Using Microbial Biofilm To Assess the Water Quality of Treated Wastewater from Deep East Texas Wastewater Treatment Plants

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Introduction

Water is a vital resource for life, playing a crucial role in daily activities, from domestic use to industrial processes. As the global population grows, water consumption increases, leading to more wastewater disposal that must be treated to ensure biosafety. Effective wastewater treatment is essential for maintaining clean water resources, promoting environmental sustainability, and safeguarding public health (Tortajada C. 2013). The treatment processes in wastewater treatment plants (WWTP) typically involve three stages: primary (mechanical), secondary (biological), and tertiary (chemical) methods. These stages work together to remove pollutants, contaminants, and pathogens, ensuring that treated water meets established quality standards. In the chemical treatment phase, chlorine (Cl_2) is commonly used for disinfection, effectively killing harmful pathogens (Amit Sonune & Rupali Ghate 2004). Sulfur dioxide (SO_2) is then introduced to dechlorinate the water, neutralizing residual chlorine and reducing its toxicity (A. Sathasivan et al. 2017). This dual approach ensures that treated wastewater is safer for discharge into the environment.

However, treated wastewater is often not 100% pure, creating an environment conducive to biofilm growth. Biofilms are aggregates of microbial cells that stick to surfaces and are enclosed in a self-producing extracellular matrix composed of proteins, polysaccharides, and nucleic acids (Chattopadhyay et al. 2022). These structures can vary in population density and are known for their resistance to antimicrobial agents, leading to chronic infections and contamination in various industries (Costerton et al.(1995)) Factors influencing biofilm formation include nutrient availability, hydrodynamic conditions, and static environments. The formation of biofilms occurs through a multi-stage process, complicating its management in different systems (Uneputty et al. (2022)).

To address the challenges posed by biofilms, innovative strategies are being explored that utilize the antimicrobial properties of nanoclusters and phytochemicals. Nanoclusters, small aggregates of nanoparticles, have shown promise in disrupting biofilm formation and reducing microbial density (Sengupta et al. (2016)). An example is the use of DNA aptamer-templated silver nanoclusters (Apt-DNA Ag-NC), formed through a chemical reaction involving the reduction of silver ions, known for their potent antimicrobial properties (Benn et.al (2010). DNA aptamers provide high specificity and binding affinity to target proteins and microorganisms within biofilms, enhancing treatment effectiveness while minimizing impacts on non-target organisms (Radwan et al. (2019). Phytochemicals derived from various plants also possess natural antimicrobial properties that can effectively combat biofilm-forming microorganisms

(Nascimento GGF et al. (2000)). Utilizing these natural compounds offers a potential alternative to traditional chemical treatments, which may contribute to the emergence of resistant microbial populations (Singh et.al (2020)).

This research investigates the effectiveness of wastewater treatment processes using biofilm formation as a key indicator. By analyzing how chemically treated wastewater can facilitate biofilm growth, which challenges the effectiveness of treatment systems, we can gain insights into the efficiency of various methods. These insights will help identify areas for improvement in wastewater management practices, enhancing treated water purity to protect public health and environmental safety.

Methods

Treated wastewater samples were collected from treatment plants in Deep East Texas, specifically from San Augustine (SAWWTP) and Nacogdoches (NWWTP) counties, focusing on those treated with sulfur dioxide (SO_2) and chlorine (Cl_2). Additionally, sterilized (ST) distilled water was prepared through autoclaving distilled water, and tap water (TW) was obtained after running the tap for 10 minutes. Bacillus thuringiensis (Bt) ATCC 33679 served as the model organism, grown overnight in liquid Luria Broth (LB) at 37ºC with shaking at 109 rpm. The optical density (OD) was measured at 600 nm using a Genesys 10S UV-Vis spectrophotometer to determine bacterial growth, with a final OD_{600} of 0.03 chosen to initiate biofilm formation. Bt was grown in 100% LB and in LB mixed with water samples at a 50%:50% v/v ratio in Eppendorf tubes, LB agar, mica, and multi-well culture plates at 37ºC for 24 hours in static conditions. After incubation, OD_{600} was measured, and the supernatants were removed and filtered for ion exchange analysis. Brightfield imaging and scanning electron microscopy were performed on the plates/mica to study the morphological structure of the biofilm.

Results and Discussion

The results obtained from this study are illustrated in Figures 1 and 2. These findings indicate the presence of Bt biofilms across various environments, including treated wastewater from the NWWTP and SAWWTP. Data collection utilizes a range of techniques, including spectroscopic studies to measure optical density, light microscopy to obtain bright field images, scanning electron microscopy (SEM), and ion chromatography analyses. Collectively, these methods provided a thorough evaluation of biofilm formation, acting as a probe to assess the purity of treated wastewater. This research offers valuable insights into the water quality of treated wastewater in Deep East Texas.

