

Original Article

Self-Healing and Biological CO₂ Capture in Concrete Using Microencapsulated Nostoc Sphaericum

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Abstract - An increase in atmospheric CO₂ coupled with concrete production presents dual challenges in modern civil engineering that threaten structural integrity and contribute to global warming. In the Andean country of Peru, where the climate is highly variable, these challenges are exacerbated by the lack of sustainable technologies both to (i) repair existing structures and (ii) mitigate CO₂ emissions associated with cementitious materials. This study evaluated the effectiveness of microencapsulated Nostoc sphaericum in both bio-mortar and concrete with respect to self-healing and CO₂ biofixation. A total of six mixes were designed with five levels of microencapsulated Nostoc sphaericum (1.75%, 2.00%, 2.25%, 2.50%, and 2.75%) and a control without additives, with a target strength of 210 kg/cm². The experimental program included the determination of workability, compressive strength, and indirect tensile strength, crack Control, and gravimetric CO₂ uptake under controlled light and humidity conditions. The 1.75% mixture exhibited the best overall performance, with an increase in compressive strength of 0.8% compared to the Control, crack closure of 85.3%, and CO₂ biofixation of 2,583 mg CO₂/cm³ (4.05 g per test tube). The increase in dosage after 1.75% was recognised a limitation because of biopolymer saturation, also due to light diffusion. The work shows how a nutritious microencapsulated cushuro is designed to generate living, self-healing, and carbon-negative concrete without the addition of external nutrients. Such an innovative biotechnological output represents a disruptive and sustainable option for the Peruvian civil construction sector, by generating higher-performance materials with a reduced environmental impact.

Keywords - Biological Co₂ Capture, Biocementitious Concretes, Calcium Carbonate, Nostoc Sphaericum, Self-Repairing Concretes.

1. Introduction

The most consumed material is concrete, which accounts for more than 10 billion tonnes to be produced each year, and it has an annual growth rate of approx 5.68% [1, 2]. Several environmental forces selectively attack the concrete material matrix. These conditions cause cracking and, therefore, carbonation, which hinders the durability and service life of the cementitious material itself. The processes then decreased structural performance [3, 4]. Cracking in Buildings, Roads, and Hydraulic Structures is an actual problem that occurs in Peru. Variations in temperature and humidity can make surface cracking in concrete buildings a common issue due to the climate [5]. Nevertheless, a global environmental threat from increasing levels of Carbon dioxide (CO₂) seems to be mounting. CO₂ is the second most important gas and a key factor in global climate imbalance [6], as there is an increasing concern over global warming. However, the last decade was characterized by an elevated atmospheric CO₂ level mainly due to the expansion of urbanization, industrialization, and agriculture; current CO₂ values are 420 ppm [7, 8]. The

degradation scenario mandates mitigation processes that combine emission reduction and carbon capture technology, along with an active place of deposition in a process called sequestration. Such processes use concrete, a widely used construction material, as a substrate for the biological capturing of gases [9]. The degradation of concrete has serious structural and environmental effects; it can lead to initial cracking or full structural failure. It has been explicitly acknowledged that the mechanisms of deterioration not only reduce concrete stiffness, increase permeability, and decrease strength but also endanger the safety and durability of structures, resulting in a high economic cost to repair existing concrete structures [10, 11]. The global crack repair mortars market was valued at USD 3.1 billion in 2024, with an annual growth rate of 7% [12]. While the market availability of these materials is on the rise, they possess serious drawbacks: little to no adhesion to wet surfaces, delayed shrinkage causing microcracks to propagate from the original repair site, and restoration of the mechanical properties of concrete that was poorly with almost never leash out [13, 14]. Some other



common specialty materials like epoxy resins, polyurethanes, and acrylics have a number of disadvantages, such as short-term performance, high rigidity or brittleness, and fast aging, which lead to reduced repair service life [15]. Hence, it signifies a call for the development of intrinsic materials that can self-regulate in response to damage, including self-healing and integrated biotechnological CO₂ capture mechanisms [16]. Microalgae and cyanobacteria were recently tested, with the promise that these biological materials improve biogenic precipitation of CaCO₃ to facilitate crack sealing in concrete [17, 18]; however, these are on a lab scale with little field testing.

These studies are inevitably limited by their reliance on exogenous nutrients and a controlled microenvironment [19]. Existing studies and self-healing approaches have lacked a self-sustaining microbial system embedded within repair materials, capable of actively promoting crack self-healing through biogenic CO₂ fixation and biological processes in cementitious systems, without the need for external nutrient supply. Alternatively, *Cushuro*, or *Nostoc sphaericum*, is an indigenous biological resource with the ability to withstand highly variable extreme conditions and perform photosynthesis [20]. These unique properties offer the potential to utilize *Cushuro* in sustainable cementitious systems, enabling its implementation in Peruvian regions where such biological resources are readily available.

Based on the authors' investigation, no studies have reported the use of *Nostoc sphaericum* microencapsulated in gelatin–alginate matrices in relation to its potential application both as a self-healing agent and as a biological CO₂ sequestration system when incorporated as a surface repair mortar and as an integral component in concrete. Therefore, this study aims to evaluate the potential of microencapsulated *Nostoc sphaericum* (*Cushuro*) in gelatin–alginate matrices, both in bio-based mortars and in concrete, to activate self-healing and biological CO₂ fixation under controlled laboratory conditions.

2. Literature Review

2.1. Background on the use of Microalgae and Cyanobacteria in Concrete Self-Healing and Biological CO₂ Capture

Biologically derived self-healing cementitious materials are capable of sealing cracks and densifying their microstructure due to the microbial precipitation of Calcium Carbonate (CaCO₃). In microalgae and cyanobacteria, this mechanism can be attributed to the photosynthetic fixation of CO₂ and its subsequent mineralization within the cementitious matrix. Recent years have demonstrated the development of microalgae and cyanobacteria as agents for self-healing and biological fixation of Carbon dioxide (CO₂), enhancing both sustainability and the passive self-healing capacity of cementitious materials. Some of these species are presented in Figure 1. In (a), through optical micrographs, the cellular

morphology of the cyanobacterium *Synechocystis* sp. PCC 6803 and the diatom *Fragilaria* sp. CCAP1029 were isolated and cultured in Egypt for use in concrete mixtures and the evaluation of mechanical, durability, and self-healing properties of concrete. *Synechocystis* increased compressive strength by 21.66%, while *Fragilaria* achieved an increase of 22.33% under the same conditions. Furthermore, SEM–EDX test results confirmed the formation of Calcite (CaCO₃) within the pores of the concrete, which was responsible for crack healing within 7 to 14 days [17]. Item (b) presents micrographs of cement mortars produced in Malaysia, where both microalgae—the blue-green microalga *Synechococcus elongatus* and *Spirulina platensis*—were cultivated in controlled laboratory ponds to evaluate their self-healing capacity.

