Research Article

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Determination of CO_2 Sequestration into Bio-concrete Bricks Pores using Fungi

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ABSTRACT

This study investigates CO_2 sequestration in bio-concrete bricks using fungal species *Purpureocillium lilacinum* and *Penicillium notatum*. Fungal biomass was oven-treated at 35 °C and incorporated into a cement–sand mortar (1:3:0.4) at a biomass-to-mortar ratio of 0.6. Bricks were cast in 70 mm^3 iron moulds, cured in water for seven days, and subsequently exposed to a nutrient solution containing calcium chloride and urea to promote microbially induced calcium carbonate precipitation (MICP). After 90 days, white $CaCO_3$ deposits formed on specimens. Characterization was performed using XRD, FTIR, and compression testing. XRD confirmed phases such as calcite, C–S–H, and portlandite, while FTIR results were inconclusive. *P. lilacinum* showed improved compressive strength (10.90 N/mm^2) compared to control. The study indicates potential CO_2 sequestration in bio-concrete systems.

Keywords: Carbon sequestration; Fungus; Bio-concrete; Urease; Calcium carbonate.

1. INTRODUCTION

The environmental issues related to conventional cement manufacturing are the reason for the increased interest in sustainable bio-cement (Mistri *et al*. 2021). The traditional method of making cement, an essential part of concrete, requires calcining limestone at a high temperature, which releases a considerable quantity of carbon dioxide into the environment (Moropoulou *et al*. 2001). One of the main causes of greenhouse gas emissions and global warming is the manufacture of cement. A significant amount of the CO_2 emissions in the world are caused by the manufacture of conventional cement. The energy-intensive procedures required in the manufacturing of clinkers are the primary cause of the cement industry's 8% share of world carbon dioxide emissions (Miller *et al*. 2018). Alternative materials and technologies that might drastically lower the carbon footprint associated with cement manufacture are being investigated by industry and researchers. Biological components are incorporated into the concrete matrix to create bio-concrete or bio-cement. This can include microbes like bacteria that cause mineral precipitation, enhancing the concrete's strength and endurance. Another option is to utilize bio-based ingredients in place of some of the conventional cement (Hoffmann *et al*. 2021). Certain bio-cement methods concentrate on absorbing and making use of CO_2 as the concrete cure. This method addresses climate change issues by lowering the concrete's carbon footprint and assisting in carbon sequestration (Adesina 2020). Using waste materials or renewable resources as component parts is common in sustainable bio-cement. This strategy is in line with the circular economy's tenets, according to which waste or byproducts from one operation may be useful inputs in another. The necessity for governments and regulatory agencies to manage carbon emissions from the building industry is becoming more and more apparent (Waris and Din, 2024). As a result, sustainable building techniques are becoming more and more important, with an emphasis on using eco-friendly materials like bio-cement. Technology advancements and ongoing study in the field of bio-cement are factors in its growing popularity. This covers the development of novel materials, cutting-edge manufacturing techniques, and enhancements to concrete's general sustainability (Tayebani *et al*. 2023). The need to address the environmental effects of traditional cement manufacturing is the driving force behind the rising interest in sustainable bio-cement. To advance sustainable building practices and lessen the more general problems brought on by resource depletion and climate change, bio-cement technology research and adoption are essential.

1.1 Myco-Concrete and Its Research History

By integrating fungi into the concrete matrix, fungus-based concrete, sometimes referred to as myco-materials or concrete, offers a novel and environmentally friendly alternative to traditional building materials. Because of its potential to improve the environmental sustainability of construction techniques, this new sector has drawn attention. Fungal mycelium, or the vegetative

