

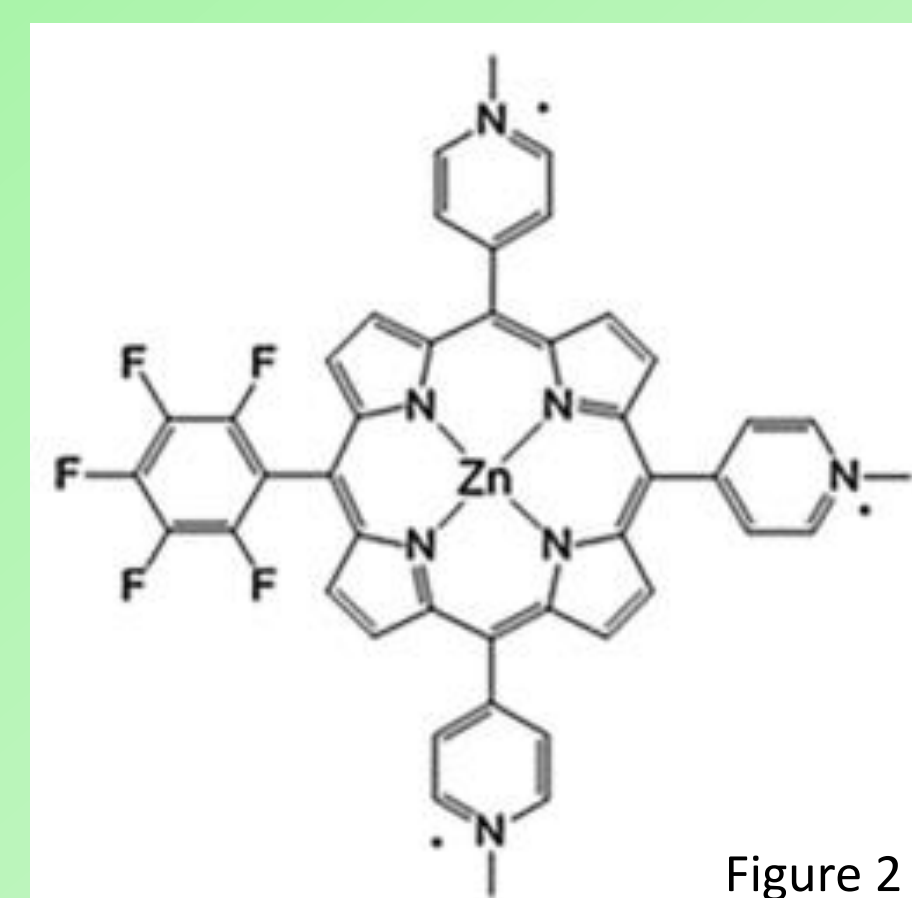
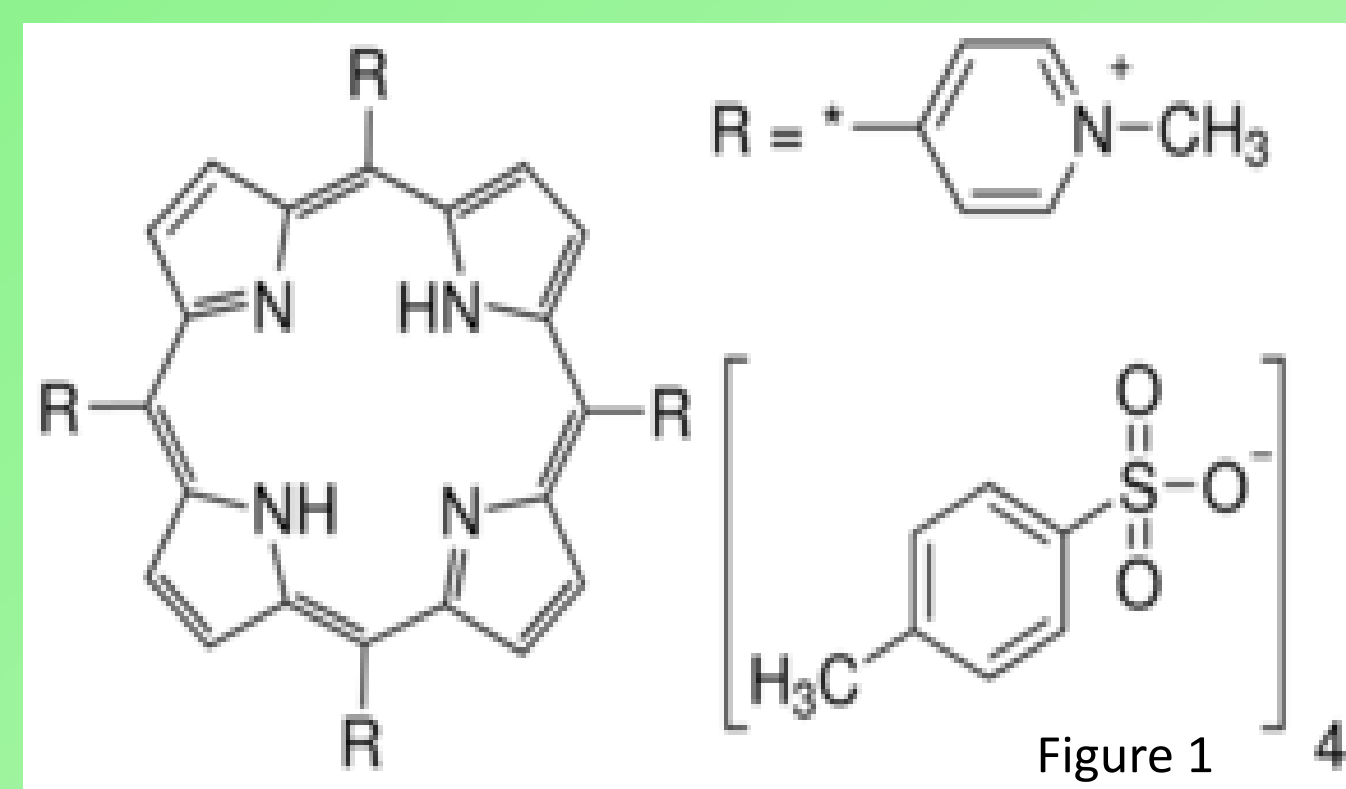
Disruption and Prevention of Biofilm Growth: The Effect of a Cationic and Novel Zinc Porphyrin on *Pseudomonas aeruginosa* Biofilm Formation on Different Substrata

