Transportation Infrastructure Precast Innovation Center (TRANS-IPIC)

University Transportation Center (UTC)

Exploring Fungal-Induced Carbonate Precipitation (FICP) for Healing Concrete Cracks
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Quarterly Progress Report
For the performance period ending *12/31/2024*

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TRANS-IPIC Quarterly Progress Report:

Project Description:

1. Research Plan - Statement of Problem
   This research aims to explore fungal-induced carbonate precipitation (FICP) to heal cracks and improve the durability of concrete. FICP is a natural biomineralization process involving calcifying fungi's metabolic activities to induce $CaCO_3$ precipitation.

2. Research Plan - Summary of Project Activities (Tasks)
   Task 1. Comparing the performances of three fungal strains on $CaCO_3$ precipitation. The research team assessed the performance of three fungal strains, *Aspergillis niger* (ATCC 9029), *Neurospora crassa* (FGSC 2489), and *Trichoderma reesei* (ATCC 13631), on mineral precipitation.

   1. Cultivation of Three Fungal Strains

   Three 500 mL Erlenmeyer flasks were prepared by adding 200 mL of grow media (potato dextrose broth, PDB) and a stir bar in each flask. The flask opening was sealed with aluminum foil and then autoclaved in Yamato Autoclave (SK101C) to sterilize the grow media. After sterilization, three fungal trains were inoculated into the corresponding flask in a biosafety cabinet (Thermo Scientific 1300 Series A2). These three flasks were then placed on the magnetic stirrer plate with a speed of 60 rpm to maintain homogeneity of the growth medium in an incubator at 28°C for 19 days to incubate fungal strains.

   2. Adding Cementation Solution to Induce Mineral Precipitation

   The cementation solution was prepared by adding 150 mL of 1M Tris Buffer, 20 g of Urea, and 29.4 g of $CaCl_2.2H_2O$ into deionized water to form 1 L solution. The pH of the solution was tuned to 6 using HCL. The solution was then filter sterilized using the 0.22 um opening filter. After 19 days of fungal growth in the flasks, 200 mL of the cementation solution was then added into each fungal culture flask to induce mineral precipitation for 7 days. Figure 1 shows the fungal solutions after 7 days of mineral precipitation.

Figure 1. fungal solutions after 7 days of mineral precipitation.

3. pH and NH_4^+ measurements

Starting from the day the cementation solution was added, 6 mL of the solution in each flask was extracted daily for seven consecutive days using a sterile syringe. The 6 mL extracted solution was then filtered using a 0.22 µm syringe filter to remove suspended fungal mycelium and stored in the 50 mL centrifuge tubes which was then stored in a freezer at -4°C for pH and NH_4^+ measurements later.

pH measurement: The stored 50 mL centrifuge tubes corresponding to different sampling date were thawed and subjected to pH measurement using a pH meter (**Thermo Scientific ORION 3 STER pH Benchtop**). The pH meter was first calibrated using a three-point calibration method with standard buffer solutions (pH 4.0, 7.0, and 10.0) prior to pH measurements. The pH results (Figure 1) show that the pH increased with time from pH=6 at the beginning to pH=~8 after 7 days of cementation solution addition.

NH_4^+ concentration measurement: After pH measurements, the concentration of **NH_4^+** was measured in each collected solution **using the Modified Nessler Method.** First, a **standard curve** between true **NH_4^+ concentrations (0.1, 0.3,** and **0.5 mM)** and measured absorbance at 425 nm **using a spectrophotometer (721-VIS spectrophotometer)** was developed. Then, the **NH_4^+ concentrations in** each **sample** was measured**.** 2 mL solution was mixed with **100μL** Nessler reagent for absorbance measurement using the spectrophotometer. The results of NH_4^+ measurements (Figure 2) show that the NH_4^+ concentration increased with time.

The *Neurospora crassa* solution demonstrated the highest pH increase and the highest NH_4^+ increase, which indicates the best potential for fungal-induced carbonate precipitation (FICP) among the three strains tested.