portion of the fungus, is commonly used as a binding agent in place of or in addition to conventional cement in the creation of fungal concrete. The mycelium of fungus is essential to the production of fungal concrete. Mycelial networks of fungi, such as those produced by species like *Trichoderma reesei* and *Pleurotus ostreatus*, are used for their adhesive properties. These fungi proliferate and bind onto organic substrates, resulting in the formation of a network that is strong and immune to damage (Van *et al*. 2021). The mycelium is cultivated on a substrate consisting of agricultural waste, such as straw, wood chips, or other materials derived from plants. When the mycelium develops, it binds the particles together, resulting in the formation of a structure that is sturdy and long-lasting. It is possible to modify this method to produce blocks, panels, or other forms that are suited for structure building. The use of fungal concrete has a few positive effects on the environment. With organic waste materials as a substrate, it eliminates the need for energy-intensive procedures that are often connected with the manufacturing of conventional cement. Furthermore, the development of fungal mycelium is a low-energy process in comparison to the fire of cement in a kiln at a high temperature, which is necessary for cement production (Manan *et al*. 2021). Furthermore, biodegradability is another characteristic of fungal concrete. It is possible for the material to be readily broken down by natural processes after it has reached the end of its life cycle. This helps to reduce the material's effect on the environment and contributes to a more circular economy (Van *et al*. 2021). In the early 2000s, the investigation of the use of fungal materials in the building industry started. Researchers began conducting experiments with a variety of fungal species and substrates to get a better understanding of the growth features of these organisms and their potential use as building materials (Bertron, 2014). Around the middle of the 2010s, there was a discernible trend towards the investigation of the structural qualities of concrete that had been caused by fungi. Researchers began investigating its potential for use in load-bearing structures and initiating the process of optimizing the growing conditions in order to achieve increased strength (Luo *et al*. 2018). Recent research focuses on the optimization of the material qualities, which include resistance to environmental conditions, durability, and strength. This requires the substrate to be refined in terms of its composition, the selection of suitable fungal strains, and the optimization of growth conditions (Tahir *et al*. 2023; Zia *et al*. 2023). Over the course of the last several years, there have been attempts made to increase the production of fungal concrete for use in commercial installations. Myco-materials have been brought into mainstream building methods via a collaborative effort comprised of academic institutes and start-up companies. Researchers in the fields of mycology, materials science, engineering, and architecture often work together to conduct research on fungal concrete. This kind of study frequently requires multidisciplinary cooperation. To make significant progress in the knowledge of fungal concrete and to create practical applications, this partnership is very necessary (Almpani *et al*. 2021). Research is now being conducted with the goal of improving the qualities of fungus concrete and expanding its usage in a variety of structural applications. fungus concrete is an exciting new frontier in the field of environmentally friendly building materials. The sector is continuously developing, with the common objective of developing ecologically acceptable alternatives to conventional building materials as the driving force behind this evolution.

1.2 Advantages of Myco-concrete

There are a few environmental benefits associated with the use of fungal bio-cement, which makes it an intriguing alternative to conventional concrete. To providing a growth medium for fungal mycelium, fungal bio-cement requires the utilization of organic substrates such as agricultural waste or forestry by-products. Fungal bio-cement helps to decrease waste by reusing waste materials, which in turn lowers dependency on resources that do not replenish themselves and adds to efforts to reduce waste. In comparison to the conventional method of producing cement, the production process of fungal bio-cement often needs a lower amount of energy generation. Consequently, it removes the need for high-temperature kiln fire, which is a significant contributor to carbon emissions in the cement manufacturing process, hence lowering the total effect on the environment (Miller *et al*. 2018).

The production of fungal bio-cement may be included into the ecosystems of the surrounding area since it is dependent on organic materials and fungal strains that are readily accessible in the area. The use of this decentralized strategy helps to minimize carbon emissions that are associated with transportation and promotes manufacturing practices that are sustainable, and community based. Fungal bio-cement is biodegradable, in contrast to ordinary concrete, which, owing to its inert nature, presents difficulties in terms of disposal and recycling because of its composition. When it reaches the end of its life cycle, fungal bio-cement may be composted with relative ease or returned to the soil, where it will naturally degrade (Adesina, 2020). By virtue of its biodegradability, fungal bio-cement reduces the environmental impact that is often associated with waste from building projects. It provides a sustainable option for construction materials, which is in line with the concepts of circular economy and the conservation of resources. When compared to the manufacture of regular cement, the production of fungal bio-cement results in a smaller, more environmentally friendly carbon footprint. The development of fungal mycelium is dependent on the temperature of the surrounding environment and does not include the high-energy processes that are linked with the

synthesis of clinker. Furthermore, during their development phase, some fungal species that are used in the creation of bio-cement have the capacity to absorb carbon dioxide from the surrounding environment. This potential for carbon capture further balances the carbon emissions that are related with building operations, which ultimately results in a decrease in greenhouse gas emissions on a statistical level. Fungal bio-cement has considerable environmental benefits in comparison to traditional concrete. These benefits include eco-friendliness, biodegradability, and a decreased carbon footprint. Fungal bio-cement is a sustainable option for building materials that contributes to efforts to mitigate climate change and promote environmental stewardship. Utilizing the natural qualities of fungus and organic waste materials, fungal bio-cement is a sustainable alternative.