David Rivetti, Nehaben Patel, Jayne B. Robinson, Karolyn M. Hansen
Department of Biology, University of Dayton

INTRODUCTION AND BACKGROUND

- The properties of biofilms differ greatly from free-living, or planktonic, bacterial cells.
- The structure and function of the biofilm matrix promotes cooperation, capture of resources, an enhanced rate of genetic exchange, and resistance to antimicrobial agents.
- Pseudomonas aeruginosa* is a gram-negative bacterium capable of forming biofilms on both biotic and abiotic surfaces.
 - Previous experiments have demonstrated effective disruption of *Pseudomonas aeruginosa* biofilm growth on abiotic surfaces (e.g. glass).
- Research Focus: Determining the efficacy of TMP and a novel zinc porphyrin in both disruption and prevention of biofilm growth on various substrata with medical and environmental applications.**

PORPHYRINS



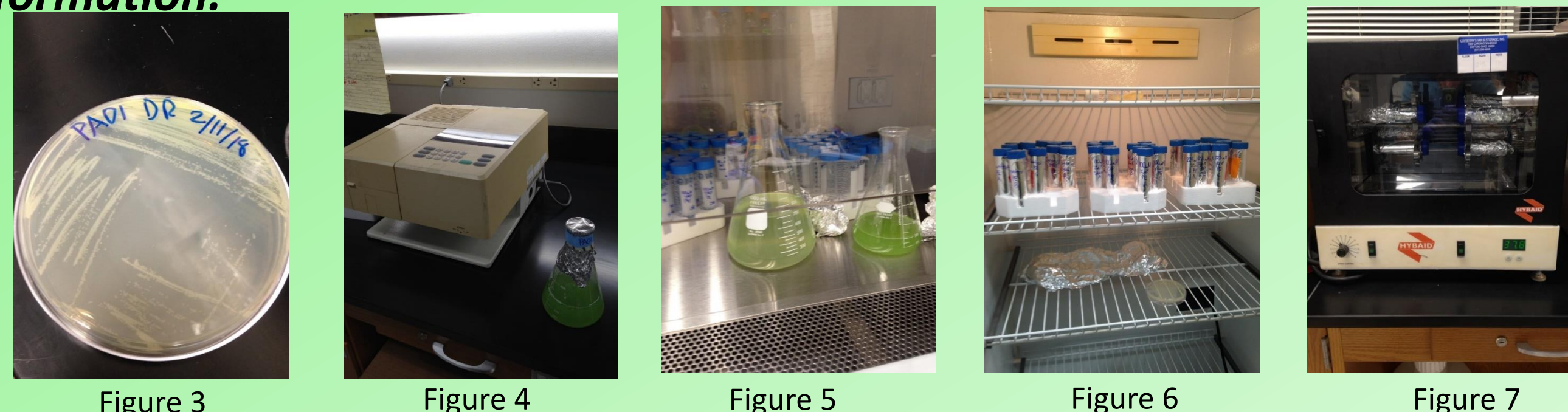
- Ringed and planar compounds
- Occur naturally and can also be synthesized
- Used in photodynamic therapy:
 - Generation of singlet oxygen (1O_2) that damages biomolecules
 - Intercalation between DNA base pairs \rightarrow damage when irradiated
- Microbial resistance not yet reported

Figure 1 displays the structure of 5,10,15,20-tetrakis (1-methyl-pyridino)-21H,23H-porphine, tetra-p-tosylate salt (TMP). Figure 2 shows the structure of the novel zinc porphyrin (5,10,15-tris(N-methylpyridyl)-20-pentafluorophenylporphyrinatozinc (II)).

