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Applying X-ray Microtomography to Study Microbial-Induced Self-Healing of Geopolymers

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Geopolymers are one of the emerging sustainable materials for the building and construction industry. They are inorganic polymers produced from aluminosilicate waste materials such as coal fly ash and have been shown to have a lower carbon footprint as compared with Portland cement. However, geopolymers are a cementitious material like Portland cement-based concrete and are thus brittle and prone to develop cracks. The use of microorganisms has recently been considered as an approach to improve the self-healing capability of geopolymers through microbial-induced calcite precipitation. The application of X-ray microtomography to observe the potential of self-healing of bio-geopolymers is explored in this study. Bio-geopolymers were produced from the alkali activation of coal fly ash and mixed with self-healing agents using a form of biochar-immobilized spores of *B. sphaericus* and *B. thuringiensis*. These bio-geopolymers were then scanned using micro-focus XCT to obtain a visualization of the microstructure of the material via a non-destructive 3D imaging technique after self-healing. Coupled with image analysis, quantification of the segmented data allowed further investigation of self-healing through comparison with the other self-healing characterization method.

1. Introduction

Geopolymer is a greener alternative to Portland cement-based concrete. The utilization of this material involves a significantly less carbon- and energy-intensive process. Geopolymer is produce from the reaction of aluminosilicate rich source, such as waste materials, and an alkaline solution (Telesca et al., 2019).
Different precursors and mixing ratios can be used to create geopolymers, allowing them to have notable physical and chemical properties that can surpass that of regular concrete, such as high compressive or flexural strength (Burciaga-Diaz et al., 2013), low shrinkage (Chi et al., 2012), acid resistance (Kwasny et al., 2018), and fire resistance and high temperature stability (Saxena et al., 2017). However, despite the potential benefits of using geopolymers, their main drawback lies on their susceptibility to crack-formation, a problem shared by many cementitious materials due to their limited tensile strength (Van Tittelboom and De Belie, 2013).
There are several methods to remedy crack formations, with epoxy injection being one of the most widely used. However, such methods are often complex, expensive, and labour-intensive (Khaliq and Ehsan, 2016). They are also difficult to perform in hard-to-reach areas or places of continuous service. These reasons provide the incentives for the development of self-healing concrete and geopolymers.
Among the several self-healing mechanisms available for cementitious materials, bio-based self-healing has been considered preferable being potentially safer and more sustainable than chemical-based mechanisms. Bio-based self-healing involves the incorporation of a biological agent, specifically a microorganism, within the cementitious matrix (Gupta et al., 2017). The process is activated when air or water enters the cracks through biomineralization and can be classified into two categories: bio-induced or bio-controlled. In biologically induced mineralization, minerals are produced due to the metabolic activities of the microorganisms and the chemical reactions associated with the metabolic by-products, specifically their interaction with the chemical species in the substrate (Dhami et al., 2013). While more precipitates can be produced in shorter periods through biologically induced mineralization, their disadvantages include the poor crystallinity of the minerals, the inclusion of impurities in their lattice structure, and the lack of control over mineral formation (Frankel &

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Bazylinski, 2003). In biologically-controlled mineralization, the microorganisms limit crystal formation due to the direct synthesis of crystals at specific locations, yielding better crystalline structures, but the yield is not as much as bio-induced mechanisms (Dhami et al., 2013).
In this study, the application of micro-focus X-ray computed tomography (XCT) was explored to study the internal microstructure of the geopolymers after self-healing and to compare the void fractions of the healed geopolymers with their corresponding ultrasonic pulse velocities (UPV). This is a continuation of a previous study that sought to compare the healing efficiencies of the geopolymers when pure cultures and co-cultures of bacteria are used and when different concentrations of biochar as the immobilizing agent are added (Doctolero et al., 2020). In an XCT, X-rays are made to pass the material, and the series of 2D projection images produced undergo tomographic reconstruction to produce a 3D image (Landis and Keane, 2010). The image can then be analyzed using image processing software to determine the void fraction in the geopolymers due to the presence of cracks or air spaces (Promentilla and Sugiyama, 2010).