2. MATERIALS AND METHODS

2.1 Cultivation of Fungi and Preparation of Culture Broth

Two fungal cultures, *Purpureocillium lilacinum* and *Penicillium notatum*, were obtained from MTCC, India. The fungi were first cultivated on YPD agar plates for 48 hours, followed by inoculation for mass culture. For each strain, 800 mL of YPD medium was prepared in conical flasks. The pH of the medium was adjusted to 7.0 as per standard guidelines. The prepared medium was sterilized by autoclaving at 121 ℃ for 15 minutes to eliminate any contamination. Following sterilization, inoculation was carried out aseptically using a sterile loop to transfer fungal cultures from Petri plates into the YPD broth. The inoculated flasks were incubated in an orbital shaker at 180 rpm and 37 ℃ for 24 hours to promote mass growth. Fungal growth and sporulation were monitored periodically using microscopic examination. In addition, growth kinetics were assessed using a spectrophotometer by measuring the optical density at 600 nm (OD_{600}). Measurements were recorded at 24-hour intervals over a period of seven days, and the obtained data were used to construct a growth curve and estimate fungal concentration.

2.2 Biomass Preparation for Introducing in Concrete Mixture

After 14 days, the fungal biomass was ready for harvesting. The culture was transferred into Falcon tubes and centrifuged at 6500 rpm for 7 minutes. After that, they were rinsed with a solution of 1X PBS and distilled water to completely remove any dirt. The biomass that was gathered was subjected to a hot air oven at a temperature of 35 ℃ for one hour to achieve a consistency that was suitable for incorporation into the mortar.

2.3 Casting of Bio-concrete

Mortar was prepared by mixing cement, sand and water in the ratio of 1:3:0.4, respectively. The harvested biomass was added to the mortar and mixed well. The ratio of biomass to mortar was 0.6. On the inside walls of the iron molds (70 mm^3) used to produce concrete bricks, a releasing agent was applied after they had been cleaned. After that, the molds were filled with the mortar, and it was left there for twenty-four hours to set up. The next day, the concrete blocks were demolded and soaked in water for a period of seven days to cure.

2.4 Characterization of Bio-concrete: Instrumentation

One day before the testing, the samples were collected from the water that was being used for curing. The compressive machine was used to load the cubical specimen on a regularly at a steady pace, and the load was recorded at the point in time when the cube completely failed. The fracture load (N) is divided by the cross-sectional area (mm^2) to get the compressive strength (MPa), which is equal to the fracture load. A Bruker ALPHA II FTIR spectrophotometer was used to record the Fourier transform infrared (FTIR) spectra of all concrete specimens were recorded over the wavenumber range of 4000–400 cm^{-1}. With the use of an PANalytical 3 kW X'pert Powder – Multifunctional equipped with CuKα radiation, X-ray diffraction (XRD) was employed to determine the presence of bio-minerals and other chemicals in concrete specimens. XRD measurements were taken at room temperature in step-scan mode using Bragg-Brentano geometry and scanning the 2θ angle from 0º to 90º to carry out qualitative analyzes of the bio concrete. With the database maintained by the International Center for Diffraction Data, the crystalline phases of each specimen were determined. After failure of the bio-concrete brick specimens in the compression testing machine (CTM), the formed cracks were clearly marked, and crack widths were measured using a scale for subsequent analysis.

2.5 Curing Media Preparation and Incubation

The goal of the curing solution generated with the help of di-hydrated calcium chloride and urea was to provide the fungal cells with the nutrients they needed to make use of carbon dioxide, carry out the MICP process, and generate $CaCO_3$ precipitates. After dipping the fractured concrete blocks in the solution, they were stored for a period of ninety days to allow them to cure. After being allowed to cure in the nutrient solution for a period of ninety days, a white precipitate was deposited over the concrete block. The FTIR analysis was used to better characterize the white ppt that had developed on the concrete. The width of the identified cracks was measured again.

3. RESULTS

3.1. UV-Vis Analysis

A growth curve was constructed by plotting absorbance against incubation time. The results showed that *Penicillium notatum* exhibited exponential growth for up to 6 days, after which it entered the decline phase. In contrast, *Purpureocillium lilacinum* showed growth up to 4 days, followed by a decline in growth thereafter.