The progressive mineralization of cracks and galling, resulting in the deposition of calcareous coating on the concrete surface, can be noticed. In an experimental study, *S. platensis* added mixtures reached a compressive strength of 72% compared with the Control, while those with *S. elongatus* only achieved 36%. A residual strength recovery of about 35% was also found after biological curing, along with a significant reduction in crack width within 14 days [18]. Item (c), in contrast, relates to UK micrographs obtained via a literature watch and experimental studies for the purpose of enhancing the potential use of fast-growing cyanobacteria as a biotechnological system for CO₂ capture and fixation. The photos depict species of *Synechococcus elongatus* UTEX 2973, PCC 11801, PCC 11901, and *Cyanobacterium aponinum* UTEX 3222 grown under controlled settings.

These strains showed homogeneous cultures, with rounded structures and smooth surfaces, and delivered a 1.83 kg of CO₂ conversion rate per kg of biomass, which is new from an industrial biorefinery in regard to their Carbon Capture, Utilization, and Storage (CCUS) benefits [19]. Last to item d, which gives us microscopic images from Greece, which is based on a microbial consortium with the cyanobacterium *Leptolyngbya* sp. Considered for capturing atmospheric CO₂, filaments of this genus are elongated and entangled, being embedded in a gelatinous extracellular matrix rich in exopolysaccharides. The agglomerating consortium showed efficiencies of CO₂ capture up to 94% under low organic load (250 mg·L⁻¹) for the reduction of dissolved COD to regulatory levels within a 2–4 days period.

The study found that consortia dominated by *Leptolyngbya* sp. can survive this CO₂ sequestration and organic pollutants removal while manufacturing some biotechnologically relevant biomass. [21]. Collectively, these studies demonstrate that microalgae and cyanobacteria enhance cementitious materials through bioinduced CaCO₃ precipitation, enabling crack sealing and CO₂ fixation. However, most approaches rely on non-native strains and controlled conditions, which limit their practical applicability.

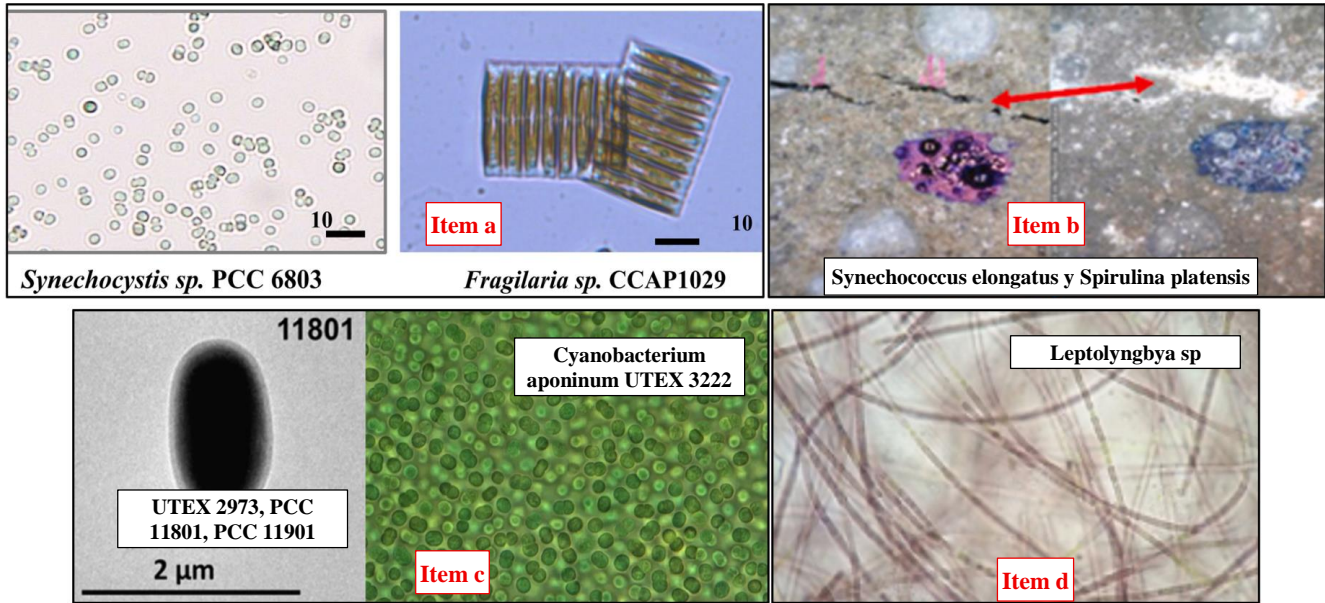


Fig. 1 Representative species used as additives in concrete

2.2. Recent Research on Nostoc Sphaericum

In this regard, specific attention is paid to *Nostoc sphaericum* (cushuro) for its biochemical properties and resistance against negative environments, as well as the self-healing ability of cementitious materials via biological CO₂ fixation—proving beneficial for long-term applications. Its impact on concrete performance, carbon capture, polysaccharide functionality, and environmental biotechnological application propels the recent interest in a valuation of its potential use as a bioactive agent for sustainable construction up to date.

The compressive strength of concrete using *cushuro* (*Nostoc sphaericum*) as a natural additive was investigated in this study by researchers at the Department of Civil Engineering, Universidad Privada del Norte, Peru. To this end, 36 cylindrical specimens (15 × 30 cm) were fabricated with three experimental dosages of *cushuro* (2.25%, 3.34%, and 4.46% by weight of cement) and one reference mix without an additive. The *cushuro* was pre-processed, went through washing and blending before being added during the process of mixing concrete according to ACI 211 guidelines.

In addition to compressive strength tests, aggregate density, moisture content, and absorption tests were conducted in accordance with ASTM standards. The standard concrete mix achieved an average compressive strength of 225.06 kg/cm² at 28 days. The mixes containing *cushuro* exhibited reductions in compressive strength of 6.4%, 9.7%, and 19.7%, respectively. It was concluded that the optimal dosage was 2.25%, as it provided compressive strength values close to the Control while improving workability. Higher dosages were detrimental to the cohesion and hydration of the concrete [22].