2. Methodology

The bacteria used as healing agents were *Bacillus sphaericus* BIOTECH 1272 and *Bacillus thuringiensis* BIOTECH 1092. These bacteria were obtained from the Philippine National Collection of Microorganisms in Laguna, Philippines. The geopolymer precursor employed was class F coal fly ash (FA) obtained from a coal-fired power plant in Bataan, Philippines. The alkaline activator (AA) was a mixture of Na_2SiO_3 and 12 M NaOH with a mass ratio of 2.5. The immobilizer utilized was rice-husk biochar obtained from the Philippine Rice Research Institute. The characterization of these materials can be found in the previous study of Doctolero et al. (2020).

2.1 Preparation of the healing agents

The healing agents used in the study were immobilized spores of *B. sphaericus* and *B. thuringiensis* in a biochar matrix. Pure cultures and co-cultures of these microorganisms were prepared with nutrient solutions of urea and $CaCl_2$. Detailed preparations were described in previous work (Doctolero et al., 2020).

2.2 Synthesis of the geopolymers

Geopolymers were synthesized following the ratios specified in Table 1. The materials were mechanically mixed in the following order: (1) fly ash, (2) activator, (3) healing agent and biochar mixture, and (4) nutrient solution. The mixtures were then cast into 50 mm cubic molds.

Table 1: Geopolymer mix ratios

Label	Type of Healing Agent Added	Healing Agent (mL)	Biochar (g)	Nutrient Solution (mL)	Alkaline Activator (g)	Fly Ash (g)
NT	*Distilled water only*	6.0	0.0	9.0	95.0	243.6
PL	Pure Culture Suspension	6.0	0.0	9.0	95.0	243.6
PM	Pure Culture Suspension	6.0	2.1	9.0	95.0	243.6
PH	Pure Culture Suspension	6.0	4.2	9.0	95.0	243.6
CL	Co-Culture Suspension	6.0	0.0	9.0	95.0	243.6
CM	Co-Culture Suspension	6.0	2.1	9.0	95.0	243.6
CH	Co-Culture Suspension	6.0	4.2	9.0	95.0	243.6

After 24 h, the cubes were demolded and subjected to oven curing at 60 °C for 1 day to induce more natural cracks via thermal stress as shown in Figure 1. This was followed by six days of ambient curing at an average temperature of 30 °C in the open air. Subsequently, the geopolymers were subjected to a dry-wet cycle (20 h underwater and 4 h air-drying) for 14 days and complete water immersion for the 14 days that followed. Ultrasonic pulse velocity (UPV) measurements (using 150-kHz transducers) were taken every 7 days to non-destructively measure the changes in the mechanical properties of the samples for the duration of the 28-day healing period.

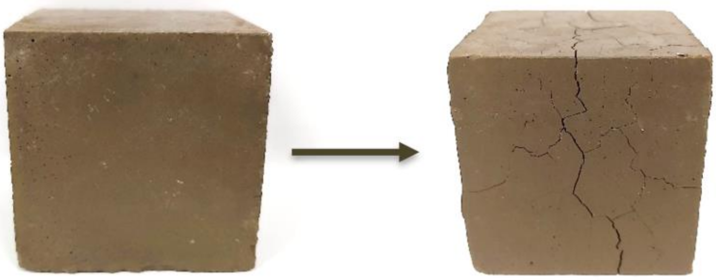

Figure 1: Thermally induced cracks in the geopolymers

2.3 XCT analysis on the healed geopolymers

At the end of the 28 day healing period, the 3D images of the healed geopolymers were obtained using a 3D X-ray Computerized Tomography Scanner (X5000, North Star Imaging, MN, USA). The XCT images were then processed using the ImageJ software to estimate the solid and void fractions of the cubes. The XCT images were exported into 1,107 slices of 8-bit grayscale images, each slice having a thickness of around 45 µm. An 820 x 820 x 820 voxels as the volume of interest (VOI) was then selected, as shown in Figure 2. The VOI was subjected to a median filter with a radius of 2.5 pixels and a bandpass filter for suppressing vertical stripes to remove visual artifacts. Otsu's thresholding was then applied to the images, resulting in a monochromatic binary image highlighting empty spaces in black as shown in Figure 3. The solid and void fractions were finally computed and compared with the UPVs of the specimens.