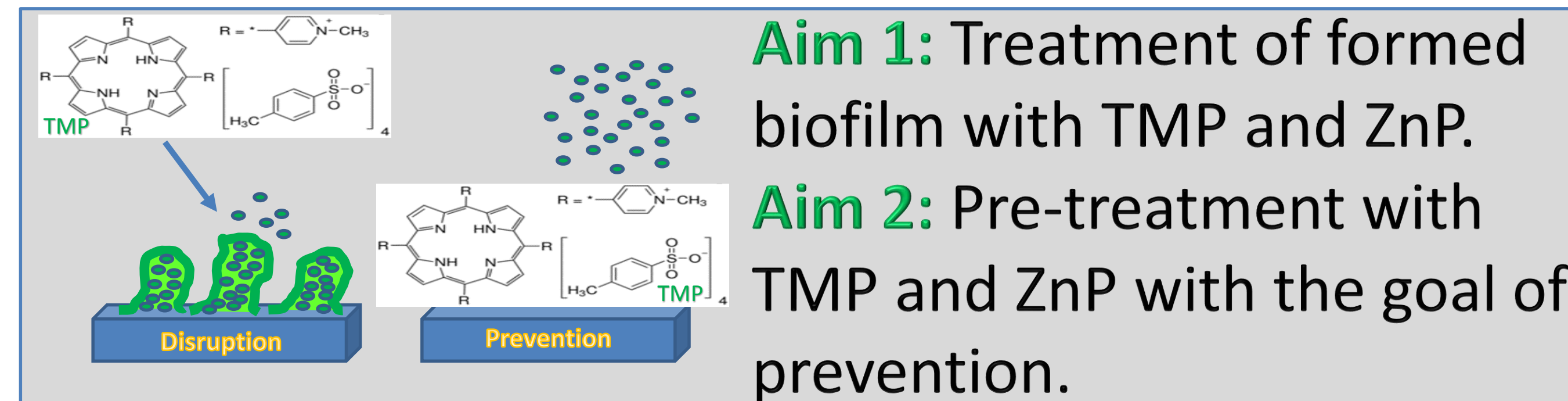
MATERIALS AND METHODS

Disruption Experiment: 2 hour exposure to TMP or ZnP following 16 hour biofilm formation.

Prevention Experiment: 2 hour pre-soak in TMP or ZnP prior to biofilm formation.



Figures 3-7: Experimental design for the disruption and prevention experiments performed. Figure 3 is a streak plate of PAO1 on LB agar. Figure 4 displays the spectrophotometric readings taken before the liquid culture was adjusted to an optical density of 0.15, which is depicted in Figure 5. The adjusted culture was incubated under dark, static conditions for 16 hours at 37°C, as shown in Figure 6. Pre-treatment with a 2 hour soak in TMP or ZnP was performed in a hybridization incubator set to 37°C (Figure 7).



