THE HEALING CAPABILITIES OF *TRICHODERMA REESEI* IN CONCRETE

Pauline Do, Natalie Ge, Nels Martin

INTRODUCTION

In the current world, societies everywhere are refilling fractures in concrete for sidewalks, buildings, and other structures by hand which can be a dangerous and costly job. The production of new concrete to fill these fractures is time-consuming as well as environmentally detrimental. During concrete production, CO_2 is released when calcium carbonate is thermally decomposed, which contributes to the greenhouse effect. *Trichoderma reesei (T. reesei)* is a fungus with the ability to secrete calcium carbonate ($CaCO_3$) as a product of its metabolic processes. *T. reesei* can be incorporated into concrete mix to heal structural damage via its metabolism. It has been proven that *T. reesei* is one of the only organisms resilient enough to survive the deleterious environment of concrete. *T. reesei* lies dormant until its incubating concrete is damaged. As the fungus is exposed to moisture and oxygen from the external environment, it will germinate, produce $CaCO_3$ to repair the fracture and then form spores again once the crack has been sealed. The purpose of this experiment is to gather more information on the efficiency of this procedure by incorporating different amounts of *T. reesei* into concrete.

MATERIALS & METHODS

Rehydrating fungi: A vial of freeze-dried *T. reesei* fungi was opened. The inner vial was removed from the outer casing along with all cotton plugs. Tap water was boiled to sterilize it. After the sterilized water cooled to room temperature, a sterile pipette was used to transfer 1 mL to the inner vial of *T. reesei* (Figure 1). The mouth of the vial was sealed using parafilm and the vial was left in a controlled environment for 24 hours to rehydrate the freeze-dried *T. reesei*.

Resuspending fungi: We combined 10 mL of nutrient broth and all of the rehydrated *T. reesei* in a glass vial (Figure 2). The fungi was left to grow for 48 hours undisturbed and then refrigerated to prevent the fungi from overgrowing.

Experimental tests: Three cups were filled with ¼ cup of water and 1 mL of *T. reesei* nutrient broth. A large bowl was used to mix 1¼ cup of concrete powder and the cup of diluted fungus. The concrete was separated into three equal parts. Each portion of concrete was placed into a circular mold (Figure 4) and spread out evenly. This was repeated two more times with 2 mL and 4 mL of *T. reesei* nutrient broth. The concrete was left to cure for 72 hours. Water was sprayed over the concrete every five days to stimulate rain. Samples from the concrete were extracted using a needle and observed under a microscope after 12 days.