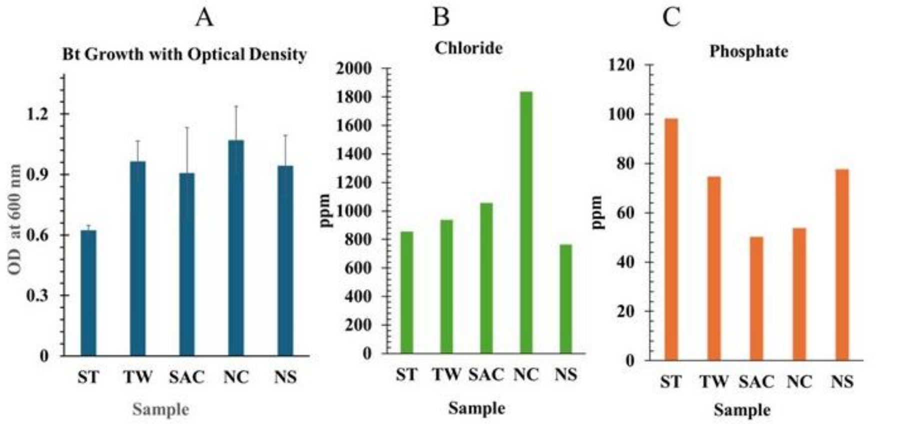

Figure 1 : Spectroscopic Studies and Ion Chromatography Studies on Microbial Biofilm Assay grown for 24 h. ST = Sterilized distilled water, TW = Tap water, SAC = San-Augustine chlorine treated water, NC = Nacogdoches chlorine treated water, NS = Nacogdoches sulfurdioxide treated water. (A) Mean optical density of supernatant of Bt solutions after 24 hours incubation before Ion Chromatography (from 3 independent measurement). (B) Chloride concentration of Bt supernatant obtained from Ion Chromatography studies. (C) Phosphate concentration of Bt supernatant obtained from Ion Chromatography studies. Chromatgographic studies on Bt samples from supernatants solution of the biofilm. IC Standards: combined seven anion standard (Thermoscientific Dionex) diluted 50x, 40x, 20x and 10x for calibration standard curve.

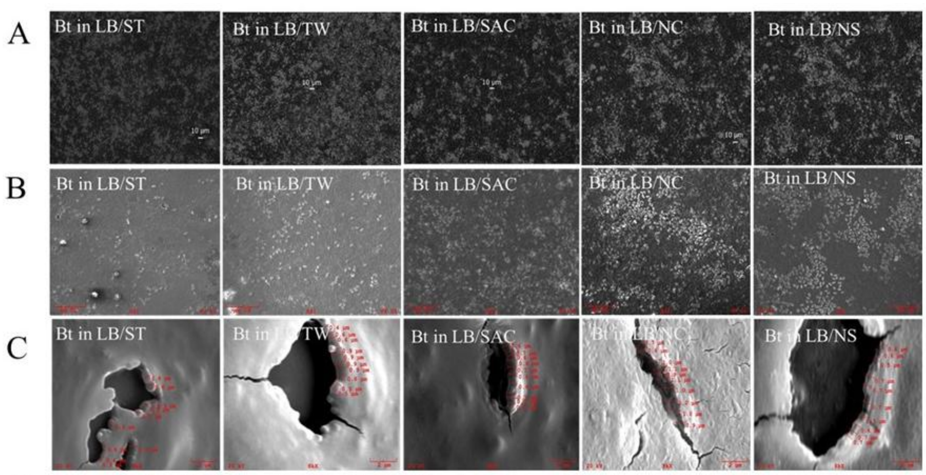

Figure 2 : Imaging Studies on Bt Microbial Biofilm Assay grown for 24 h. Brightfield Images were obtained using a light microscope. (A) Brightfield Images of Biofilms at 400x Magnification to compare the growth of biofilm in Cl_2 and SO_2 treated wastewater. (B) Scanning electron microscope (SEM) images of Biofilms at 4000x magnification(C) Scanning electron microscope (SEM) images of Biofilms showing the thickness of the biofilms measured at 8000x magnification at 45^0 tilted angle. The thickness of biofilm in Bt in LB/ST = 0.46 ±0.12 μm, Bt in LB/TW = 0.7 ±0.2 μm, Bt in LB/SAC = 0.65 ±0.09 μm, Bt in LB/NC = 0.95 ±0.13 μm and Bt in LB/NS = 0.63 ± 0.08μm.