Researchers from Tongji University in China, specializing in materials engineering, drew inspiration from natural lichens and conducted studies on indigenous self-healing concrete through microbial symbiosis. A symbiotic combination of filamentous fungi (*Trichoderma reesei*), acting as biological repair agents in concrete, and diazotrophic cyanobacteria (*Nostoc punctiforme* and *Anabaena inaequalis*) was developed. The fungi attract Ca²⁺ ions, promoting the precipitation of Calcium Carbonate (CaCO₃) that seals microcracks, while the cyanobacteria fix atmospheric CO₂ and N₂, generating organic compounds that nourish the fungi and facilitate crack sealing. The system exists, relying on light, air, and safe minerals without external nutrients or organic identity, by establishing a self-contained ecosystem. Results demonstrated that the engineered concrete ecosystem functions like a living repair material via CaCO₃ formation in an experimental symbiotic system of *Anabaena* and *Nostoc*. This study opened new perspectives in biotechnology for the development of living materials capable of evolving toward carbon and CO₂ reduction, thereby promoting sustainability in ecological construction engineering through active regeneration and resilient reuse [23]. Likewise, researchers from the School of Food and Biological Engineering conducted research to evaluate the capacity of microalgae of the *Nostoc* genus to absorb Carbon dioxide (CO₂) and their potential as an ecological substitute for synthetic urea in agriculture. To do this, they cultivated nitrogen-fixing strains of *Nostoc* sp. in tubular photobioreactors under controlled conditions of light, temperature (25 °C), and CO₂ concentration of 0.3% in order to experimentally measure the rate of carbon sequestration. Two application methods were compared: a slow-release fertilizer (S2), which incorporated the algal biomass directly into the soil, and another based on

biogas digestate (S3), obtained by anaerobic digestion of the biomass to release available nutrients. The results showed that *Nostoc* microalgae achieved a capture rate of 2030.4 g CO₂/kg of dry biomass, and that replacing one ton of synthetic urea with these alternatives reduced the global warming potential from 5.4 to 2.3 t CO₂-eq in S2, achieving a net capture of -1.1 t CO₂-eq in S3 when using the biogas generated as an energy source. The study showed that microalgae of the *Nostoc* genus have high biotechnological potential for mitigating climate change, as they act simultaneously as nitrogen fixers and natural CO₂ absorbers, establishing themselves as a sustainable alternative to conventional chemical fertilizers [24].

Researchers from the Functional Foods Research Group at the University of Lima, in collaboration with the University of Leeds (United Kingdom) and the University of Murcia (Spain), conducted the extraction and characterization of polysaccharides from *Nostoc sphaericum* (cushuro) to evaluate its potential as a natural hydrocolloid agent. Cushuro samples from Cotaparaco (Recuay, Ancash) were subjected to acid (PCAcE) and aqueous (PCAqE) extraction methods assisted by ultrasound and microwave techniques. FTIR, HPAEC-PAD, and SEC-MALS-RI-UV/Vis were used to analyze their composition and properties. From them, the comparison result indicated acid extraction resulted in higher performance (49%), greater purity (83.3%), enhanced antioxidant activity (73%), and higher WHC (3.0 g/g), also displayed “weak gel” rheological behavior with a branched structure able to retain moisture from a stable network. Such information indicates that *Nostoc sphaericum* has a water-retaining polysaccharide matrix with high resistance to drying, suggesting its application as a way of reducing microcracking caused by drying in cementitious materials like concrete and promoting self-healing mechanisms inherent to the system, widely reported elsewhere [25].

Bio-remediation potential of hydrocarbon-contaminated soils from the Lurin district was studied using cyanobacteria *Nostoc sphaericum* (cushuro). The State, October 2023. In this sense, soil samples with different degrees of contamination were collected, and the microorganism was tested in a fractionated way. The study took readings on selected physicochemical soil parameters, namely pH, temperature, moisture content, electrical conductivity, and Total Petroleum Hydrocarbon (TPH) concentration. Methodology- It was a laboratory-based study where the *Nostoc sphaericum* biomass was inoculated in the samples and studied over 45 days of duration to check the efficiency of the process. The results presented up to 94.55% TPH reduction, also improving the quality of soil that showed *Nostoc sphaericum* capacity for adsorbing and degrading organic pollutants. These results justify the fact that *Nostoc sphaericum* exhibits high values of biological tolerance and resistance, and even acts under adverse conditions by absorbing and converting environmental compounds, making it an interesting biotechnological agent

for environmental recovery processes on a sustainable basis in civil engineering applications [26].

Researchers from the Institute of Plant Biochemistry and Photosynthesis of the Spanish National Research Council (CSIC) and the University of Seville, Spain, investigated how the Cyanobacterium *Nostoc* sp. post-transcriptionally regulates Carbon dioxide (CO₂) fixation in response to nitrogen-deficiency stress. In order to accomplish this, they were able to grow strains of *Nostoc* sp. PCC 7120 in the presence and absence of nitrogen, and analyzed the expression of a small regulatory RNA (sRNA, NsiR4). Methods: The NsiR4 RNA and the mRNAs of the genes *glpX* and *pgk*, which are responsible for encoding enzymes sedoheptulose-1,7-bisphosphatase/fructose-1,6-bisphosphatase (SBPase) enzyme and Phosphoglycerate Kinase (PGK), respectively, that play a central role in the Calvin cycle, were quantified through genetic/molecular assays, as well as recombinant plasmids expressed in *Escherichia coli* cells to confirm their interaction. The results illustrated that overexpressing NsiR4 lowered the levels of both enzymes and the photosynthetic activity of CO₂ fixation, verifying its function as a repressor in carbon assimilation. This research showed that *Nostoc* sp. has a very well-developed and flexible biological apparatus, indicative of a tightly-regulated metabolism that connects the carbon fixation with nitrogen availability to maintain its vigorous metabolic activity. Additionally, it proves its ability to be used for natural solutions for CO₂ capture technologies and environmental applications [27].

