Fiber Treatment Effects on Bioreactor Bulk Fluid Trends

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Abstract

In order to facilitate the exploration of worlds beyond the borders of our planet, it is necessary to maintain sustainable levels of clean water. The remediation of water via Membrane Aerated Bioreactors (MABRs) is one such method, and the focus of this study. MABRs rely on healthy biofilms grown on hollow fiber membranes to clean non-potable water. These biofilms can take weeks to months to establish. Therefore, various fiber treatments and two inoculums were evaluated for their effect on rapid biofilm formation. Fiber treatments are as follows: sanding of the fibers with 1500 and 8000 grit sandpaper, immersion of the fibers in a 1% hydrofluoric acid solution for 12 seconds and 15 minutes, and the immersion of the fibers in a Fluoroetch® solution for 18 seconds and 5 minutes. The two inoculums utilized were sourced from healthy, established MABRs; Texas Tech University (TTU) MABR "TRL5" and Kennedy Space Center (KSC) MABR "R3". Data attained from direct bacterial cell counts of the reactor bulk fluids via fluorescent microscopy, suggests that the fluoroetching treatment combined with the TTU inoculum show the greatest biofilm creation.

I. Introduction

The need for clean water terrestrially continues to be a concern and a challenge. Part of those challenges center on the required instrumentation and chemicals used to produce clean potable water. As we prepare to explore beyond our planet, these concerns intensify. Payload weight concerns would most likely limit the amounts of instrumentation and clean water that can realistically and initially be transported aboard the space craft. The Delta 4 Heavy rocket manufactured by Space X for instance, can raise approximately 23 metric tons into orbit at a cost of 19 million dollars per ton, or about 8,600 dollars per pound (John K. Strickland, 2012). Given these enormous dollar amounts, cargo weights must be strictly monitored, and can become inhibitory. Environmental and human health concerns dictate the amounts of hazardous and or caustic chemicals which scientist are willing to transport. Therefore, much research has been done to determine methods that bypass the use of these materials and keep payload weights low.

Space travel requires the employment of a compact water processing system which has a very short start-up time, and is able to survive launch. The remedy to these concerns is to be found in membrane aerated bioreactors (MABRs). Basically MABRs consist of gas permeable membranes, or fibers, which allow oxygen to diffuse throughout the bulk fluid inside the reactor, providing aerobic conditions an attachment sites for biofilm growth. This facilitates oxidation by the biofilm which is attached to the outside of the fiber. Two of the major challenges presented by space travel are the attachment of the biofilm, which is simply a community of microbial organisms used in the oxidation and breakdown of pollutants, and the amount of time it takes to actually get the reactor itself operating at a steady state, which is the actual establishment of a healthy microbial community of organisms (E. Casey, 1999). In essence, a quick efficient start up is required. To this end, various fiber treatments, meaning the manipulation of the fiber or

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membrane through either chemical or physical techniques, were explored to see which generated the most robust and expedient biofilm communities. The various treatments are intended to increase surface area along with make the fiber surface more conducive for bacterial attachment—so more room for heartier communities. Another dimension to the equation is what to feed the communities to expedite attachment and then growth. The feed or inoculum was obtained from various sites, of which two in particular performed exceptionally well. The inoculums sourced from the Texas Tech University (TTU) bioreactor, and from the Kennedy Space Center (KSC) bioreactor, produced the most well-conditioned or fit biofilm communities.

Sanding and the fluoroetch treatments facilitated the greatest biofilm attachment. This was evidenced by relatively low bacterial counts in the bulk fluids, and high counts on fluid samples taken actually from the fibers themselves. The actual characterization of the bacterial communities has yet to be elucidated. This however is trivial when examining the efficacy of the various fiber treatments.