Our studies found that treated wastewater can promote the growth of biofilm-forming microbes, with Bt serving as a model organism. The optical density (OD) of the supernatant (see figure 1A) was a reliable indicator of bacterial growth, directly correlating with biofilm formation. Ion chromatography studies (see figures 1B and 1C) on the supernatants highlight the role of bacteria in releasing phosphate to enhance biofilm mechanical stability (Flemming et al., 2016), with chloride excretions showing a direct significance with biofilm formation. Imaging studies, as shown in Figure 2, revealed that brightfield microscopy highlighted lighter spots, indicating the development of microbial communities within the biofilm. SEM further illustrated the vertical growth and complex surface structure of the biofilm, providing a deeper understanding of its architecture. Additionally, ongoing strategies aim to mitigate biofilm formation using phytochemicals in basil and mint extracts are underway to further improve the efficiency of wastewater treatment processes and mitigate the adverse effects of biofilms, ultimately contributing to sustainable water management practices in Deep East Texas.

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References

1. Tortajada C. (2013). Water management in Singapore. In Water Management in Megacities Routledge (pp. 45-58).
2. Amit Sonune, Rupali Ghate, (2004). Developments in Wastewater Treatment Methods, Desalination, Volume 167, Pages 55-63.
3. A. Sathasivan, B.S. Herath, S.T.M.L.D. Senevirathna, G. Kastl, (2017). Dechlorination in Wastewater Treatment Processes, Editor(s): Duu-Jong Lee, Veeriah Jegatheesan, Hao Huu Ngo, Patrick C. Hallenbeck, Ashok Pandey, Current Developments in Biotechnology and Bioengineering, Elsevier, Pages 359-380.
4. Chattopadhyay, I., J, R. B., Usman, T. M. M., & Varjani, S. (2022). Exploring the Role of Microbial Biofilm for Industrial Effluents Treatment. Bioengineered, https://doi.org/110.1080/21655979.2022.2044250 (Accessed March 13, 2022)
5. Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R., & Lappin-Scott, H. M. (1995). Microbial biofilms. Annual review of microbiology, 49, 711–745. https://doi.org/10.1146/annurev.mi.49.100195.003431.
6. Uneputty, Aron & Dávila-Lezama, A & Garibo, Diana & Oknianska, A & Bogdanchikova, N. & Hernández-Sánchez, José Federico & Susarrey-Arce, Arturo. (2022). Strategies applied to modify structured and smooth surfaces: A Step Closer to Reduce Bacterial Adhesion and biofilm formation. Colloids and Interface Science Communications, Volume 46. https://doi.org/10.1016/j.colcom.2021.100560 (Accessed January 2022)
7. Sengupta, Bidisha & Sinha, Sudarson & Garner, Bianca & Arany, Istvan & Corley, Christa & Jr, Keith & Brown, Elrica & Ray, Paresh. (2016). Influence of Aptamer-Enclosed Silver Nanocluster on the Prevention of Biofilm by *Bacillus thuringiensis*. Nanoscience and Nanotechnology Letters, 8(12), 1054-1060. https://doi.org/10.1166/nnl.2016.2243 (Accessed December 1, 2016)
8. Benn, T., Cavanagh, B., Hristovski, K., Posner, J. D., & Westerhoff, P. (2010). The Release of Nanosilver from Consumer Products Used in the Home. J. Environmental quality, 39(6), 1875–1882.
9. Radwan, I. M., Gitipour, A., Potter, P. M., Dionysiou, D. D., & Al-Abed, S. R. (2019). Dissolution of Silver Nanoparticles in Colloidal Consumer Products: Effects of Particle Size and Capping Agent. J. Nanoparticle research: an interdisciplinary forum for nanoscale science and technology, 21(7), 1–155.
10. Nascimento GGF, Locatelli J, Freitas PC, Silva GL, (2000). Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic-resistant bacteria. Brazilian J. Microbiol vol. 31: issue 4; pp: 247-256.
11. Singh, N., Jaiswal, J., Tiwari, P., & Sharma, B. (2020). Phytochemicals from Juice as Potential Antibacterial Agents. The Open Bioactive Compounds Journal, 8(1):1-6.

12. Flemming, H.-C.; Wingender, J.; Szewzyk, U.; Steinberg, P.; Rice, S. A.; Kjelleberg, S. (2016). Biofilms: An Emergent Form of Bacterial Life. Nature reviews. Microbiology, 14 (9), 563–575.

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