Polyethylene (PE) Stainless Steel Glass Slide

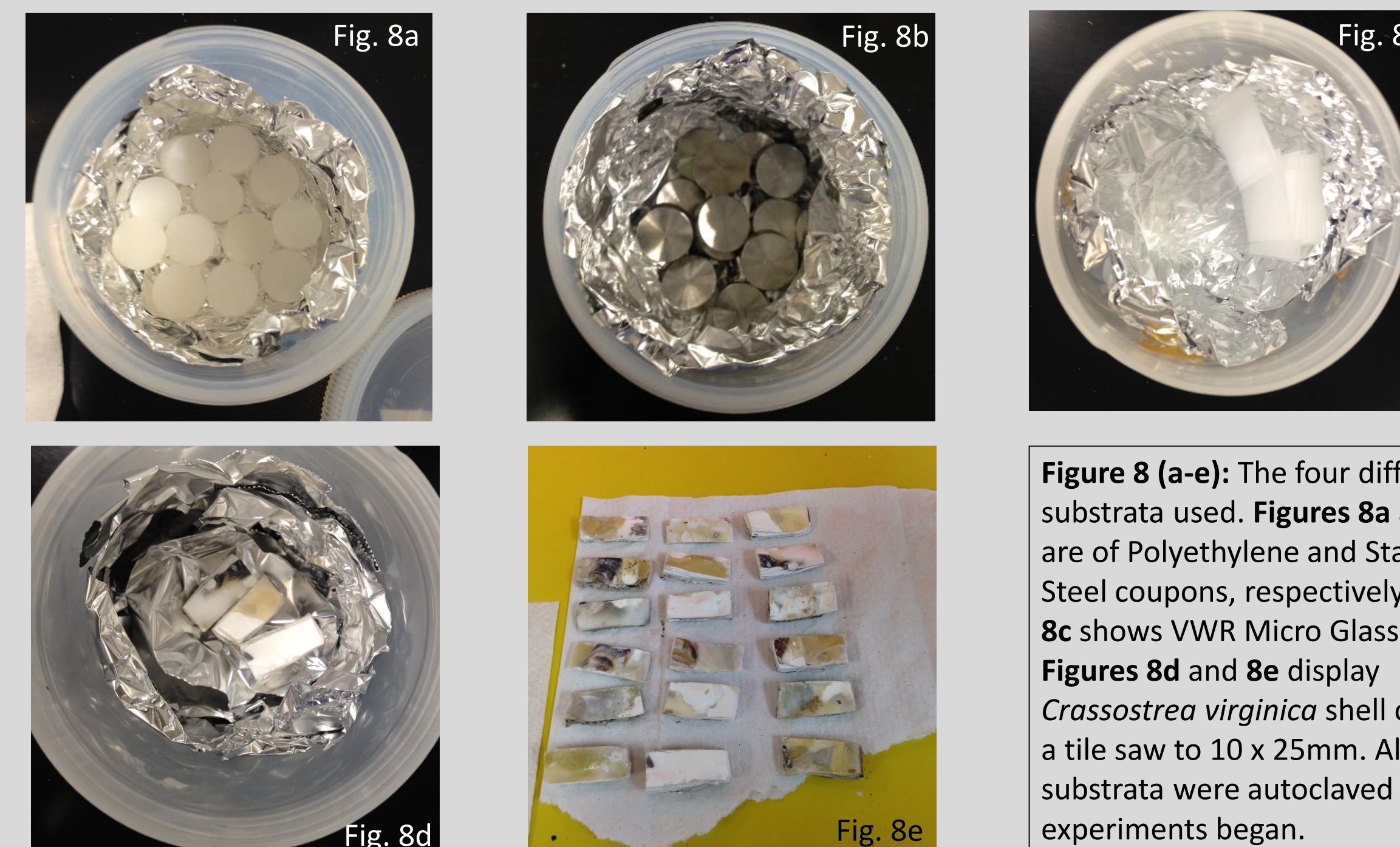
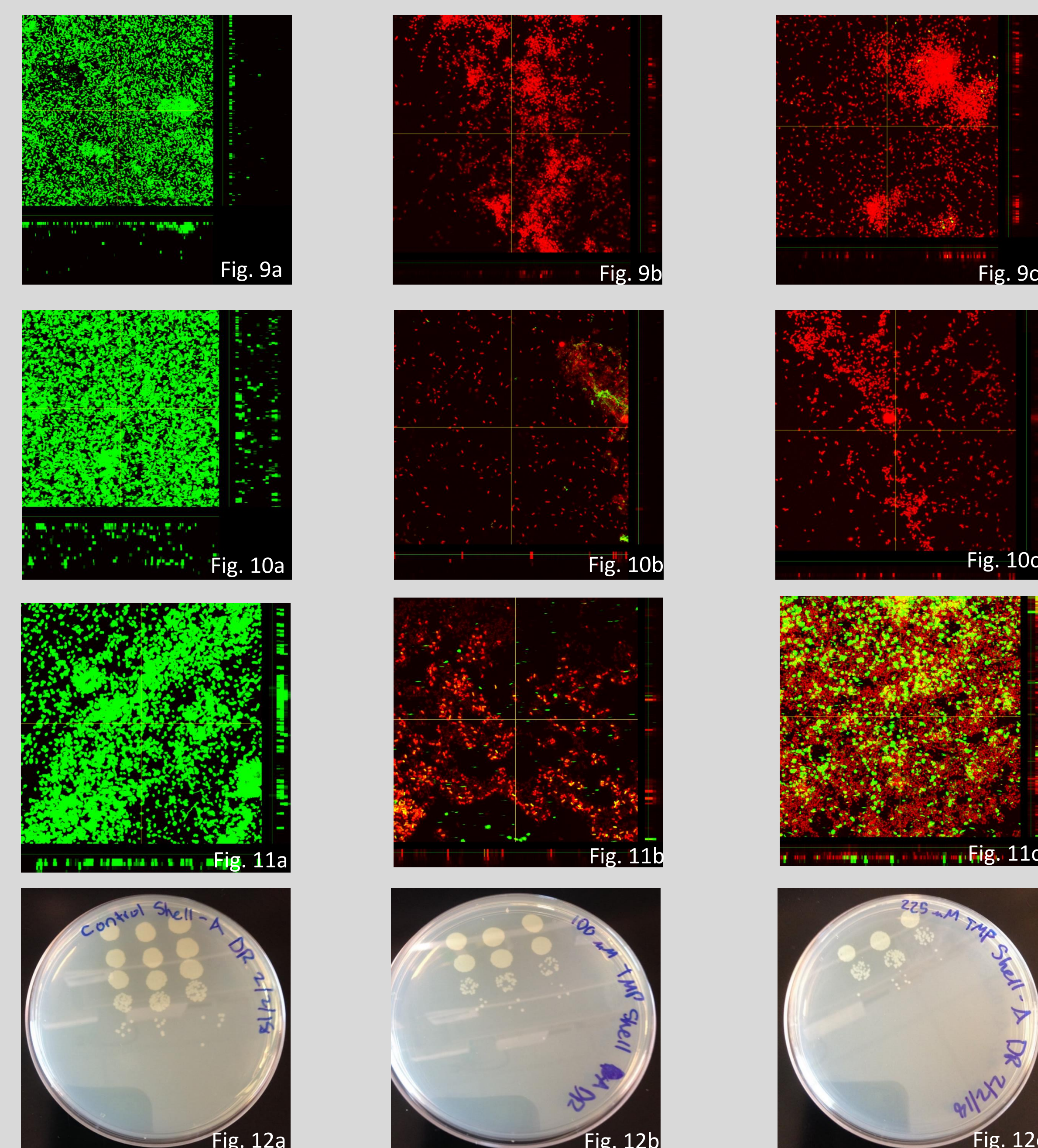


