# Ground Testing of Biofilm Formation on Spaceflight-Relevant Materials


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## Abstract


Biofilm formation has been observed on board spacecraft as well as altered bacterial behavior in different in vitro experiments, such as increased final cell counts. The formation of biofilms can decrease the efficiency and lifetime of equipment and can increase the risk of pathogen transmission. Furthermore, microorganisms in biofilms tend to have an increased resistance to disinfectants, antibiotics, and environmental stresses, and can cause human diseases and infections. In preparation for a biofilm experiment to be performed on the International Space Station where biofilm formation will be studied, different substrata materials and microbial organisms were assessed on the ground. *Penicillium rubens*, and *Pseudomonas aeruginosa, were* cultured on carbon fiber, stainless steel, aluminum 6061, titanium Ti-6Al-4V, polycarbonate, silicone, quartz and cellulose membrane coupons. The tests were performed on the same hardware planned to be used on spaceflight – BioServe's 12-Well BioCell. Test variables included growth media, temperature and time. The data produced from this ground-based testing, presented here, will serve to inform the spaceflight experiment design.


## Objectives

The overarching objective of this spaceflight investigation is to characterize biofilm growth during one experiment using different spaceflight-relevant microbial species and material substrata. For now, the objectives for ground control testing are as follows:

1. Characterize the growth of *Pseudomonas aeruginosa* and *Penicillium rubens*
2. Determine what media will be used for spaceflight
3. Determine the BioCell's gas-permeable material to ensure biofilm will not form on the film

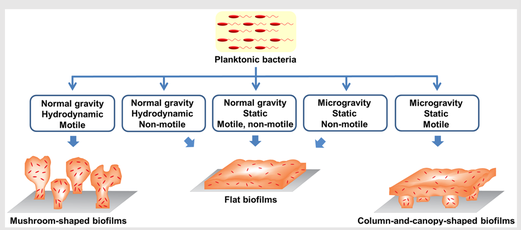
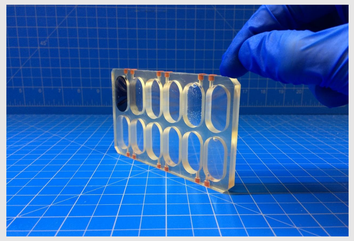



**Illustration summarizing *P. aeruginosa* biofilm architecture during spaceflight, (Kim et al., 2013)**

## Methods and Results

**Shown on the left is a 12 well commercial, off the shelf (COTS) well plate and on the right is a 12 well BioServe Biocell. Both plates were used to characterize the growth of the organisms.**



Inoculate the organisms in the COTS well plate

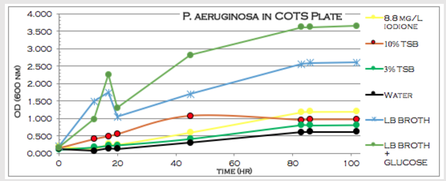
Take aliquots from the cultured media over a period of time and collect absorbance data with a photometer at 600 nm

The growth curves for each media were plotted together in one plot for comparison.

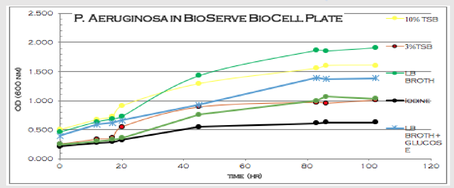
Inoculate the organisms in a BioServe BioCell

Place the entire BioCell in a photometer and collect absorbance data at 600 nm periodically over a period of time

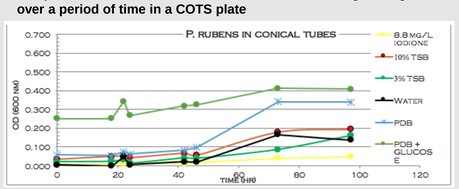
The growth curves for each media were plotted together in one plot for comparison.



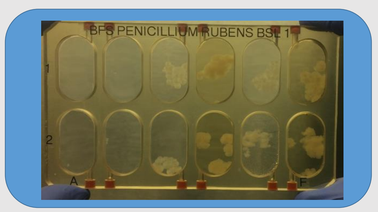

**The plot shows all the different media used for *P. aeruginosa* growth over a period of time in a COTS plate**



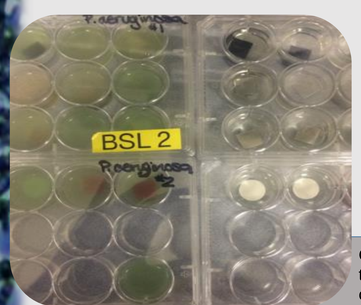

**The plot shows all the different media used for *P. aeruginosa* growth over a period of time in a COTS plate**



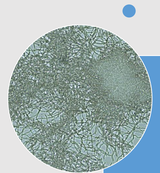

**The plot shows all the different media used for *P. rubens* growth over a period of time in conical tubes**



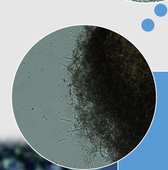

*P. Rubens* reproduces through the production of brush-shaped conidiophores. Therefore, the fungi formed cloud-like structures which proved difficult to quantify growth with the photometer. Upon close examination, we found exactly how the *P. rubens* forms non-homogenous solutions




Organisms have also been incubated on the materials as a part of preliminary testing of adhesive compatibility and media combinations. Thus far, cellulose membrane has confirmed biofilm growth as expected.



The figure is an image taken with a Nikon microscope (such as the one found on the ISS) of suspended homogenized *P. rubens* in Potato Dextrose Broth within a BioServe BioCell.



The figure is an image taken with a Nikon microscope (such as the one found on the ISS) of suspended non-homogenized *P.rubens* in Potato Dextrose Broth w ithin a Bioserve Biocell.

## Summary

The characterization of *P. aeruginosa* and *P. rubens* in various media and with various hardware has shown the following:

1. The BioServe BioCell has overall lower rates of growth with more restricted acceleration rates. Although the BioCell film allows for gas permeation, the lower access to oxygen (with respect to a culture in an open well) translates into lower growth rates and lower final cell counts.
2. *P. rubens* reproduction with brush-shaped conidiophores leads to variability in absorbance data.
3. Although LB Broth proved to be the best growth media for *P. aeruginosa,* it was also the only media to encourage biofilm formation on the Type C film of the BioServe BioCell.
4. The best growth media for *P. rubens* was shown to be Potato Dextrose Broth.
5. Cellulose Membrane has been confirmed to form a biofilm on its surface while incubated with P. aeruginosa

## Future Directions

The next steps are to ensure that long-term storage conditions at 4℃ does not detrimentally effect growth in media (in case of launch delays), ensuring that the media and film combinations are compatible, and designing an experimental timeline for spaceflight.

**Shown on the left is a SABL unit used by BioServe for the storage and maintenance of experiments during spaceflight. This experiment will be stored in a SABL unit on the ISS**

## Acknowledgments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## References


**(2013). Spaceflight promotes biofilm formation by Pseudomonas aeruginosa. *8*, e62437.**