II. Materials and Methods

Direct bacterial counts were performed using a live /dead backlight bacterial viability kit which employs molecular probes, acridine orange which is nucleic acid selective fluorescent stain was also used (LaShelle McCoy, 2009). The actual counts were conducted on a Zeiss Axioskop2 plus® microscope which was coupled with an X-cite® series 120 fluorescence excitation light source. Weekly samples were harvested unpreserved and 1mL aliquots for each of the 8-16 reactors were delivered to the laboratory. Upon receipt, staining occurred within a 4 hour window. The Staining procedure for the bacterial viability is as follows:

- 990µL of filtered de-ionized water is added to 10µL of well mixed sample
- The solution is vortexed for approximately 3-5 seconds
- 1.5µL of Syto9 is added to each of the diluted samples
- 1.5µL of Propidium Iodide is added to each of the diluted samples
- Each sample is then well mixed (vortexed) and allowed to stand at room temperature for 15mins.
- (note) Reagents are light labile so exposure to light should be limited.
- The stained aliquots are then filtered and mounted on microscope slides for observation.

The acridine orange staining procedure is as follows:

- 990µL of filtered de-ionized water is added to 10µL of well mixed sample
- The solution is vortexed for approximately 3-5 seconds
- 100µL of 0.1% acridine orange solution is added to each sample and then vortexed
- Samples are allowed to stand at room temperature for 15mins.
- Stained aliquots are then filtered and mounted on microscope slides for observation.

Counts are then conducted using fluorescent microscopy and a hand operated tallying tool where each individual colony is enumerated (see figure 2). Cell viability slides also known as the live/dead slides require that a distinction be made between cells which are alive, stained green and cells which are dead, stained red (see figure 3).

III. Results

Looking at the data presented in figure 4 below, it becomes apparent that the Texas Tech University inoculum provided the largest yields and most fit biofilm communities. Direct bacterial counts revealed that, of the tested treatments, sanding evidenced in figure 5 and fluoroetching depicted in figure 7, produced the highest biofilm fiber yields—attachment.