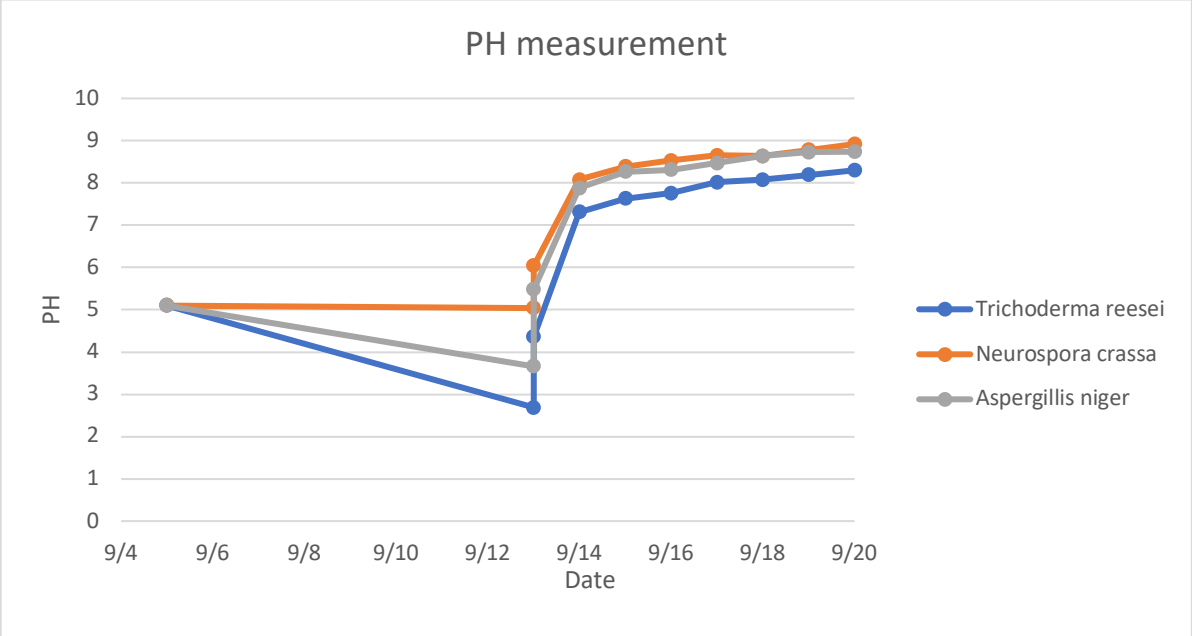

Figure 2. pH measurements of three fungal strain solutions.