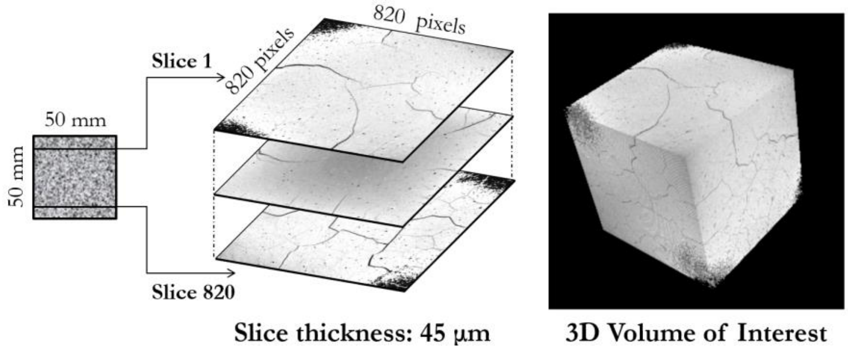

Figure 2: A reconstructed 3D image of a geopolymer cube from its VOI

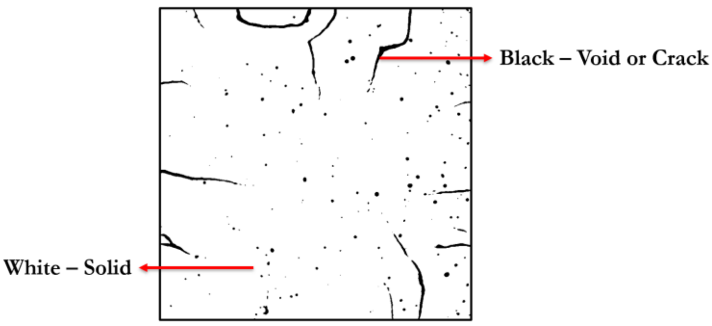

Figure 3: Binary image of a slice of a geopolymer cube

3. Results and discussion

The physical sealing of cracks in the representative geopolymers can be seen in Figure 4, where cracks ranging from 0.10 to 0.65 mm were sealed. The ultrasonic pulse velocities (UPV) of the healed geopolymers in this study were then compared with their solid fractions. Note that the current study is then limited to a proof-of-concept to detect evidence of healing through the filling of the cracks or voids present on the microstructure in a non-destructive fashion. Other macroscopic behaviors such as change of mechanical properties in terms of compressive strength in a longer time horizon for example will be the subject of future work.

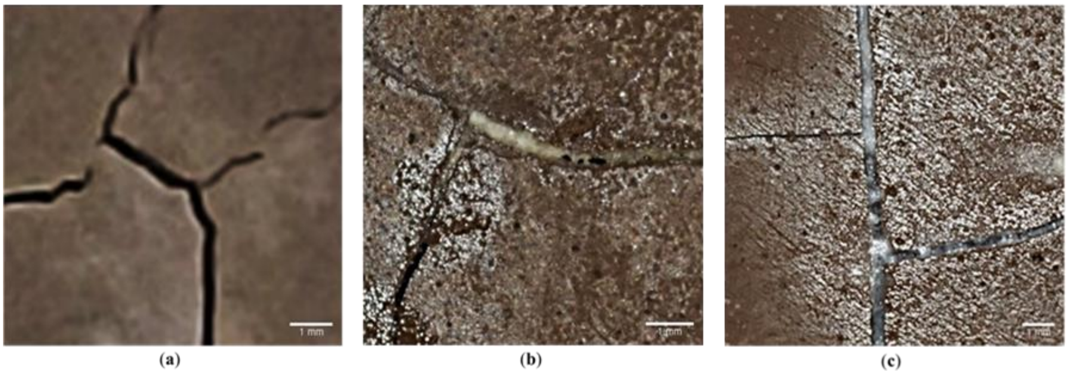

Figure 4: Crack sealing in a geopolymer after 28 healing days—(a) no healing agent; (b) using a pure culture of B. sphaericus, and (c) using co-culture of B. sphaericus and B. thuringiensis