Within this context, although self-healing and Biological CO₂ Capture through the use of microalgae and cyanobacteria in cementitious materials has been previously studied with great advances, there are very few experimental studies being conducted that utilize native species in Peru. Earlier works have concentrated on non-native strains like *Synechocystis* sp., *Synechococcus elongatus*, and *Spirulina platensis*, grown under controlled conditions, fed by external inputs, therefore limiting their real-world application. Further, the majority of studies on CaCO₃ bioprecipitation are primarily qualitative in nature and do not establish clear quantitative relationships among relevant biological and functional factors such as microbial concentration, CO₂ fixation efficiency, and the mechanical performance of concrete. This gap highlights the opportunity to assess *Nostoc sphaericum*, an abundant Andean cyanobacterium in biotechnological applications for sustained CO₂ fixation and self-repair of cracks in cementitious matrices. To this end, the current study experimentally assesses the potential of microencapsulated *Nostoc sphaericum* embedded into gelatin-alginate matrices used within repair mortars and directly mixed with concrete for mechanical performance, self-healing, and CO₂ capture.

3. Materials and Methods

In this Section, we present the materials and experimental methods used to assess their performance in self-healing and

biological CO₂ fixation for biocement-based concretes by means of microencapsulated *Nostoc sphaericum* (cushuro). Fresh biomass was harvested in Huancayo, pretreated, and cultured before being microencapsulated by complex coacervation with gelatin and sodium alginate to allow the microorganism to be viable in the basic environment of the concrete. The mixtures for the preparation were with cushuro dosages ratio to cement weight (1.75%, 2.00%, 2.25%, 2.50% and 2.75%) and finally, one more mixture without additives as Control corresponding to a compressive strength of 210 kg/cm². This section describes the materials and methods used to assess microencapsulated *Nostoc sphaericum* (cushuro)- and then that a biocement-based concretes showed self-healing and biological CO₂ fixation. In Huancayo, fresh biomass was harvested, pretreated, cultured, and microencapsulated by complex coacervation using gelatin and sodium alginate to preserve vigor in the alkaline medium characteristic of concrete. Concrete mixtures containing cushuro dosages of 1.75%, 2.00%, 2.25%, 2.50%, and 2.75% (where % is respective to the cement weight with a control mixture free from additives) were prepared aiming to obtain compressive strength of 210 kg/cm². The specimens were cured in controlled conditions and tested for slump, temperature, compressive strength, indirect tensile strength, crack monitoring, and gravimetric CO₂ absorption. This study was focused on assessing the effect of microencapsulated cushuro addition on mechanical performance, self-healing ability, and biogenic carbon capture potential of the material.

3.1. Concrete

In civil engineering, concrete is a composite material that consists mainly of a binder, fine and coarse aggregates, and water; it can withstand both compressive and flexural stresses as the combination of the three produces a mass that constitutes most present-day structures [28, 29]. For this

study, the concrete mix was designed for compressive strength of 210 kg/cm² at 28 days according to National Building Regulations (NBR) Standard E.060 “Reinforced Concrete” [30]. Dosages are shown in Table 1.

Table 1. Concrete mix design F'c = 210 kg/cm²

F'c (kg/cm ²)	210
a/c	0.45
Added size (inches)	½”
Volume dosing	1:2:2
Cement (bags)	9.73
Coarse sand (m ³)	0.52
Crushed stone (m ³)	0.53
Water (m ³)	0.186

3.1.1. Cracks in Concrete

Cracks are one major sign of the structural degradation of concrete as cracks start and develop progressively under mechanical loads, thermal changes, or environmental effects, impairing rigidity, durability, and load-bearing capacity [31]. Figure 2 shows the main types of cracks that occur in concrete. Item (a) represents cracks due to plastic or surface shrinkage, with a typical opening of less than 0.05 mm, caused by rapid evaporation of water before setting. Item (b) corresponds to cracks due to shrinkage from drying of hardened concrete (0.05 to 0.20 mm), caused by moisture loss and movement restriction. Item (c) shows thermal cracks (0.20 to 0.30 mm), caused by temperature gradients between the core and the surface of the element. Item (d) illustrates cracks due to steel corrosion (0.30 to 0.50 mm), caused by the expansion of iron oxides in the reinforcement. Finally, item (e) shows structural cracks (> 0.50 mm), resulting from bending, shear, or compression stresses that exceed the strength capacity of the element [11, 32].

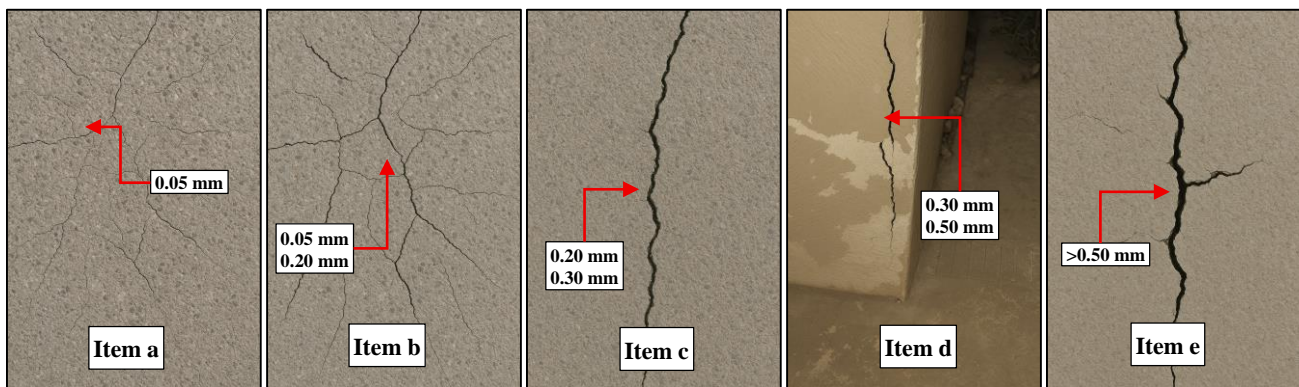


Fig. 2 Classification of cracks

3.2. *Nostoc sphaericum* NS

Cushuro (*Nostoc sphaericum*) is a blue-green cyanobacterium that forms dark green gelatinous spheres, commonly found in high Andean lagoons in Peru above 3000 meters above sea level. It is considered an autotrophic photosynthetic organism, capable of fixing atmospheric

nitrogen and synthesizing organic compounds of high biological value [33]. Various studies describe it as a natural source of functional proteins, minerals, and essential amino acids, with antioxidant, nutritional, and bioactive properties. In addition, its composition includes polysaccharides and bioactive peptides that give it techno-functional

characteristics such as solubility, foaming capacity, and water retention, allowing its application in the food and biotechnology industries [25, 34].