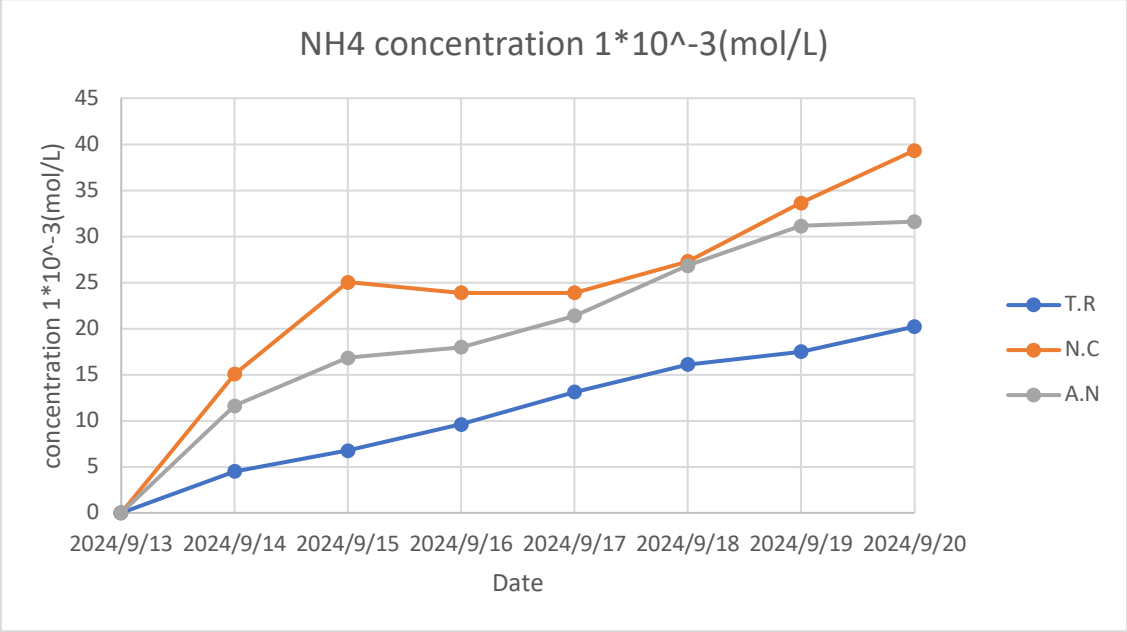

Figure 2. NH_4^+ results of three fungal strains.

4. Sample Collection for SEM, EDS, and XRD Analysis

After completing the tests of mineral precipitation, the three fungal solutions were filtered using a 500 mL vacuum filtration unit equipped with a 0.22 μm filter membrane (fisherbrand 500 mL Bottle Top Filter) to collect the retained fungal mycelia material on the filter. The vacuum pump was operated at a pressure of 0.08MPa to ensure efficient filtration. During filtration, additional 250 mL of deionized water was added into the filtration funnel to rinse the retained material on the membrane three times in order to remove the residual chemicals in the retained fungal mycelia materials on the filter. Then, the retained materials on the filter membrane were carefully collected using a sterile spatula and transferred into sterile storage containers, which was placed in a 60°C drying oven for 48 hours to dry the samples.

5. SEM, EDS, and XRD Analysis

Due to the non-conductive nature of the fungal mycelia samples, each fungal sample must undergo an 8-minute coating process prior to SEM analysis. After coating with a thin layer of platinum (EMS550X Sputter Coater), the samples were fixed on the aluminum stubs using conductive carbon tape for SEM imaging. A scanning electron microscope (Thermo Fisher Scientific Quanta 3D FEG, S/N: D8805) was used. The accelerating voltage was set to 5-20 kV, depending on the sample's conductivity and resolution requirements. The microstructural features of the samples were observed and captured. During SEM imaging, EDS analysis (integrated with the SEM) was also conducted to analyze the elemental compositions in the samples. The EDS spectra were taken from multiple regions of interest on the sample to investigate the distribution and types of elements present in the samples. Figures 3 to 5 show the SEM images of three fungal mycelium samples. Figure 6 shows the EDS analysis for *Neurospora crassa*

samples, which shows the presence of calcium minerals in the samples. EDS results for the other two strains are currently in progress.

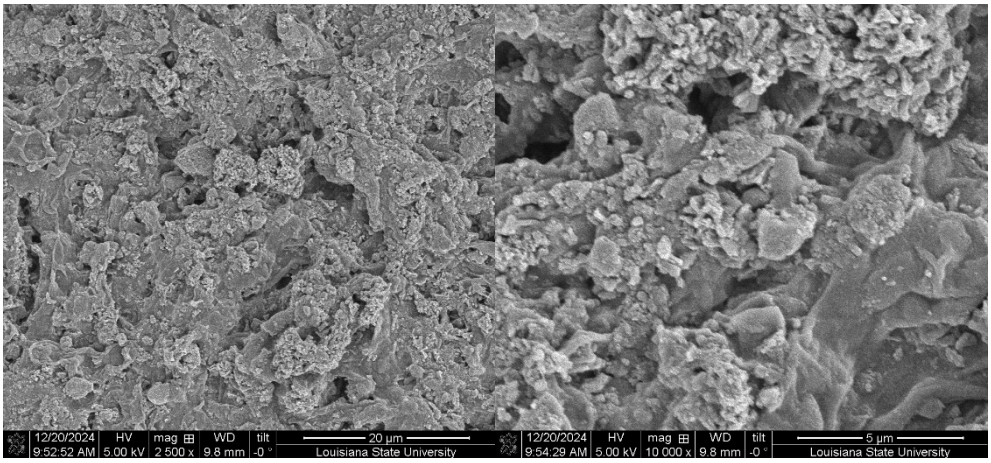

Figure 4. SEM imaging of Trichoderma reesei (ATCC 13631).