The change in the UPVs of the geopolymers is shown in Figure 5, along with the healing efficiencies of the specimens. For the geopolymer with no added healing agents, the change is minimal as compared to those geopolymers with pure culture and co-cultures of bacteria. The increased compactness of the geopolymers, as evidenced by the change in their UPVs, is attributed to biologically induced mineralization, despite the highly alkaline environment present in the substrate. Biologically induced mineralization occurred because as air and water entered the cracks, the dormant bacteria became vegetative again. In their vegetative state, the bacteria metabolized the available nutrients, and the release of the metabolic byproducts facilitated the production of the minerals which then sealed the cracks.

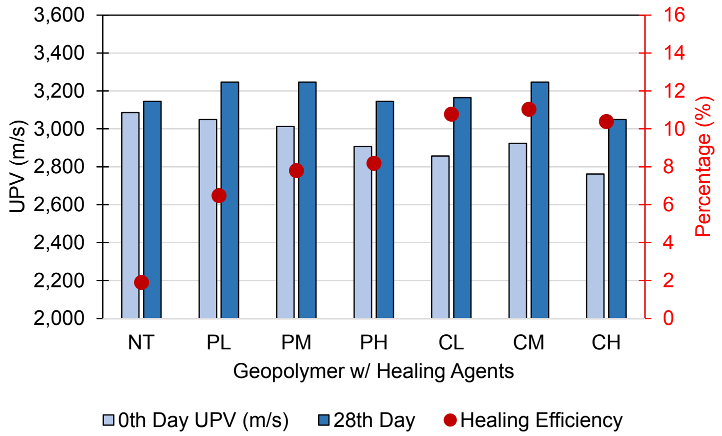

Figure 5: Initial and final UPV and healing efficiency of the geopolymers

It may be observed in Figure 5 that the use of co-cultured bacteria yields higher healing efficiencies by at least 60 % of that of pure cultured bacteria. A possible reason for this phenomenon is that in most natural environments, biofilm is formed from such microbial consortia. In a biofilm, bacteria gather on a surface and secrete molecules called extracellular polymeric substances (EPS) that form a protective array. This enables the bacteria to stabilize their local environment, resist stresses, and easily adapt to adverse conditions. Moreover, through chemical signaling called quorum sensing, they can coordinate their metabolic activities, making the community even more efficient and resilient (Ikuma et al., 2013). This also consequently makes a multispecies community harboring biomineralizing bacteria possess greater mineral precipitation capacity (Jang et al., 2020). In the present study, the use of co-cultures possibly enhanced the formation of biofilms, allowing the bacteria to better endure the very alkaline conditions in the geopolymers than a single strain alone could bear. Since *B. sphaericus* is ureolytic and *B. thuringiensis* is non-ureolytic, they could exhibit less competition in a biofilm state and cooperate in inducing the precipitation of minerals for crack repair.

The 28th day solid and void fraction estimates of the geopolymers from XCT analysis are shown in Figure 6 in order of increasing solid fraction, and a plot of the UPVs at the 28th day of the geopolymers versus their corresponding solid fractions is provided in Figure 7.

Generally, as the solid fraction increases, the UPV also increases. This observed trend indicates that the more compact material is, the higher is its expected UPV. This happens because the production of minerals reduces the air gaps in the microstructure of geopolymers. Since UPV involves measuring the speed at which ultrasonic waves travel through a material, it follows that the more compact a specimen is or the fewer voids it has, the faster the waves can travel. It is for this same line of reasoning that a higher UPV is often associated with a higher compressive strength as well. Future work will include the measurement of such mechanical properties to understand further the self-healing capability of geopolymers.