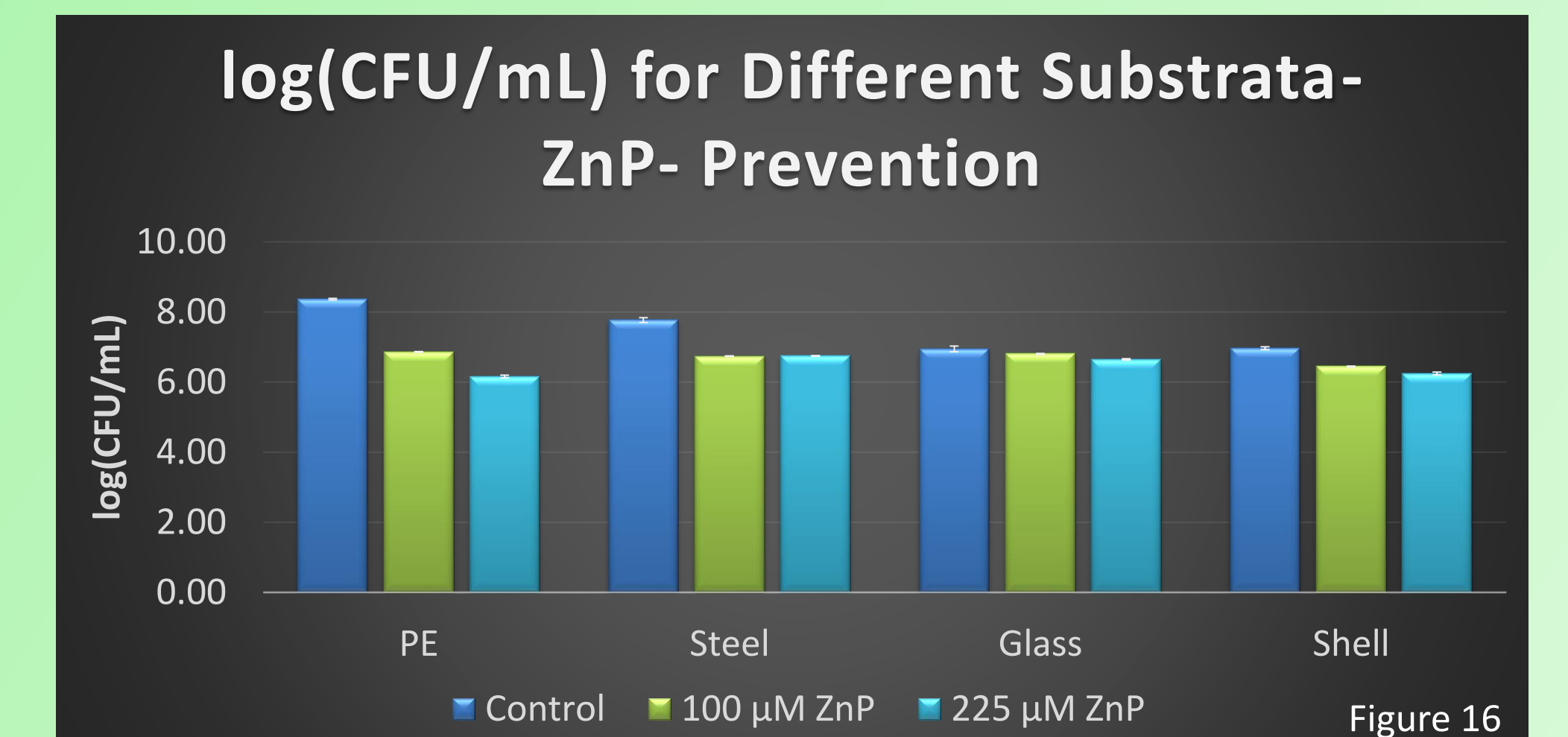
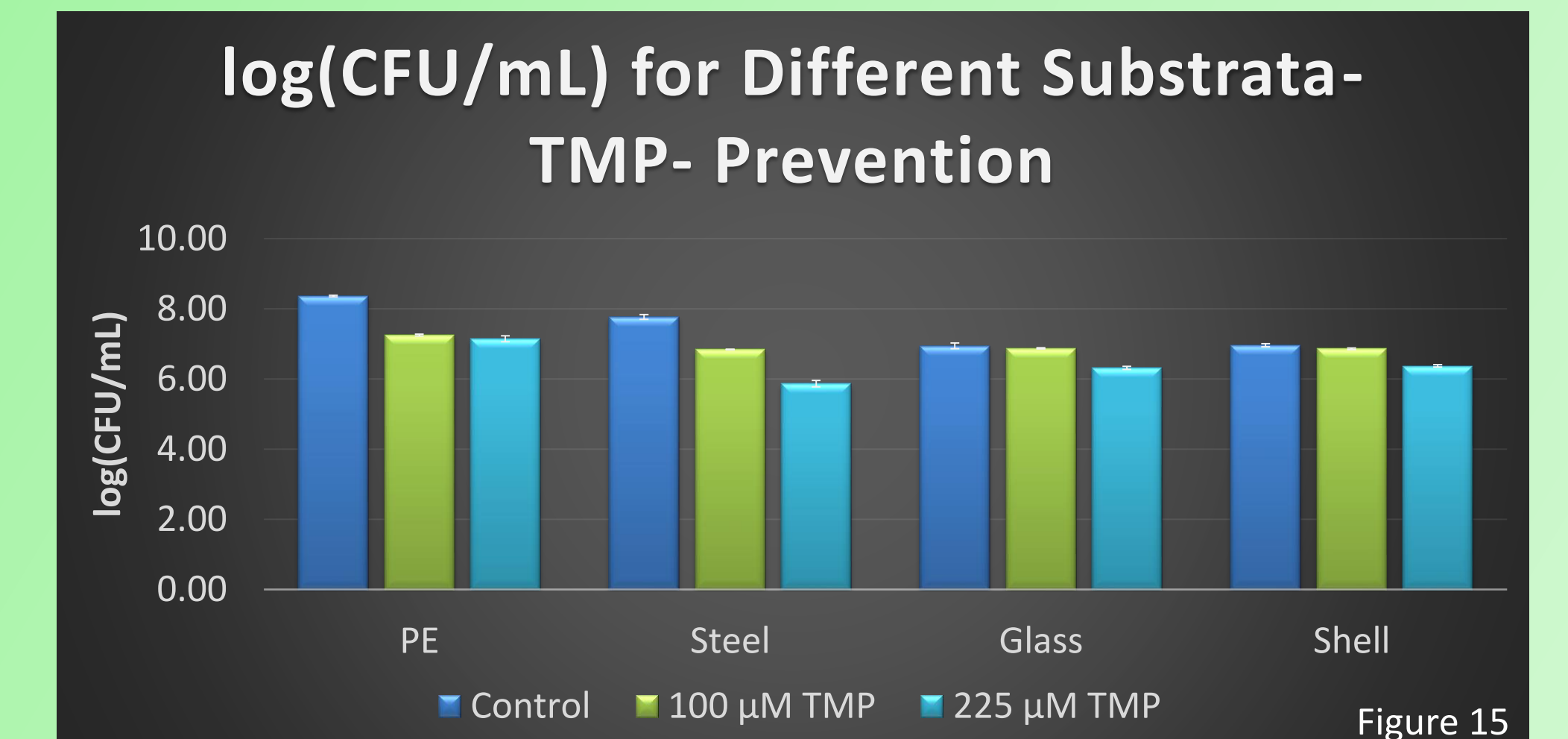
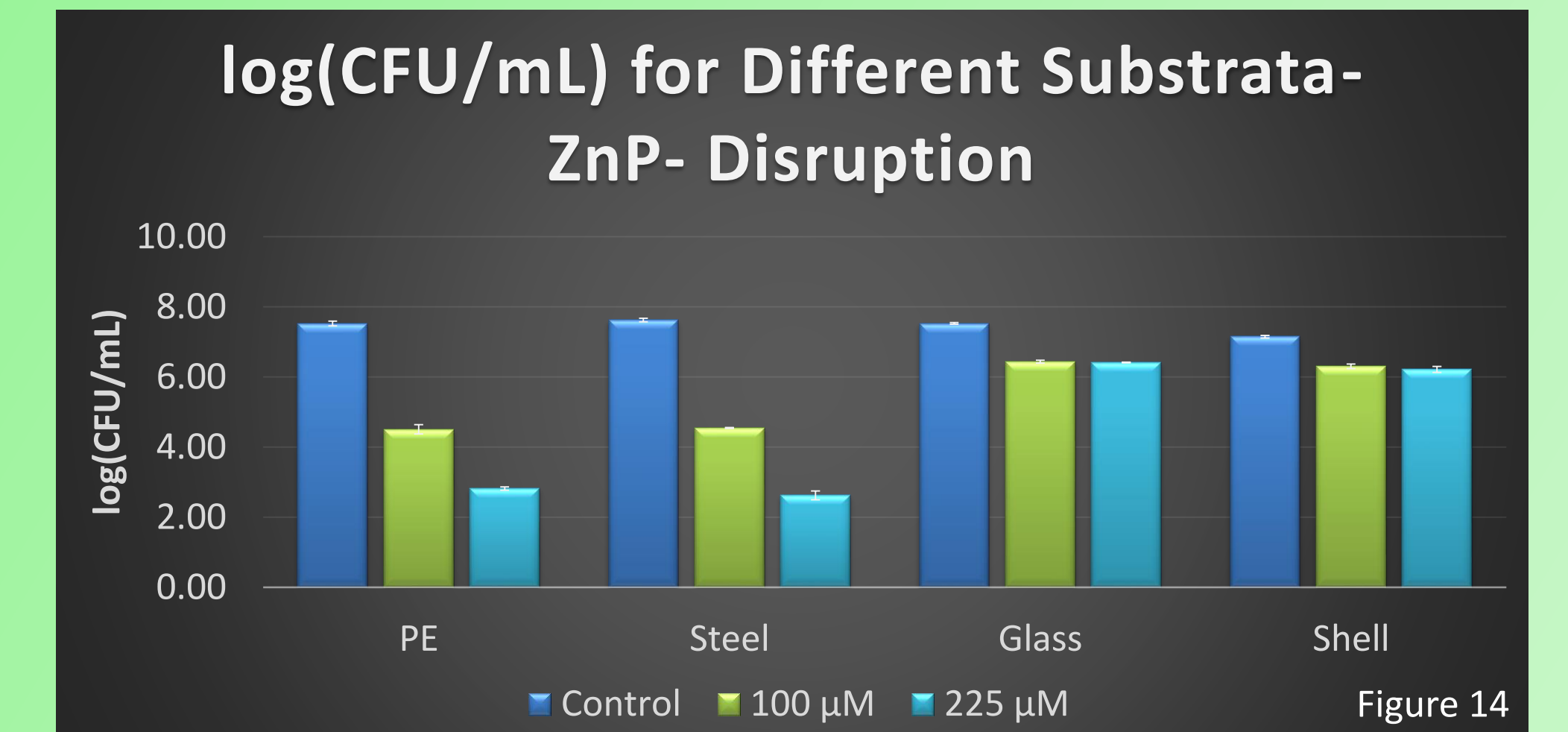
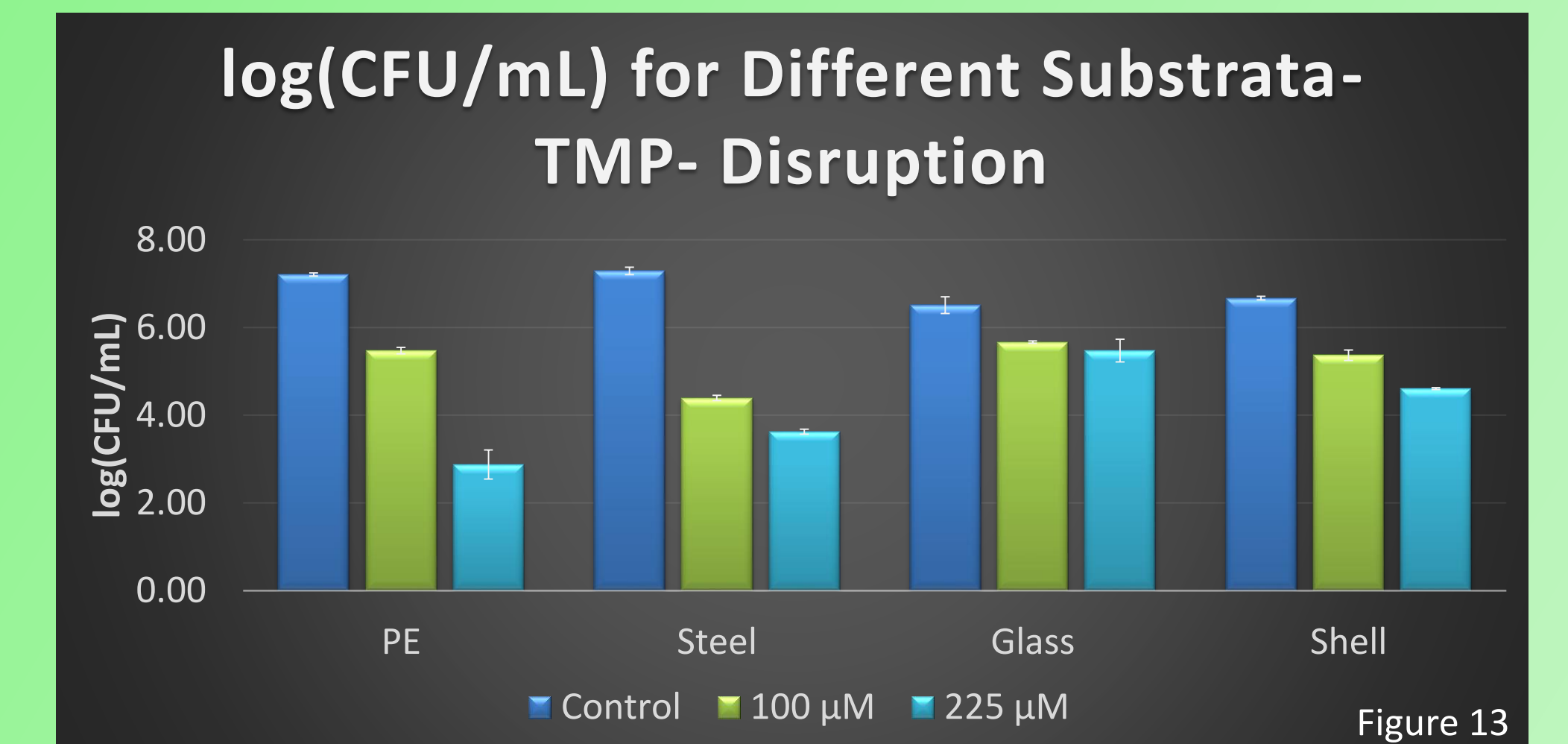
Figure 8 (a-e): The four different substrata used. Figures 8a and 8b are of Polyethylene and Stainless Steel coupons, respectively. Figure 8c shows VWR Micro Glass Slides. Figures 8d and 8e display *Crassostrea virginica* shell cut with a tile saw to 10 x 25mm. All substrata were autoclaved before experiments began.

