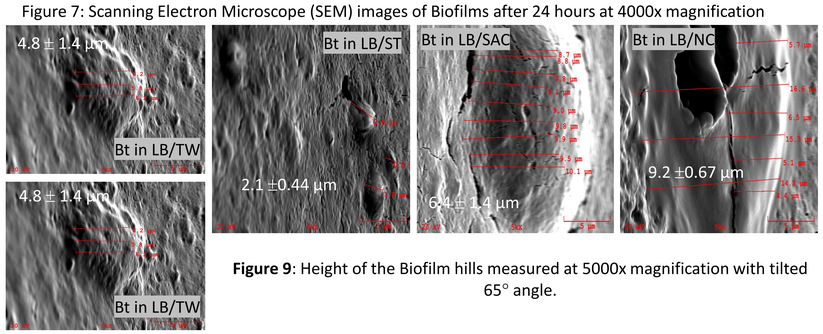

Figure 9: Height of the Biofilm hills measured at 5000x magnification with tilted 65° angle.

Ion Chromatographic Studies

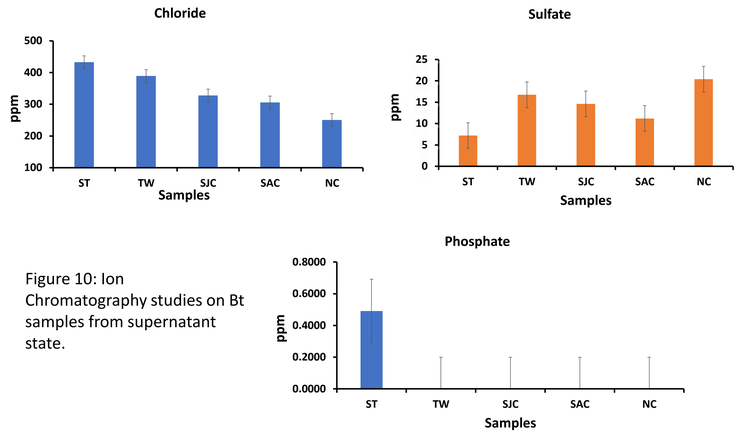

Figure 10: Ion Chromatography studies on Bt samples from supernatant state.

Table 1: Ambient levels of anions (ppm) in water samples measured using Ion Chromatography

Anions	ST only (ppm)	TW only (ppm)	NC only (ppm)
Fluoride	0.0044	0.2085	0.1763
Chloride	0.0076	5.1150	23.1313
Nitrite	0.0000	0.0644	0.4317
Bromide	0.0001	0.0047	0.0125
Nitrate	0.0013	0.6458	5.7370
Sulfate	0.0258	15.0853	21.1365
Phosphate	0.0296	0.0442	2.5591

Observations and Conclusions

- OD of the supernatant is a good indicator of the amount of biofilm formation.
- Lighter spots in images from the Brightfield Microscope indicates the vertical growth of the biofilm.
- SEM images showed the height, thickness, and surface structure of the biofilm.
- Bacteria absorb phosphate to increase the mechanical stability of biofilm.
- No significant change in sulfate level after biofilm formation. Sulfate does not influence significant biofilm formation.
- Chloride excretions is inversely proportional to biofilm formation.

Future Studies

- Biofilm formation with other microbes : *Pseudomonas aeruginosa*, *Candida albicans*.
- Effectiveness of DNA-aptamer templated silver nanoclusters against biofilm formation and growth.
- Effectiveness of phytochemicals against biofilm formation and growth.

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