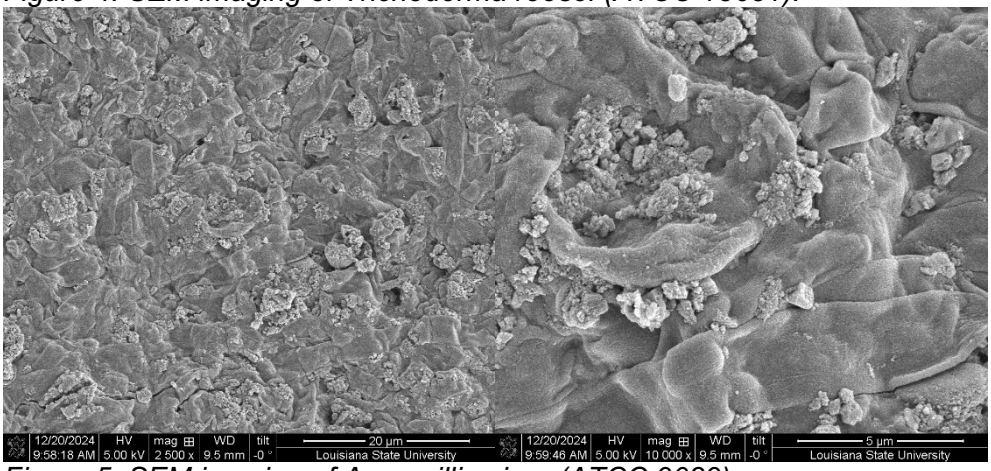

Figure 5. SEM imaging of Aspergillis niger (ATCC 9029)

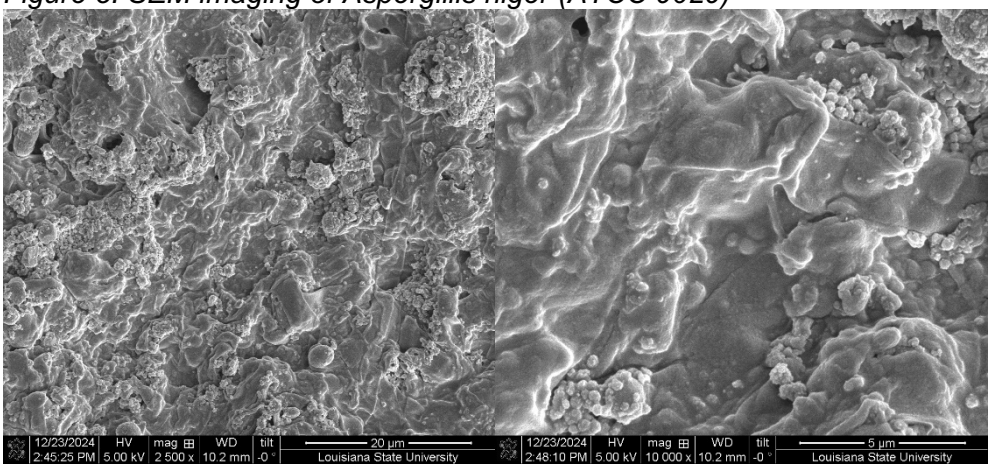

Figure 6. SEM imaging of Neurospora crassa (FGSC 2489).