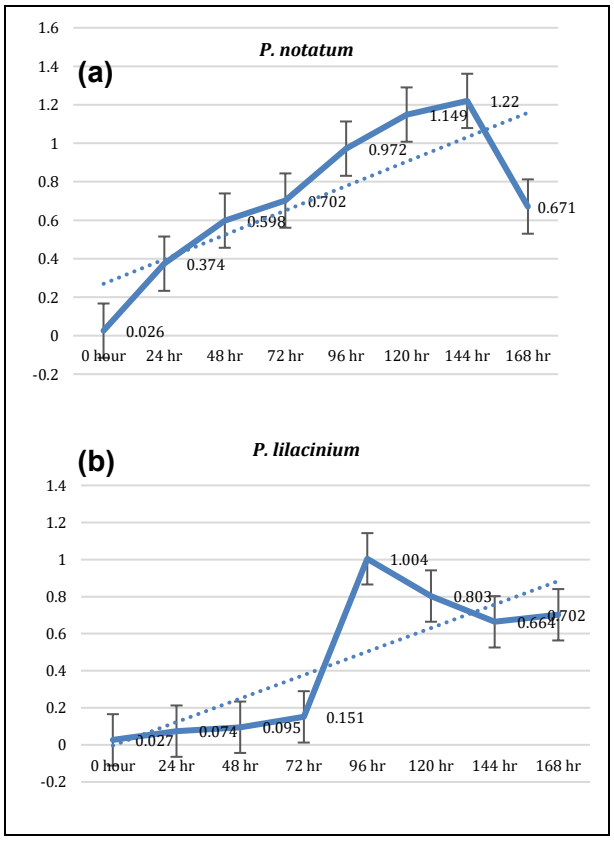


Fig. 1: The graphs depict the growth curves for a period of 7 days (a) Species: *P. notatum* (b) *P. lilacinum*

3.2 Compressive Strength Test

The values of the compressive strengths of the control and microbial specimens are listed in Table 1. These values were obtained after seven days of curing in fresh water. The findings indicate that the compressive strength of the myco-concretes of the two fungus species increased after they had been aged for a period. The enhanced binding that occurred because of the mycelial network and the calcite precipitation that was caused by the fungus is the reason for this; these minerals contributed to the filling of the pores that were present within the matrix. This was observed by many researchers such as Mondal and Ghosh, (2018) and Siddique, (2013). Despite the fact that a higher cell concentration leads to an increase in the precipitation of calcites, the optimal enhancement of compressive strength does not necessarily correspond to a high value of microbial cells. Following the occurrence of a peak value, a decrease in the strength is documented.

Table 1. Compressive strengths of the control specimen and specimen with fungal strains *P. lilacinum* and *P. notatum* after 7 days of curing

Sample Name	Total Load Obtained (kN)	Compressive Strength
Control	10	4 Nmm^{-2}
Purpureocillium lilacinum	42	10.90 Nmm^{-2}
Penicillium notatum	16	4.15 Nmm^{-2}

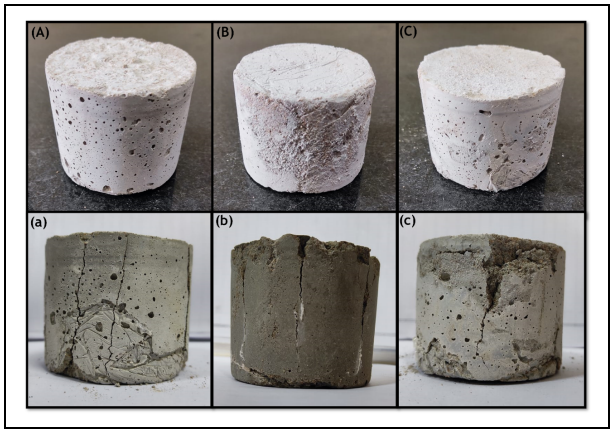


Fig. 2: Comparison between the compressive behaviors of the control, *P. lilacinum* - based bio-concrete and, *P. notatum* based bio-concrete. (A) Control concrete sample (B) *P. lilacinum* based concrete (C) *P. notatum* based concrete (a) Control concrete sample after CTM (b) *P. lilacinum* based concrete after CTM (c) *P. notatum* based concrete after CTM

The FTIR and XRD profiles of the concrete samples are shown in Fig. 3 and Fig. 4. In the 1420–1450 cm^{-1} range, bands corresponding to the bending vibrations of carbonate ions can be observed. In fungal-influenced concrete, these bands are useful for identifying the presence of calcium carbonate formation within the matrix.

However, for the prepared samples, the FTIR data is inconclusive, as all specimens exhibited similar molecular bonding features, making it difficult to distinguish fungal effects based on spectral differences alone.