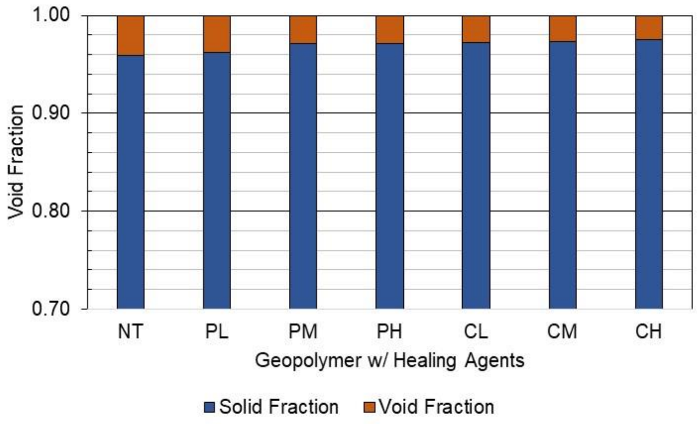

Figure 6: Solid and void fractions of the geopolymers based on image analysis

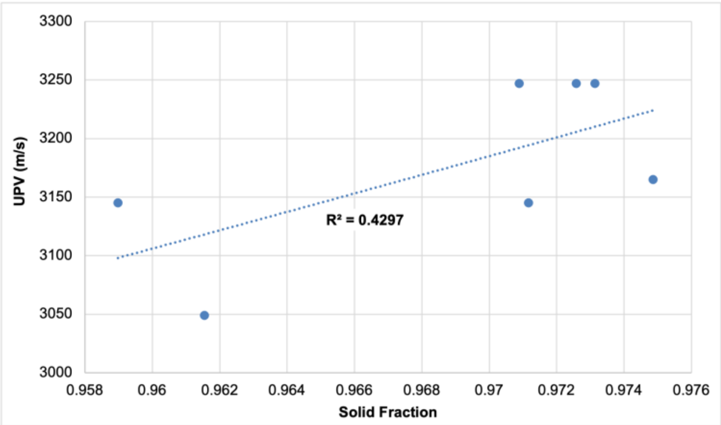

Figure 7: UPV versus the solid fraction of the geopolymers

The value of the coefficient of determination (in Figure 7) suggests that the relationship may not be linear. Noise in the images and the low resolution of the equipment are other possible contributors to the poor linear fit. It is also likely that the UPV equipment was able to capture the subtle changes in the quality of the specimens. Other considerations include the potential differences between the density of the biomineral deposited and the density of the geopolymer itself. Nonetheless, the positive relationship still indicates that the UPV increased due to the filling in of the cracks or voids in the geopolymers with the solid precipitates from XCT analysis.

4. Conclusions

This research aimed to explore the potential of using microfocus X-ray computed tomography as another non-destructive method to evaluate the self-healing of geopolymers. Preliminary results suggest a positive correlation between UPV and the measured solid fraction from XCT. This indicates that as the solid fraction or the compactness of the material increases due to crack filling, the UPV consequentially increases. Although this study only used the data for the healed specimens, it has shown that XCT can non-destructively be used to track the progress of self-healing of materials. The application of XCT analysis on a greater number of specimens coupled with an in-situ test to derive a more accurate relationship between the material's solid fraction and UPV is recommended. Further studies on the self-healing of geopolymers may also involve a qualitative analysis of crack filling that will allow easy visualization of the specimens before and after the healing process. To pave the way for the application of this product in the construction industry, larger specimens may be tested and subjected to more practical situations. Under these circumstances, the validity or extent of self-healing under laboratory conditions versus practical settings at a longer time horizon can be compared. The use of non-destructive tests to obtain such comparison would thus be greatly advantageous.

Nomenclature

AA – alkaline activator
CH – co-culture with 0.70 g/mL of biochar
CL – co-culture with 0 g/mL of biochar
CM – co-culture with 0.35 g/mL of biochar
FA – fly ash
NT – no healing agent
PH – pure culture with 0.70 g/mL of biochar
PL – pure culture with 0 g/mL of biochar
PM – pure culture with 0.35 g/mL of biochar
UPV – ultrasonic pulse velocity, m/s
VOI – volume of interest
XCT – X-ray computed tomography

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