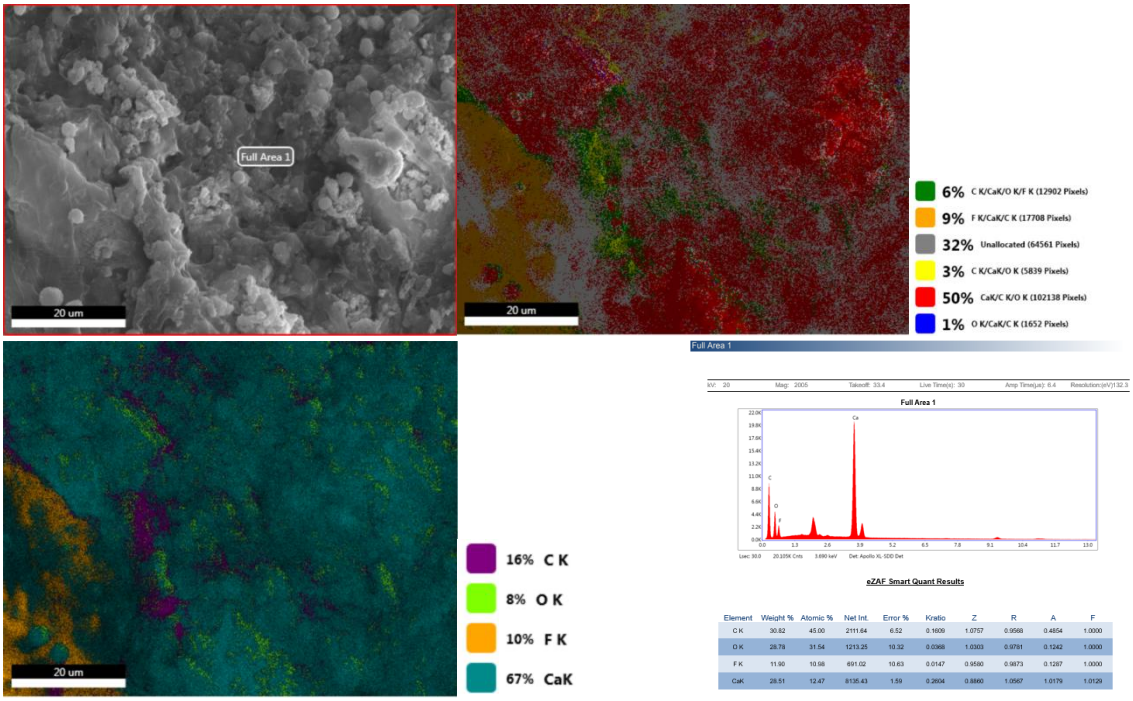

Figure 7. EDS results of Neurospora crassa (FGSC 2489).

Task 2. Investigating the performance of three fungal strains to induce $CaCO_3$ precipitation on the mortar surface.

1. Sample Preparation

Based on the results of Task 1, we selected two fungal strains that can precipitate calcium minerals, Aspergillis niger (ATCC 9029) and Neurospora crassa (FGSC 2489), for task 2 research. For each fungal strains, three petri dish samples were prepared, including two petri dish samples with cement to evaluate their growth on the cement surface and one petri dish sample without cement to evaluate their growth in the growth media without cement. Another blank petri dish sample with cement and the growth media was prepared to assess potential contamination. For petri dish samples with cement, a cement paste was prepared using a mass ratio of OPC: Sand: Water = 1:1.5:0.4. The paste was poured into sterilized petri dishes (VWR Sterilized, size: 100 × 15 mm) to reach half the height of each dish. The samples were then cured for 28 days at 25°C and 60% relative humidity to ensure complete hardening. After the curing period, the petri dishes were sterilized using an autoclave set to 121°C and 15 psi for 20 minutes. Once sterilization was completed, the petri dishes were allowed to cool to room temperature before being transferred into a biosafety cabinet for subsequent handling under sterile conditions.

2. Fungal Inoculation and Cultivation

In the biosafety cabinet, 10 mL of sterilized Potato Dextrose Broth (PDB) liquid medium was added to each petri dish. The PDB medium was then inoculated with 2 mL of the fungal suspension of each fungus. Aseptic conditions were maintained throughout the inoculation process to prevent contamination. Each petri dish was sealed with parafilm to allow gas exchange while preventing cross-contamination. The sealed petri dishes were

incubated in an incubator set to 28°C, with regular monitoring of the samples. The duration of incubation and any observable changes in the petri dishes were recorded using a camera. Figures 8, 9 and 10 show the condition of fungal growth process in the petri dishes with time. In the top row of each figure, the three petri dishes correspond to *Aspergillus niger*, while the bottom row corresponds to *Neurospora crassa*. The petri dish on the right of cement petri dishes in both rows served as a blank control sample without cement. The cement petri dish on the very right is also a control sample with cement and the growth media (without fungal strain) to assess potential contamination.