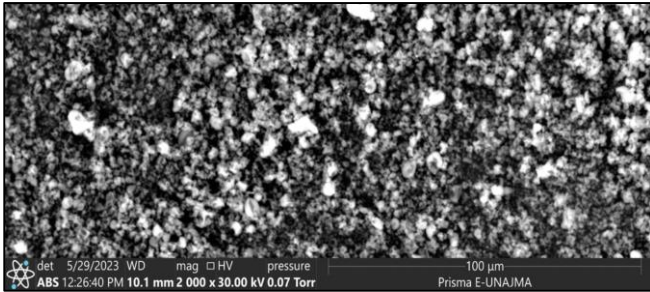


Fig. 3 SEM analysis of nostoc Sphaericum [35]

Table 2 summarizes the main physicochemical and functional properties of Nostoc sphaericum (cushuro). It indicates that the protein content of cushuro (37%), essential amino acids, and digestibility levels of over 80% are all due to its relative metabolic activity. It also has very high levels of calcium (1357 mg/100 g) and magnesium (126 mg/100 g), which are necessary for the creation of CaCO₃. As a polysaccharide, it contains carboxyl and sulfated groups with the potential to serve as nucleation sites that induce carbonate bioprecipitation. These characteristics, together with its photosynthetic and CO₂ fixing capacity, confirm the potential of Nostoc sphaericum as a biotechnological microorganism suitable for carbon capture and mineralization in cementitious matrices.

Table 2. Physicochemical and functional properties of Nostoc Sphaericum

Property	Value	Functional Implication
Total protein content	37 g/100g	Urease and carbonic anhydrase enzymes to catalyze CO ₂ hydrolysis and form CO ₃ ²⁻ ions [34].
Calcium (Ca)	1357 mg/100g	Direct source of Ca ²⁺ for the formation of biogenic CaCO ₃ during induced precipitation [34].
Magnesium (Mg)	126 mg/100g	Promotes carbonate nucleation [34].
Iron (Fe)	26.2 mg/100g	Contributes to ionic balance in bioprecipitation [34].
Total carbohydrates	57.9 g/100 g	Organic carbon source that consumes CO ₂ and generates CaCO ₃ as a byproduct [34].
Total polysaccharides	49%	It acts as a nucleation center for CaCO ₃ and adsorbs dissolved CO ₂ [25, 36].
Antioxidant capacity (DPPH)	73% (PCAcE)	Promotes the conversion of CO ₂ into carbonates through oxidation-reduction processes [25].
Water Holding Capacity (WHC)	3.0 g/g	Increases the viscosity of the medium and prolongs the residence time of dissolved CO ₂ [36].
Essential amino acids (EAA/NEAA)	0.85	High efficiency in carbon fixation and biomass generation [34].
Photosynthetic pigments	Chlorophyll-a, phycocyanin, and carotenoids	Capture solar energy for the reduction of CO ₂ to organic carbon; facilitate photobiological mineralization processes [34].

3.2.1. Obtaining Nostoc Sphaericum

Nostoc sphaericum (cushuro) was obtained in the vicinity of the “Modelo” market in Huancayo, an area where this natural product is widely sold, as shown in Figure 4.

During acquisition, it was verified that the material retained its characteristic gelatinous structure, with no signs of decomposition or visible contamination.

It was then transported in sealed polyethylene containers, avoiding direct sunlight and maintaining a controlled ambient temperature (20 °C), in order to preserve the integrity of the natural polysaccharides present in its matrix, which are essential for its future application as an additive in concrete.



Fig. 4 Acquisition of nostoc sphaericum

3.2.2. Preparation of Cushuro for Addition to Concrete

The procedure for adding cushuro (*Nostoc sphaericum*) to concrete was based on previous research on the use of microorganisms and cyanobacteria encapsulated in biopolymer matrices to improve the durability and self-repairing properties of cementitious materials [24, 37, 38].

Pre-treatment and Washing of Cushuro

Figure 5 shows the pre-treatment and washing of *Nostoc sphaericum* (cushuro), a process in which the material was subjected to controlled washing in order to remove surface impurities, plant debris, and mineral particles prior to cultivation and experimental incorporation. In item a, 100 g of fresh *Nostoc sphaericum* was weighed, and 300 ml of distilled water was prepared, maintaining a ratio of 1 part biomass to 3 parts water (weight: volume). In item b, the cushuro was rinsed three times with distilled water at room temperature (25 °C), for 5 minutes per cycle with gentle manual agitation to preserve the integrity of the colonies. Step c - The excess water was removed by decantation and filtration through a 0.5 mm mesh, which allowed the removal of fine particles without breaking its mucilaginous structure. Finally, (d) the washed *Nostoc sphaericum* was placed in sterile glass bottles and stored at 4 °C for 24 h until liquefaction and homogenization.

Cultivation and Processing of Cushuro

Figure 6 presents the cultivation and conditioning of

Nostoc sphaericum, which is performed to provide adjustable metabolic activity and viability of biomass before being added to the cementitious material [24]. In item a, in order to provide a homogeneous source of some essential nutrients necessary for cyanobacterial growth, the inorganic mineral medium was prepared by dissolving per litre (of distilled water) 0.02 g Sodium Carbonate (Na_2CO_3), 0.04 g calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 0.04 g dipotassium phosphate (K_2HPO_4), and 0.08 g magnesium sulphate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$). In item b, the medium was dissolved completely and ready to use. pH was then checked and adjusted to 7.8 (item c) for good growth conditions. For example, for item d, *Nostoc sphaericum* was inoculated at 10% of the volume of the medium (100 g biomass/L) to evenly distribute it in suspension. Cultures in item e were kept at 25 °C with constant light of $120 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Philips Master LED 6500 K) for a period of ten days. Natural aeration was facilitated with partially uncovered containers, ensuring the fixation of atmospheric CO_2 and N_2 . The biomass at the end of the cultivation period was greenish, indicating high metabolic activity. Finally, in f, the biomass was emulsified at 1:3 (weight: volume) of cushuro: distilled water in a professional blender; the process took 2 minutes at medium speed. Finally, in item g, a viscous and homogeneous suspension called *Nostoc sphaericum* solution was achieved used for the subsequent stage of protective microencapsulation.