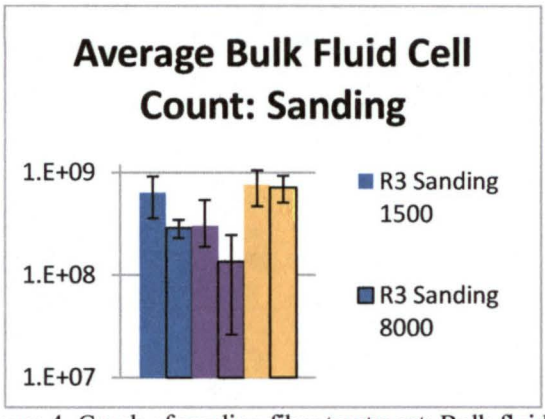

Figure 4. Graph of sanding fiber treatment: Bulk fluids

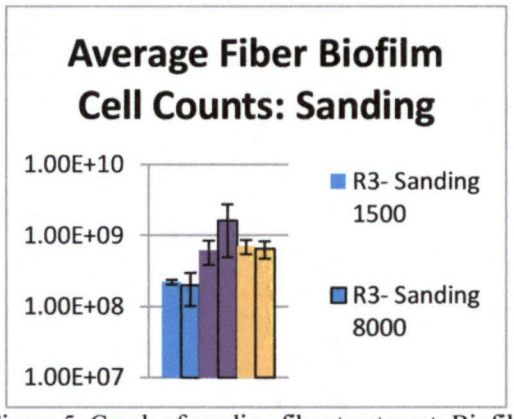

Figure 5. Graph of sanding fiber treatment: Biofilm

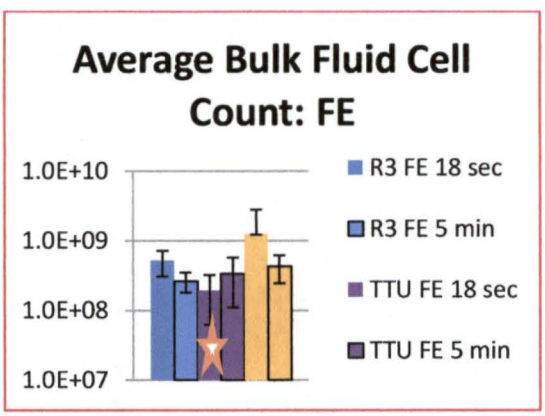

Figure 6. Graph of Fluoroetch treatment: Bulk fluids

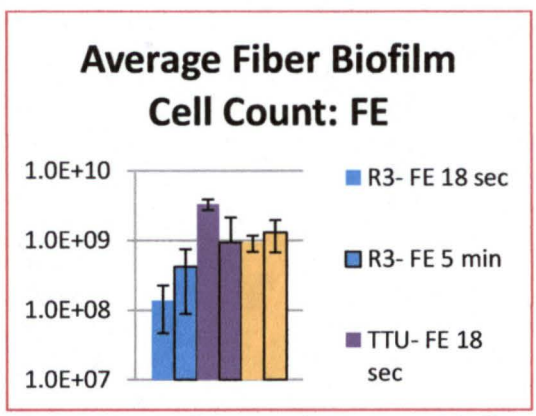

Figure 7. Graph of Fluoroetch treatment: Biofilm

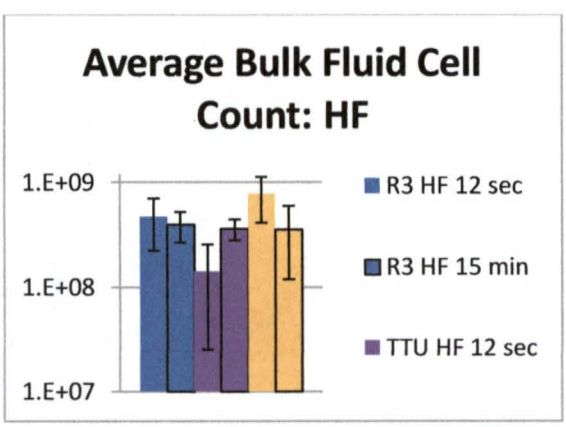

Figure 8. Graph of HF[4] treatment: Bulk fluids

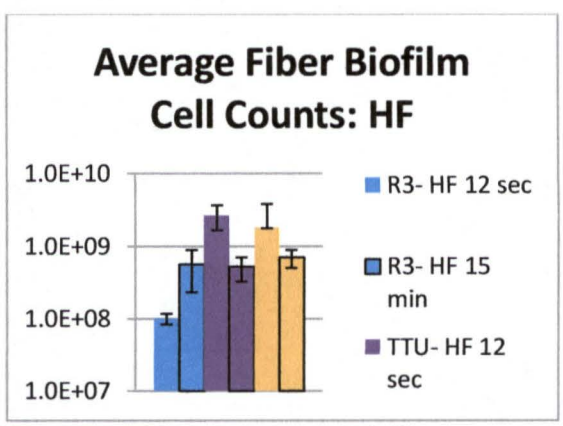

Figure 9. Graph of HF treatment: Biofilm

[4] HF=Hydrofluoric acid

IV. Conclusion

According to the data collected, it would appear that using the Texas Tech University's inoculum and either sanding or more preferable, fluoroetching (though pricing may be prohibitive) fiber treatments result in the best microbial attachment. The data suggests that when the bulk fluid bacterial counts are high as seen in the hydrofluoric acid (HF) treatment, the corresponding fiber bacterial counts are low. That is to say, if the biofilm is weakly attached due to the type of membrane treatment, the biofilm will ablate into the bulk fluid (see figures 8 & 9). Conversely, if the membrane treatment is efficacious, the biofilm attaches readily and the overall cell counts in the bulk fluid are low, and the corresponding fiber counts are high.

V. Acknowledgements

I would like to take this opportunity to thank my mentors LaShelle McCoy and Dr. Janelle Coutts for all of their understanding and patience with me. I would also like to thank the inspiring women who work in the education office, Ms. Rose Austin, Mrs. Benita DeSuza. Thanks to the faculty of Atlanta Metropolitan State College for allowing me to participate in this amazing opportunity. Finally, thank you to my fellow interns; I have truly enjoyed this outstanding summer experience.