In addition, when we proceeded on to XRD analysis, we were able to identify a few minerals from the diffraction patterns. These minerals included alite (C_3S), belite (C_2S), portlandite ($Ca(OH)_2$), CSH (calcium-silicate-hydrate), calcite ($CaCO_3$), and quartz (SiO_2) for both the cured concrete samples of both species. Quartz is represented by the peak at 29 degrees, which is found in graphs a, b, and c and is essentially identical to each other.

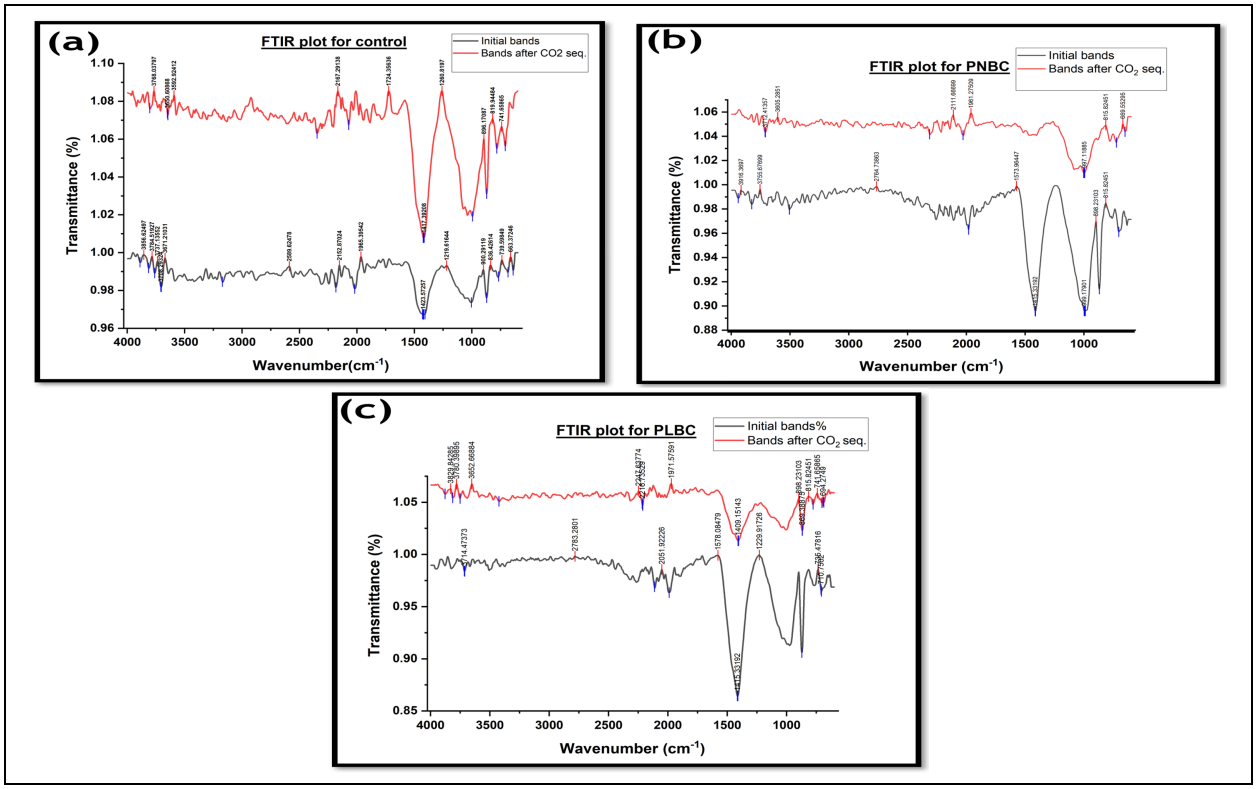


Fig. 3: Graphical data of FTIR characterization test: (a) Control sample of concrete (b) Penicillium notatum based concrete (PNBC) and (c) *Purpureocillium lilacinum* based concrete (PLBC)