Fig. 5 Pre-treatment and washing of the cushuro

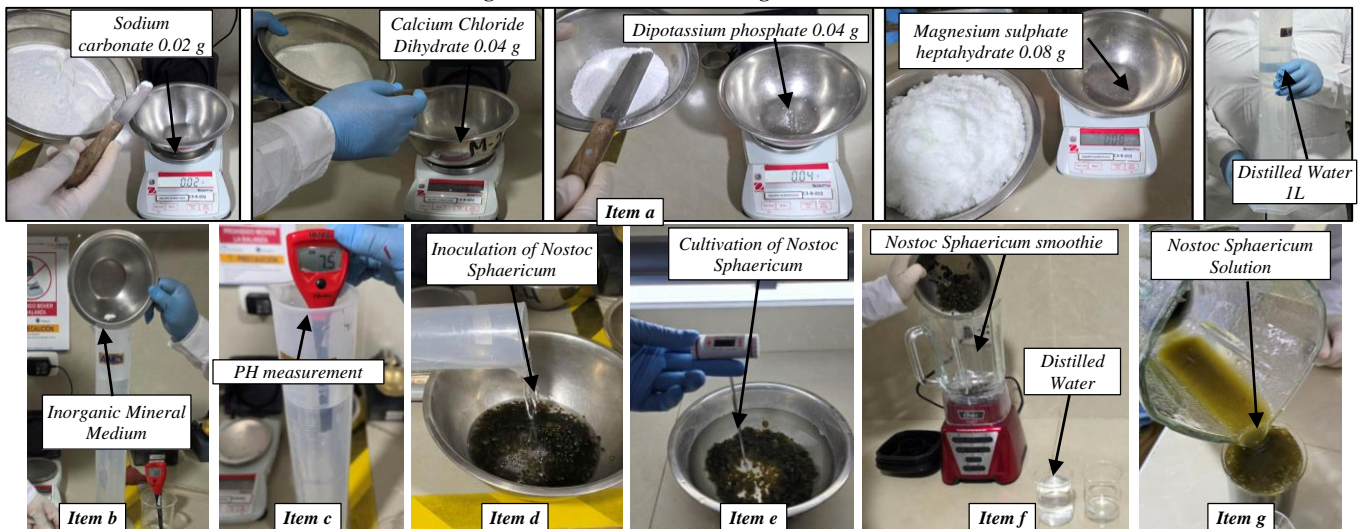


Fig. 6 Cultivation and processing of cushuro

Microencapsulation of Cushuro

Nostoc sphaericum was microencapsulated through the complex coacervation method [39] to produce a biopolymeric membrane that encapsulates the cyanobacterial cells, which should improve their stability in the alkaline and high-temperature environment of concrete. To this end, unflavoured gelatine was used as a positively charged polymer, and Sodium alginate (NaAlg), given its anionic nature, was employed due to its excellent biocompatibility and its ability to form a stable three-dimensional structure. The microencapsulation process of *Nostoc sphaericum* is designed to protect the active biomass within a mixed polymer matrix of gelatine and sodium alginate (Figure 7). In part a, two polymer solutions were prepared: a solution of unflavoured gelatine by mixing 6.75 g in 120 mL of Distilled water (dW) at 50 °C, and a solution of sodium alginate by mixing 2.25 g in 100 mL under the same thermal conditions. Both were stirred continuously for 15 minutes at 300 rpm, maintaining a homogeneous and stable mixture with a gelatin: alginate ratio of 3:1 before making polyelectrolyte complexes. For item b, the prepared solutions were maintained in a water bath until they completely dissolved into biopolymers at a constant temperature. Next, item c) was prepared by adding fresh *Nostoc sphaericum* solution (60 mL from the previous culture) to a hot gelatin solution at a 1:4(v/v) ratio of biomass and

polymer mixture. Ultraturrax is used to homogenise the resultant emulsion at 12,000 rpm for 3 minutes to achieve uniform dispersion of microbial colonies into the protein resin. In item d, the sodium alginate solution was slowly added to the gelatin-cushuro emulsion at 50 °C with stirring at 180 rpm, in order to initiate biopolymer interactions together. Following this, in item e), the pH was controlled to 4.0 by sequential addition of 0.1 M diluted acetic acid and measured as reported in f) with a digital potentiometer. This modification gave rise alternately to the implementation of complex coacervation due to the electrostatic attraction between protonated ammonium groups of gelatin with carboxylate groups from alginate, creating a stable Three-dimensional (3D) network that confined the *Nostoc sphaericum* cells. Finally, in item g, the mixture was gradually cooled to 10 °C under continuous stirring for 30 minutes, and then stored at 4 °C for 12 hours in darkness to consolidate the microcapsules formed. The wet capsules were separated by decantation, washed with sterile distilled water to remove acid residues and salts, and then frozen at -80 °C for 24 hours. The material was finally freeze-dried at -40 °C for 72 hours, yielding dry, stable, spherical microcapsules with a slightly rough surface and an average size between 100 and 400 µm, retaining the characteristic green colour of the encapsulated cushuro.