Figures 8 to 10 show that the two fungal strains were able to grow in the petri dishes with just the growth media with time. However, these two fungal strains can not grow on the surface of the cement mortar samples. The reasons could be due to the high pH, water absorbed by cement hydration, or cement chemicals that affected the growth of fungal mycelium.

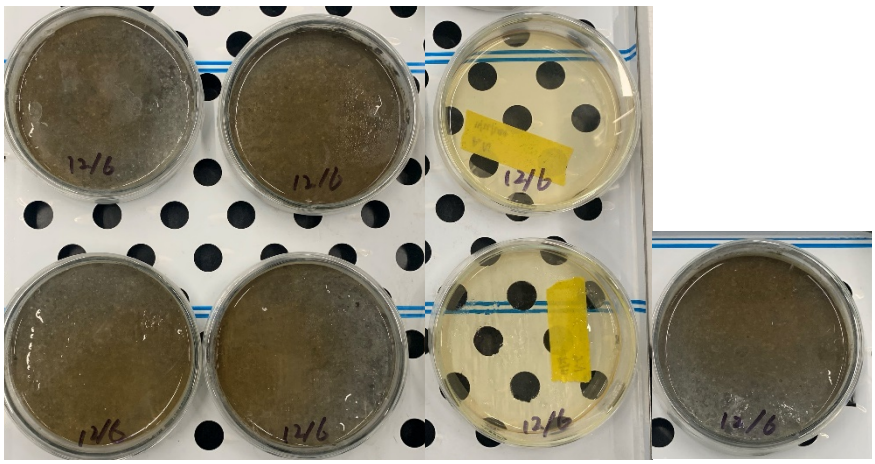

Figure 8. 12/6 imaging of fungi in petri dish.

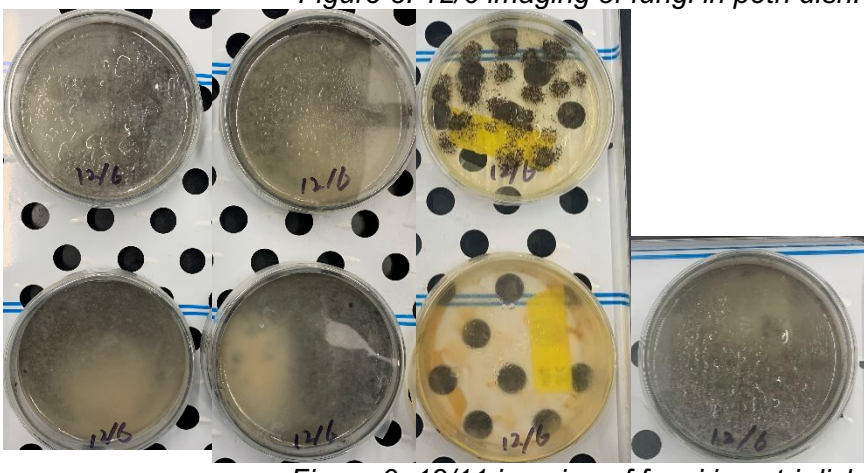

Figure 9. 12/11 imaging of fungi in petri dish.