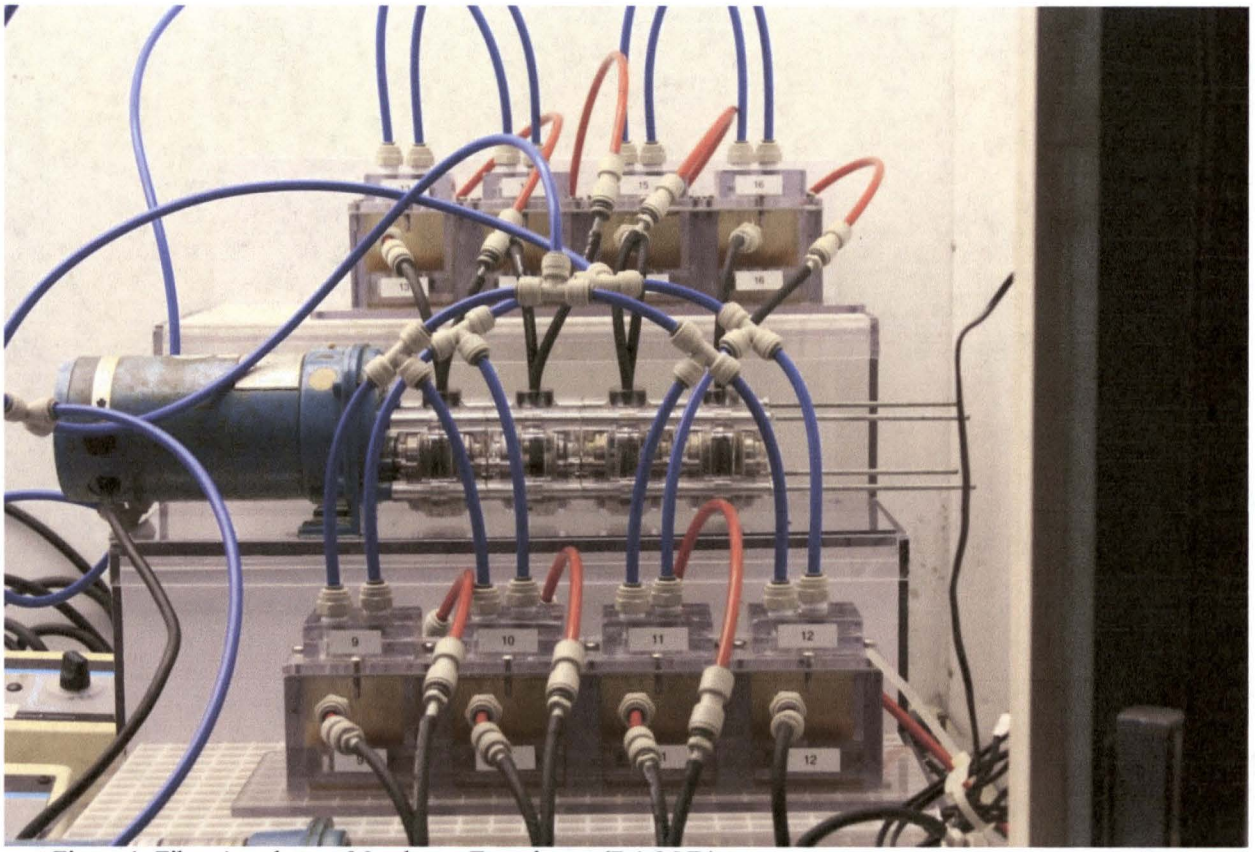

Figure 1. Fiber Attachment Membrane Experiment (F.A.M.E.) *photo courtesy of Dr. Janelle Coutts ESC*