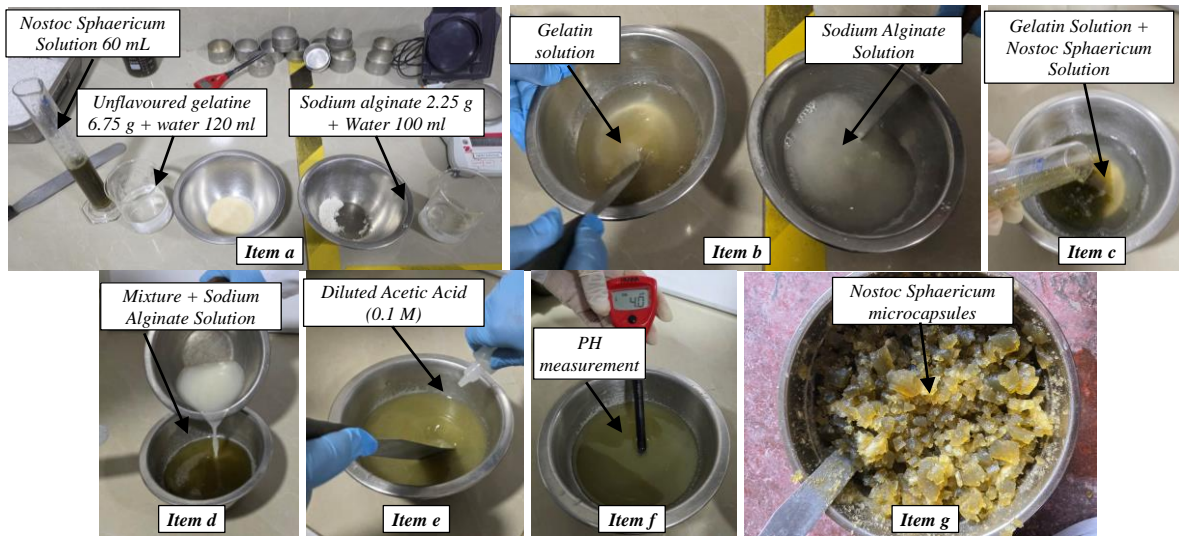


Fig. 7 Microencapsulation of *nostoc sphaericum*

Incorporation of Microencapsulated Nostoc Sphaericum into Concrete

For the addition of cushuro (*Nostoc sphaericum*) to concrete, a study conducted in Peru was used as a reference, which identified that a dosage of 2.25% relative to the weight of the cement maintains compressive strength close to the standard and adequate workability [22]. In this study, five experimental dosages of 1.75%, 2.00%, 2.25%, 2.50%, and 2.75% relative to the weight of cement were evaluated, as shown in Table 3. To evaluate the effectiveness of microencapsulated *Nostoc sphaericum* (cushuro) in the self-

repair of concrete, two different experimental methods were applied. In the first, called surface application, the microencapsulated cushuro was applied directly to the cracked area of hardened test specimens in the form of mortar with a dosage of 1:4, ensuring the infiltration of the biological material into the crack by capillarity. The purpose of this modality was to analyze the biogenic activation and precipitation capacity of Calcium Carbonate (CaCO₃) when the microencapsulated system comes into contact with air, moisture, and light. In the second modality, called incorporated application, the cushuro microcapsules were

added during the mixing stage of the fresh concrete, allowing for their homogeneous distribution in the mass before pouring. This procedure sought to evaluate the resistance of the microencapsulated system to the alkaline and thermal environment of setting, as well as its viability for subsequent activation in the event of microcrack formation during service, and, finally, its CO₂ absorption capacity.

Table 3. Quantity for concrete mix

Percentage (%)	Quantity for mortar (kg)	Quantity for concrete (kg)
1.75%	11.156	7.237
2.00%	12.750	8.271
2.25%	14.344	9.304
2.50%	15.938	10.338
2.75%	17.531	11.372

3.3. Fresh Concrete Tests

3.3.1. Slump and Temperature

The slump test is a standardized test used to determine the consistency and workability of fresh concrete. The slump value and flow diameter reflect the degree of fluidity of the mixture. This test is governed by the international standard ASTM C143 [40, 41]. On the other hand, the concrete temperature test is a test designed to record the thermal evolution of the material during its hydration and/or carbonation processes, allowing the heat released by internal chemical reactions to be evaluated. It is regulated by the ASTM C1074 standard [42, 43]. Figure 8 shows the procedure performed for each of them.

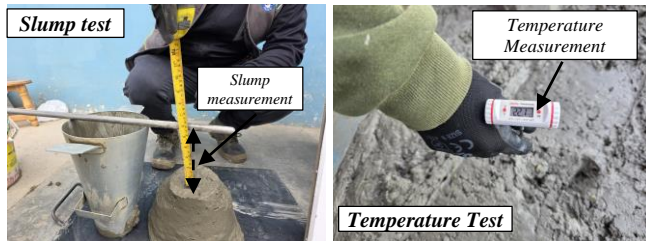


Fig. 8 Slump and concrete temperature

3.4. Hardened Concrete Tests

3.4.1. Compressive Strength and Indirect Tensile Strength Test

The concrete compressive strength test is a fundamental test for evaluating the material's ability to withstand axial loads without failing. It is the main indicator of its quality, durability, and structural behaviour, and its procedure is standardized in ASTM C39 [44, 45].

Likewise, the indirect tensile strength test for concrete is a widely used method for evaluating the material's ability to resist tensile stresses. Given its brittle nature and low ductility, its execution is internationally standardized by ASTM C496 [46, 47]. Figure 9 shows the procedure performed for both tests.

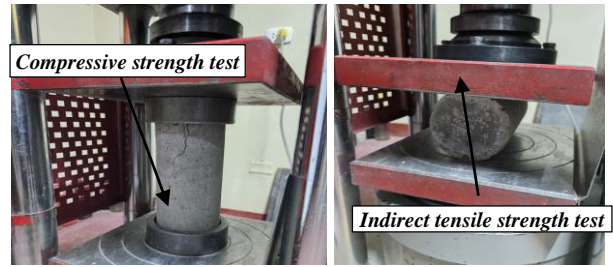


Fig. 9 Indirect compression and tensile strength test

3.4.2. Crack Control

The crack control procedure was based on a methodology aimed at reproducing controlled conditions to evaluate the biological process of crack sealing in concrete. This methodology involved both inducing cracks in hardened test specimens and preparing specimens with self-repairing agents incorporated during mixing [48]. Two types of samples were used in this research. In the first case, hardened concrete test tubes available in the laboratory, measuring 100 mm in diameter and 200 mm in height, were selected. Controlled cracks were induced in these tubes by applying a mechanical load equivalent to 60% of the design strength (f'c). A biological mortar with *Nostoc sphaericum* (cushuro) microcapsules was then applied to these specimens in order to analyze their surface sealing capacity, as shown in Figure 10, item a. In the second case, new, fresh concrete test specimens with a diameter of 100 mm and a height of 200 mm were prepared, incorporating the microcapsules directly into the mixture. These were cured for 28 days and then cracked under the same conditions as shown in item b, with the aim of comparing the self-repairing performance between the two application approaches.