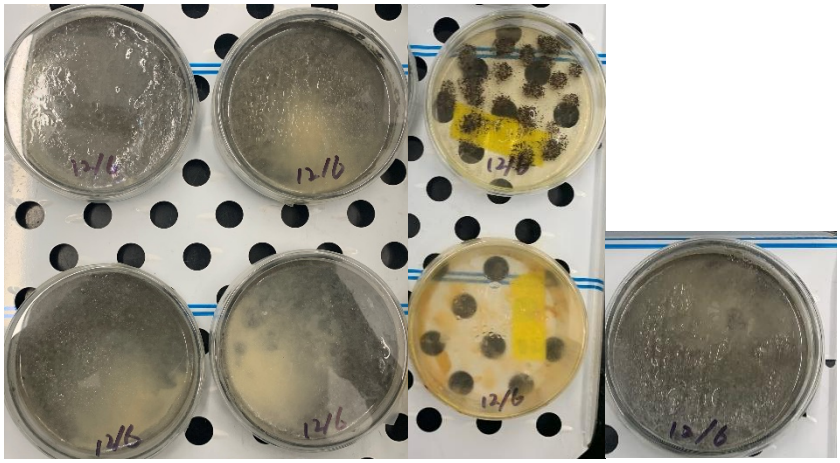

Figure 10. 12/16 imaging of fungi in petri dish.

Task 3. Healing artificial concrete cracks using the optimized fungal strain.

The research team first investigated the effect of bacteria healing ability on cement mortar samples. Then, the research team will prepare cement mortar samples for fungal healing in the next reporting period, which will be used to compare the healing ability of cement mortar samples.

We plan to prepare four sample types to evaluate self-healing capability of bacteria, including untreated samples (without fiber and bacteria), fiber samples (without bacteria), samples with bacteria (without fibers), and fiber samples with bacteria. These sample types will be used to evaluate the effects of fiber and bacteria on the strength and self-healing ability for cement mortar samples. So far, the research team has finished the untreated samples and fiber samples. The results of these samples are shown below. Next, the team will prepare the rest samples with bacteria.

1. Procedure of Concrete Mixing

The experimental design and material proportions were carefully planned to meet the requirements of the study. All materials were weighed as accurately as possible, with the error controlled within ±0.3 grams. Relevant experimental equipment for concrete mixing was prepared prior to the procedure. The cement paste was prepared using a mass ratio of ordinary Portland cement (OPC): Sand: Water = 1:1.5:0.4.

Figure 11. mix of cement mortar samples

The concrete mixing process was conducted as follows in accordance C305-06. The cement and sand were mixed at low speed for 1 minute. A spoon was used to ensure the materials at the bottom of the mixing container were fully incorporated. Water was gradually added while mixing at low speed for 0.5 minutes. The mixing speed was then increased to medium, and the materials were mixed for an additional 1 minute, with periodic use of a spoon to incorporate the bottom materials. The fiber was added, and the mixture was stirred at high speed for 1 minute. A spoon was again used to ensure complete mixing of the bottom materials. Subsequently, the mixture was further stirred at high speed for 6 minutes to achieve a uniform consistency.

2. The compressive strength test procedure follow the ASTM C39/C39M standard. Three duplicate samples were tested for days 3, 7 and 28 of curing. The results of compressive strength for untreated and fiber samples are shown in Figures 12 and 13. Figures 12 and 13 show that the compressive strength increased with curing time.

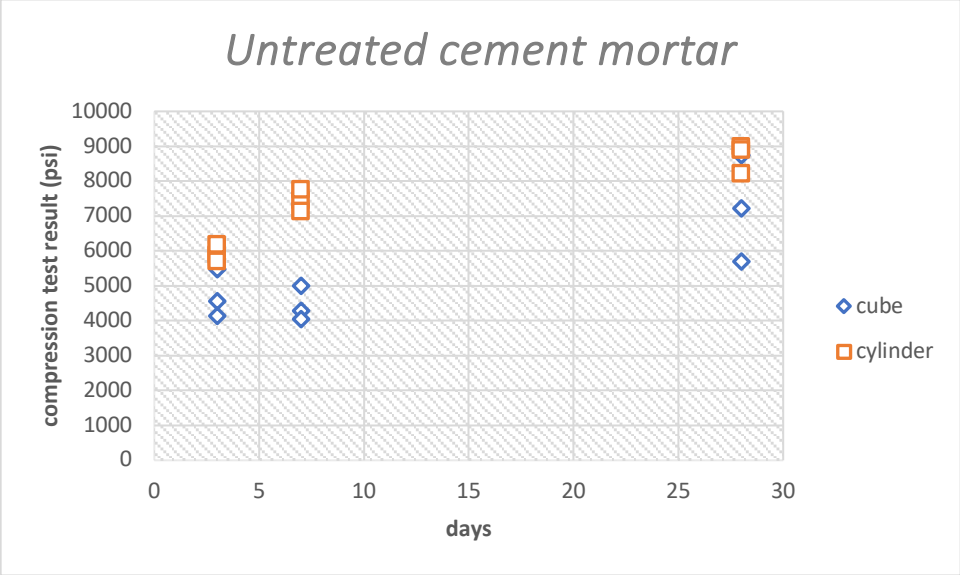

Figure 12. Compression test result of untreated cement mortar.

