Battling Bacterial Biofilms: AHP as an effective replacement for Chlorine-Based Disinfectants

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Abstract

Microbial biofilms have been a growing concern in the healthcare and surface care industry, as they are more difficult to kill by common disinfectants. A complex network of extracellular polymeric substances in the biofilm composition that protects the residing bacteria when exposed to antimicrobial agents. There are a selection of biofilm growth methods for in-lab testing of antimicrobials against biofilms, where the CDC and MBEC methods are the two most commonly used methods. The current study has looked into determining if merging of the two prominent techniques can provide a more desirable protocol for biofilm growth and testing. Additionally, two antimicrobial agents have been used to assess the strength of the grown biofilms; a standard sodium hypochlorite (SHC) solution as a known biofilm killing agent, and diluted Accelerated Hydrogen Peroxide® (AHP®) concentrate formula as a novel agent against biofilms. Results indicate that the combination method is capable of providing sufficiently grown biofilms while also maintaining the benefits of both methods. Both antimicrobial agents tested were also successful at inactivating biofilms grown using the combined method.

Intro & Methods

ASTM E2799-12 (MBEC Assay method) and ASTM E2562-12 (CDC Biofilm Reactor method) are the two prominently used methods to grow biofilms of bacteria, more specifically of *P. aeruginosa* (ATCC 27853) in lab settings for testing against possible biofilm killing antimicrobial agents. The CDC method employs a 1L reactor vessel where a series of metal disks are attached to vertical rods. The vessel continuously flows with fresh bacterial growth medium while the rods spin in the vessel to create a high sheet environment for biofilm growth. The MBEC method on the other hand employs a plastic lid with 96 pegs and a corresponding base of 96 well plate. Each well is filled with nutrient growth media in order for the biofilm to grow in the pegs while the plate is on an agitator.

The CDC method produces biofilm that is grown under high sheer conditions whereas the MBEC method produces biofilm that is grown under low sheer conditions. On the other hand, the MBEC method allows for the simultaneous and high throughput testing of multiple biocides at multiple concentrations with replicates on a single plate, making it an efficient screening tool. Figures 1 through 4 below show how we proceeded with merging the two methods by implanting the rows of pegs from MBEC method into the CDC reaction vessel:

Figure 1. The MBEC Growth Plate

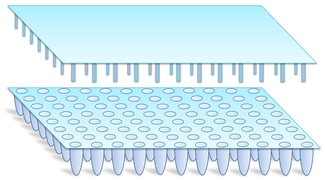
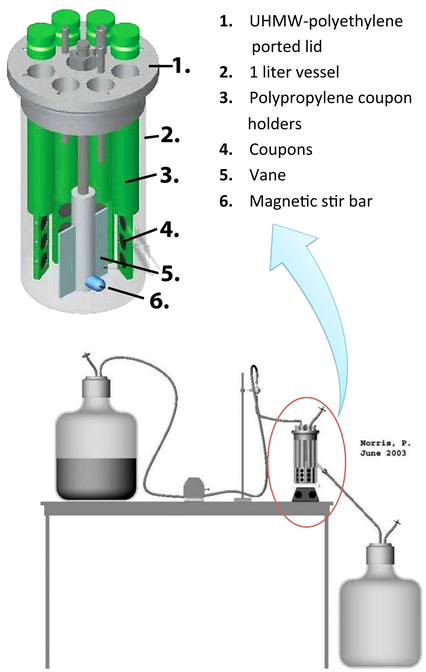


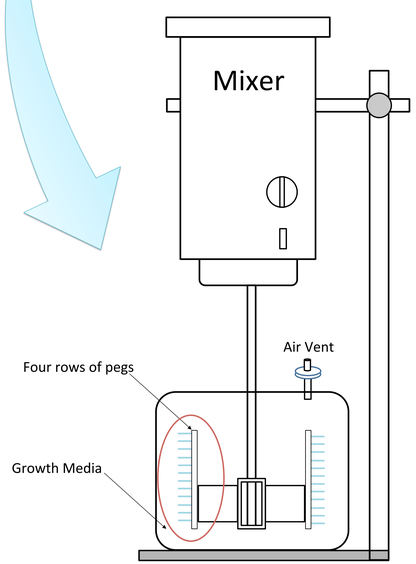
Figure 2. The CDC Reaction Vessel



1. UHMW-polyethylene ported lid
2. 1 liter vessel
3. Polypropylene coupon holders
4. Coupons
5. Vane
6. Magnetic stir bar

Figure 3. One Row of Pegs

Figure 4. Modified Growth System



Results

Table 1: Recovered Bacteria post growth

Recovered CFU/ mL (log10):	Replica 1	Replica 2	Replica 3	Replica 4	Replica 5	Replica 6	Mean
MBEC	5.67	5.67	5.29	5.92	6.51	6.51	**5.93**
Merged	5.20	5.15	5.45	5.41	5.00	5.25	**5.24**

Chart 1: Bacterial Log Reduction Using MBEC Method at 45 minutes

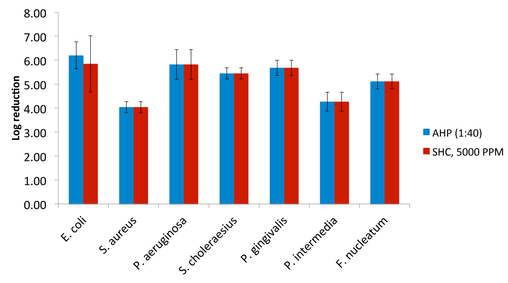
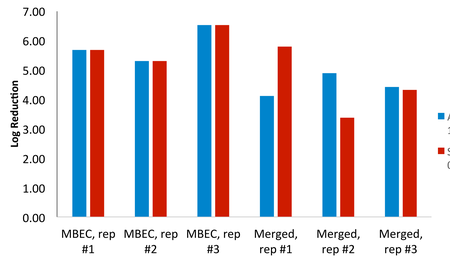


Chart 2: Bacterial Log Reduction Using the Merged Method at 45 minutes



Discussions & Conclusion

It is known that the CDC biofilm growth method provides a high sheer environment for the biofilm to grow. It is known that biofilm grown under high sheer methods is denser and therefore would provide higher resistance against penetration of antimicrobial agents. This experimentation was able to demonstrate that the CDC and MBEC methods can be merged in order produce biofilm under high sheer conditions while also keeping the option of simultaneous testing of multiple biocidal agents (up to 96) in a single plate. Bacterial recovery results show acceptable levels of biofilm formation using the MBEC and Merged method. Using the MBEC method, both AHP® and hypochlorite showed to be equally effective against bacterial biofilms. The level of efficacy against biofilm of *P. aeruginosa* grown using the Merged method is demonstrated to be very similar (mean log reduction for AHP: 4.47±0.22; SHC: 4.49±0.70). Microbial reduction from both methods also confirm that it is generally harder to kill biofilm grown under high sheer conditions.

Disclaimer: Authors of this poster are/*were employees of Virox Technologies Inc.
Third party lab used for conducting the experiment is Innovotech®.

References:

ASTM E2562 − 12 Standard Test Method
ASTM E2799 − 12 Standard Test Method