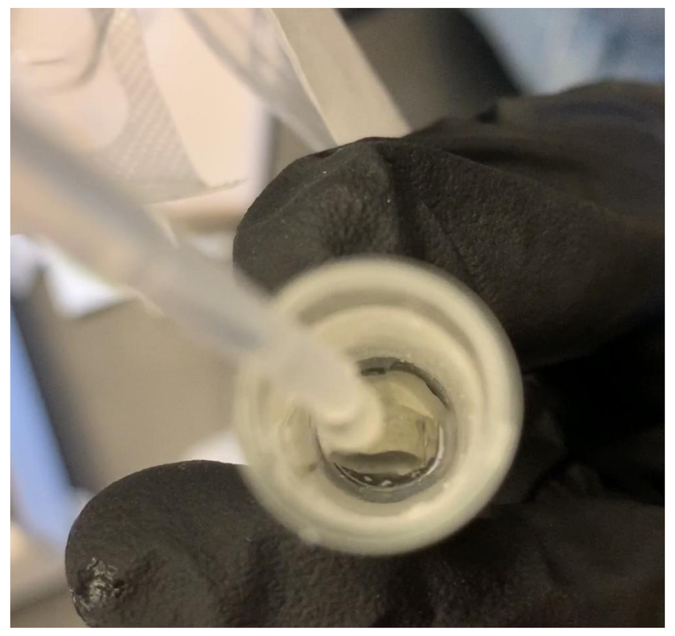

Figure 1: Rehydrating fungi

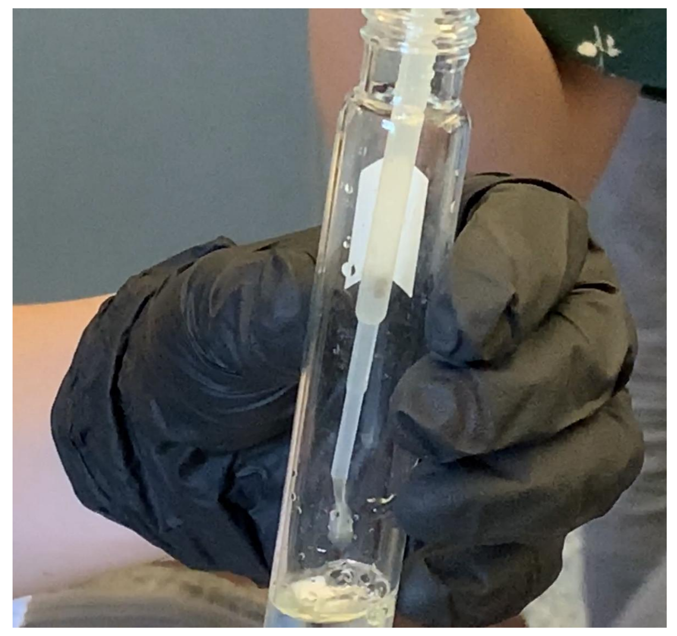

Figure 2: Resuspending fungi

Figure 3: *T. reesei* nutrient broth

Figure 4: Filling molds

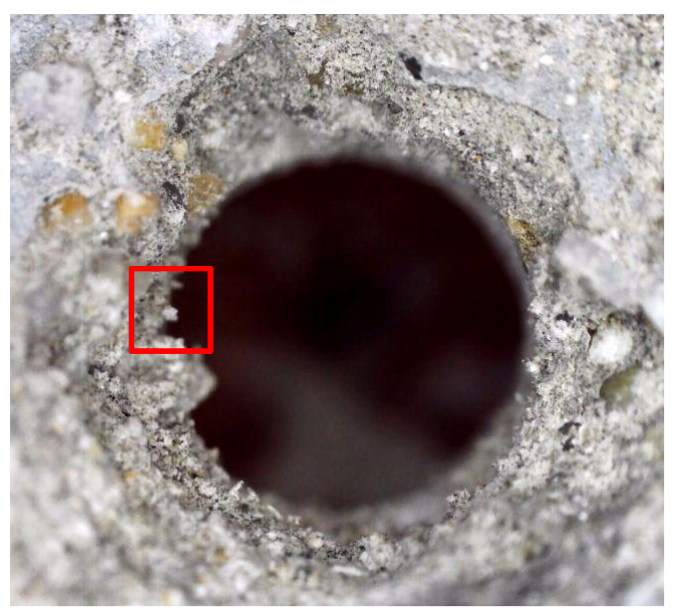

Figure 5: 4 mL test, day 10

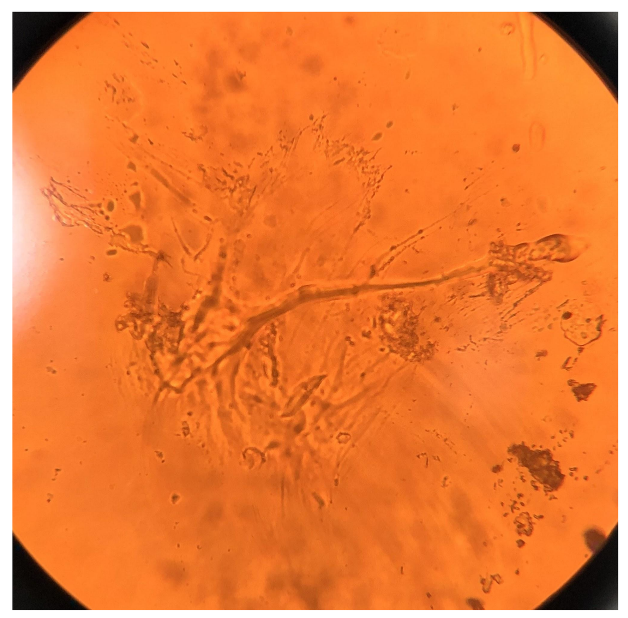

Figure 6: 4 mL test sample 40x

Figure 7: Control 2, day 10

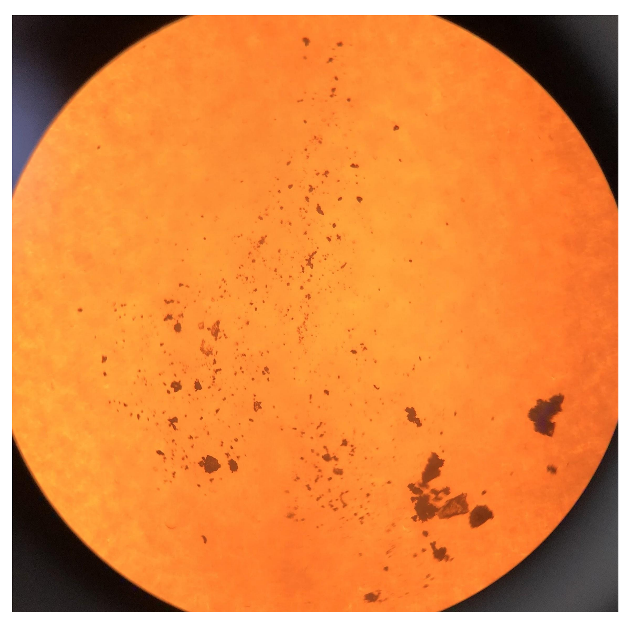

Figure 8: Control 2 sample 10x

DISCUSSION

Though no numerical data was collected, small specks of white (indicated by the red box) were observed along the rim of one of the tests containing 4 mL of *T. reesei* nutrient broth (Figure 5). A sample was scraped off using a needle and placed on a microscope slide. Figure 6 shows an image taken through a microscope at 40x magnification. The branch-like growth in the center is similar in appearance to Figure 9 and resembles germinating fungi. In contrast, Figures 7 and 8 show only rocks and sediment from the concrete. The controls and the tests containing 1 and 2 mL of *T. reesei* did not show any germinating fungi. Though *T. reesei* is germinating within the concrete, the area that was actually regrown was small; it would take a longer period of time for the fungi to actually refill the hole. The slow growth of the fungi could be due to the cold conditions under which the concrete was kept. The concrete was moved, which could