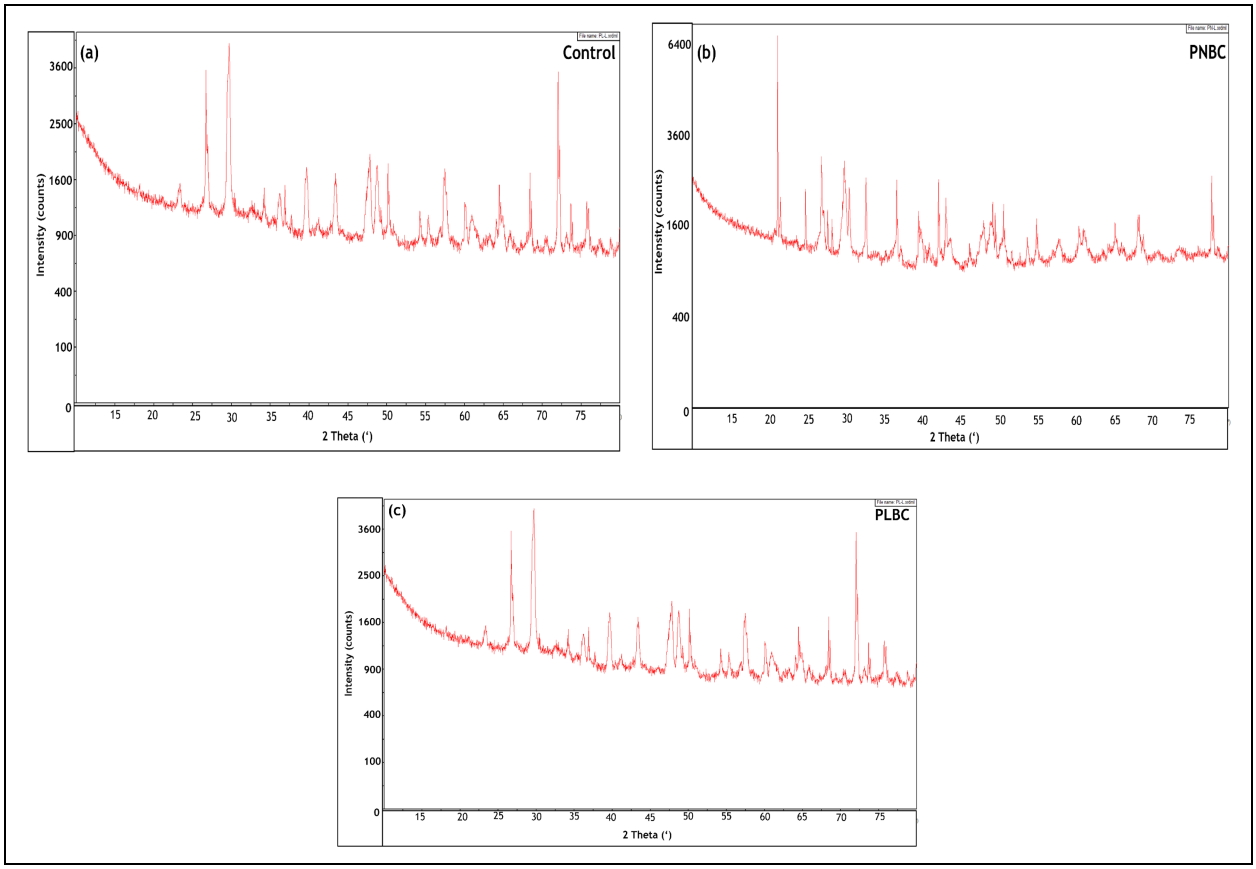


Fig. 4: Graphical data of XRD characterization test: (a) Control sample of concrete (b) PNBC and (c) PLBC

4. CONCLUSION AND FUTURE PERSPECTIVE

The present study demonstrates that the compressive strength of myco-concrete specimens incorporating two different fungal species shows improvement after a defined ageing period. This suggests a potential beneficial role of fungal activity in enhancing the mechanical performance of concrete under controlled conditions. However, an excessive level of microbial activity may adversely affect the concrete matrix, leading to a reduction in compressive strength due to possible structural disruption. The relatively modest improvement in strength can be attributed to the direct incorporation of fungal cultures into the concrete matrix without prior acclimatization or optimization. The highly alkaline environment of cementitious systems likely created unfavorable conditions for fungal proliferation, thereby limiting their effectiveness. Nevertheless, evidence of calcium carbonate formation observed after cracking and curing in a nutrient medium, as confirmed by X-ray diffraction analysis, indicates that microbially induced calcium carbonate precipitation) has occurred, contributing to carbon dioxide sequestration and $CaCO_3$ deposition.

Further studies are required to optimize fungal integration strategies, improve compatibility with the cement matrix, and perform comprehensive characterization to validate and enhance calcium carbonate formation and its long-term contribution to concrete performance.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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