CONFOCAL IMAGING AND PLATE COUNTS- TMP DISRUPTION EXPERIMENTS



Figures 9-11: Confocal images of: (9a) PE Control, (9b) PE 100µM TMP, (9c) PE 225µM TMP, (10a) Steel Control, (10b) Steel 100µM TMP, (10c) Steel 225µM TMP, (11a) Glass Control, (11b) Glass 100µM TMP, and (11c) Glass 225µM TMP. Figure 12: Plate counts for: (12a) Shell Control, (12b) Shell 100µM TMP, and, (12c) Shell 225µM TMP.

RESULTS



Figures 13 and 14 display the data from the disruption experiments following 2 hours of exposure to TMP (13) or ZnP (14). Figures 15 and 16 display the data from the inhibition experiments consisting of 2 hours of pretreatment with TMP (15) and ZnP (16).

DISCUSSION AND FUTURE DIRECTIONS

- Both porphyrins were more effective in disruption than prevention. TMP resulted in greater disruption on shell compared to ZnP.
- Future Directions: Test additional time points for TMP and ZnP exposure, conduct additional cytotoxicity studies, and test the efficacy of combination treatments with other antimicrobial agents.

ACKNOWLEDGMENTS

•Dr. Karolyn Hansen and Dr. Jayne Robinson (Advisors)
•Funding: University of Dayton Honors Program, College of Arts and Sciences Dean's Summer Fellowship 2017
•University of Dayton Department of Biology
•Nehaben Patel (Graduate Student)