have disrupted the fragile growth of the fungi. Issues faced when implementing our research into society include the cost of propagating the fungi as purchasing an ampoule of *T. reesei* is close to $400. However, the cost of repairing concrete annually is estimated to be far greater so an investment in the beginning may cut costs in the long term.

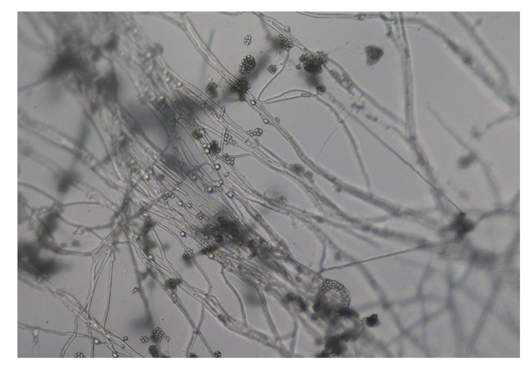

Figure 9: *Trichoderma reesei* culture 100x (Research Gate)

CONCLUSION

In the final analysis, 4 mL of fungi did begin to heal the hole, but further research and more time would be necessary to corroborate our findings. Under a microscope, fungi was found growing in the concrete. Though the process of filling in the cracks is relatively slow, naturally occurring fractures would most likely be much smaller and easier to fill than the holes that were drilled. Additionally, weather would be a large factor in the success of fungi growth as cold temperatures could slow germination. All in all, the optimal proportion of fungi to concrete as well as growth time and strength will need to be determined in future experiments.

ACKNOWLEDGEMENTS

We would like to thank Mrs. Abraham, Mrs. Roberts, Dr. Rick Nipper, and Sedia Biosciences Lab for their guidance with collecting materials and organizing our experiment. We would also like to thank the MST Magnet Program and Camas High School for funding our research.