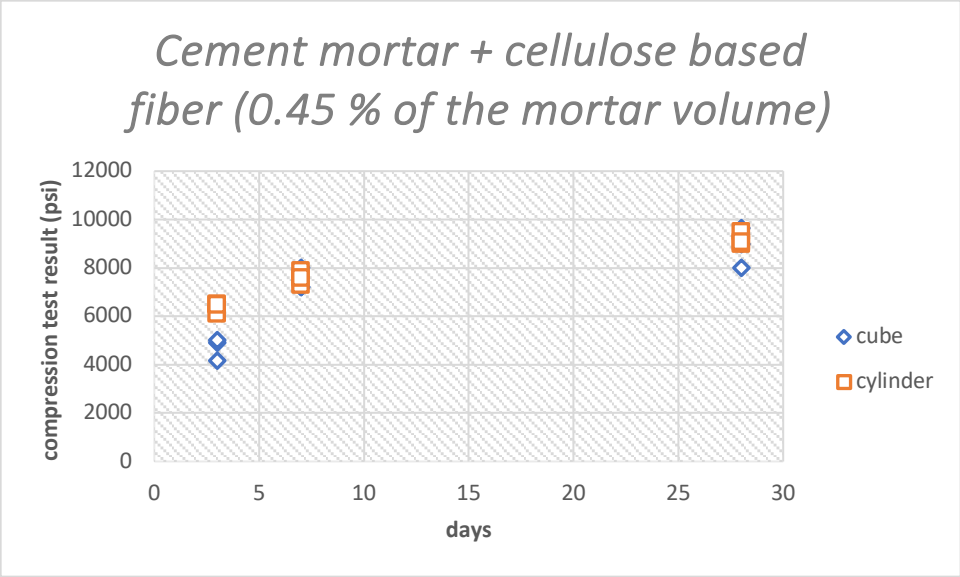

Figure 13. Compression test result of cement mortar with cellulose fiber.

Project Progress:

3. Progress for each research task
   Task 1 progress [100% completed].
   Task 2 progress [100% completed]
   Task 3 progress [30% completed]

4. Percent of research project completed
   70% of total project completed through the end of this quarter

5. Expected progress for next quarter
   Finish 90% of the tasks. In the next quarter, the research team will continue to investigate the healing capability of fungi and bacteria on cement mortar cracks. Compression tests, S-and P-wave velocity measurements, and crack size measurements will be performed to evaluate the healing performance.

6. Educational outreach and workforce development
   Planning outreach activity
   LSU ENGAGE (research demonstrations for middle school students)
   LSU college of engineering
   March 2025

7. Technology Transfer
   None

Research Contribution:

8. Papers that include TRANS-IPIC UTC in the acknowledgments section:
   Lin, H. (2025). "Harnessing Fungal Mycelia for Sustainable Soil Improvement: Opportunities and Challenges." Biogeotechnics, Under preparation.

Park, J.S., and Lin, H. (2025). "Role of Trichoderma Virens Mycelium in Enhancing Erosion Resistance of Low Plasticity Silt." Biogeotechnics, Under preparation.

9. Presentations and Posters of TRANS-IPIC funded research:
   TRANS-IPIC September Monthly Research Webinar, title: Exploring Fungal-Mediated Carbonate Precipitation for Healing Concrete Cracks, Presented by Dr. Hai Lin

10. Please list any other events or activities that highlights the work of TRANS-IPIC occurring at your university (please include any pictures or figures you may have). Similarly, please list any references to TRANS-IPIC in the news or interviews from your research.
    None

References:

None