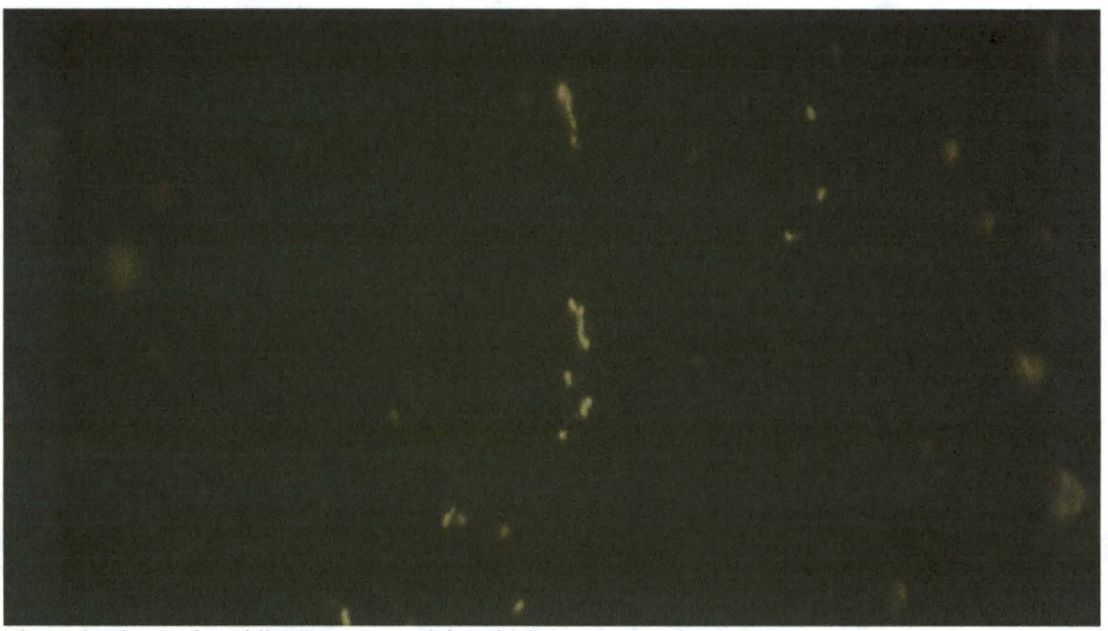

Figure 2. Photo of Acridine Orange nucleic acid fluorescent stain 1000x *photo courtesy of R.E.*

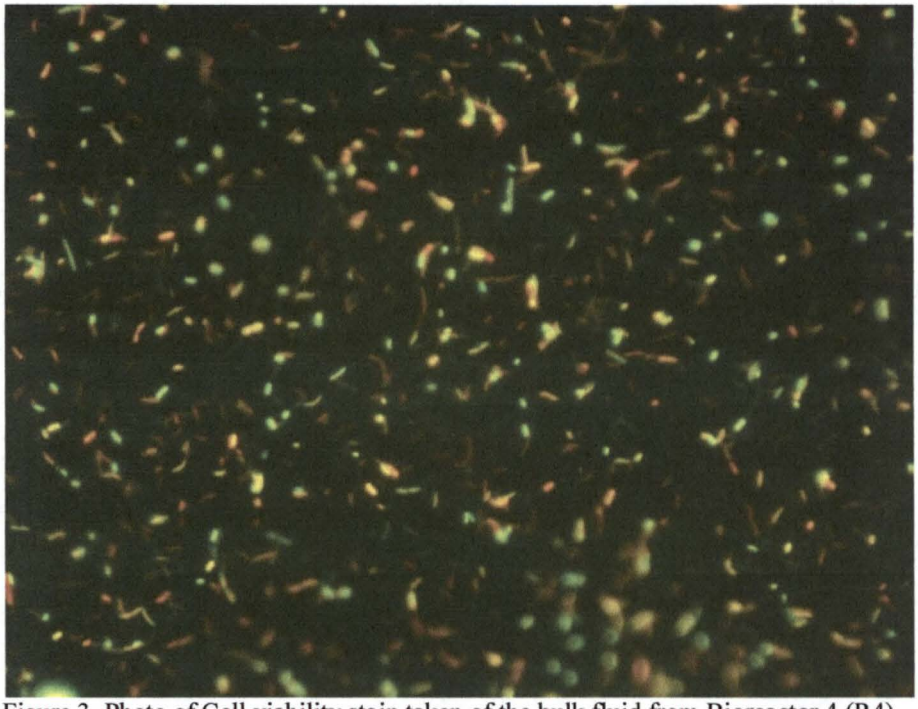

Figure 3. Photo of Cell viability stain taken of the bulk fluid from Bioreactor 4 (R4)
Photo courtesy of R.E.

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