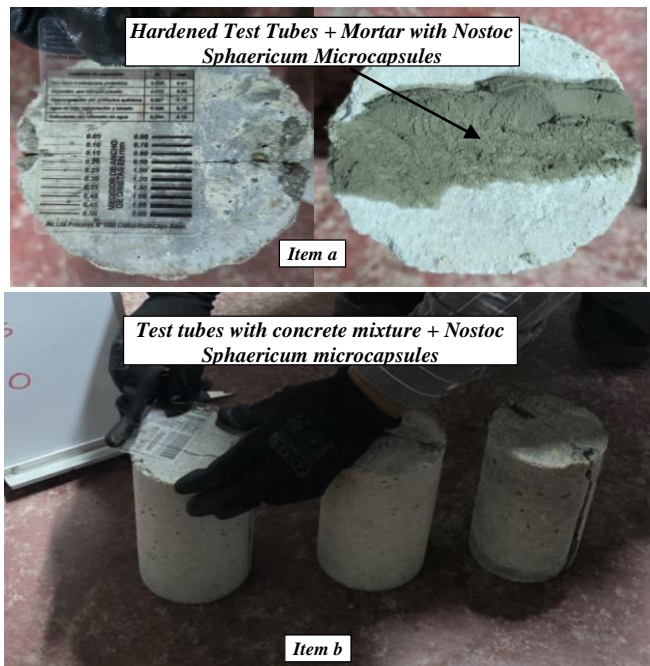


Fig. 10 Crack control

3.4.3. Biological Activation of Microencapsulated Cushuro

The activation of microencapsulated *Nostoc sphaericum* (cushuro) is based on its photosynthetic and autotrophic capacity, which allows it to resume its metabolism upon contact with light, air, and environmental humidity. Once the microcapsules are rehydrated, the cyanobacterial cells reactivate enzymes such as urease and carbonic anhydrase, catalyzing the transformation of dissolved CO₂ into Carbonate ions (CO₃²⁻). These ions react with the Calcium (Ca²⁺) available in the cementitious matrix, leading to the biogenic precipitation of Calcium Carbonate (CaCO₃). The result is the formation of a dense mineral film that seals microcracks and partially restores the impermeability of the concrete [23].

In the cracked test tubes treated with biological mortar, the system was activated immediately upon exposure of the surfaces to light emitted by a 50 W cool white LED lamp, adjusted to provide a light intensity of 5000 lux and maintain

a relative humidity above 60%, as shown in Figure 11. These conditions encouraged photosynthesis of the cyanobacterium (*Nostoc sphaericum*) and nucleation of Calcite (CaCO₃) crystals within these cracks [49]. On the other hand, in the test tubes made with cushuro incorporated into the mixture, the activation of the system was delayed, manifesting itself after the 28-day curing period, when the formation of microcracks allowed air and water to enter the concrete. This exposure rehydrated the latent microcapsules, stimulating the biological fixation of CO₂ and the progressive precipitation of CaCO₃ within the porous system. In both modalities, the biogenic self-repair process remained active during the weeks following cracking, provided that the aforementioned lighting and humidity conditions were maintained. Light intensity was verified using a Benetech GT1011 digital lux meter, while relative environmental humidity was controlled with an RD MT18 digital hygrometer.



Fig. 11 Biological activation of microencapsulated cushuro

3.4.4. CO₂ Absorption Control

The quantification of Carbon dioxide (CO₂) fixation in the test tubes was carried out using an indirect gravimetric-volumetric method shown in Figure 12, adapted for biocementitious materials with internal incorporation of *Nostoc sphaericum* (cushuro), in order to avoid thermal alteration of the biological system [50]. In item a, the test tubes cured for 30 days in a controlled environment were superficially scraped, extracting approximately 2 g of material from the areas with the highest deposition of visible crystals, mainly in the cracks sealed by bioprecipitation. In item b, the

collected samples were weighed using an analytical balance with a precision of ±0.0001 g to ensure consistent measurements. Subsequently, in item c, the samples were subjected to controlled drying at 50 °C for 24 hours in a convection oven in order to remove surface moisture without denaturing the polysaccharides present in the biocement matrix. For part d, use 0.1 N HCl for both the acid solution and in the reaction vessel. Referring to item e, the dry sample was cautiously introduced and stirred in the acid under a fume hood, thus beginning CO₂ that is expelled from bioprecipitated CaCO₃. In item f, the mixture was stirred magnetically to

ensure complete reaction of $\text{CaCO}_3 + 2\text{HCl} \rightarrow \text{CaCl}_2 + \text{H}_2\text{O} + \text{CO}_2\uparrow$. For item g, the excess of HCl was back-titrated using 0.1N NaOH and phenolphthalein (indicator) as a breakpoint identifier. Lastly, a titrated solution with a constant pink colour (h) was obtained, and the volumes of sample and blank NaOH were recorded for calculation purposes to determine the acid that carbonates consumed. Using the stoichiometric ratio presented in equation 1, the equivalent mass of fixed CO_2 was defined as follows:

$$m_{\text{CO}_2} = 0.44 \times m_{\text{CaCO}_3}$$

Here, the factor 0.44 is related to the mass fraction of CO_2 in CaCO_3 . The results were expressed as the mean of $\text{mg CO}_2/\text{cm}^3$ normalised with respect to the test tube volume, each one being compared to control test tubes (test tubes without bioburden).



Fig. 12 CO2 absorption control procedure

4. Results

4.1. Slump and Temperature

As seen from the slump values (Table 4), these were found to progressively decrease as levels of various microencapsulated cushuro (CM) added increased. The standard recorded a slump of 6 inches, while the mixtures with 1.75%, 2.00%, 2.25%, 2.50%, and 2.75% reached values of 5 3/4, 5.5, 5 1/4, 4 3/4, and 4 1/4 inches, respectively. The decrease indicated a progressive modification of fluidity caused by the increase in system viscosity induced by polysaccharides through the effect exerted on their matrix, Nostoc sphaericum. Alginates and exopolysaccharides worked as biopolymer compounds, these compounds acted by functioning as natural thickening agents generating higher water retention capacity, the result of that was to decrease the mobility of fresh concrete. In contrast, with the increase of biological content, the temperature of concrete exhibited a small bump from 22 °C in the baseline to 23.6 °C in the CM 2.75% mixture. This behaviour was explained through the exothermic effect caused by the initial cement hydration reactions and, additionally, possible residual metabolic activity of the encapsulated cushuro that may have promoted a modest acceleration in the

setting process. In summary, results demonstrated the maintainability of suitable consistency with moderate dosages (2.25% and below), whereas higher concentrations affected workability because of a general rise in the viscosity within the system.

Table 2. Slump and temperature

Description	Slump (inches)	Temperature (°C)
Control	6	22
CM 1.75%	5 3/4	22.1
CM 2.00%	5.5	22.5
CM 2.25%	5 1/4	22.8
CM 2.50%	4 3/4	23.4
CM 2.75%	4 1/4	23.6

The slump of the mixtures with cushuro and the control mixture is reflected in Figure 13, which shows a decrease between 4.2% (CM 1.75%) and 29.2% (CM 2.75%), compared to that of the standard, while confirming a downward trend in workability as microencapsulated biomass

increased within these CC composites cementitious paste specimens. The temperature increased from 0.5% for CM 1.75% to 7.3% for CM 2.75%, indicating a direct correlation of biological content and heat of hydration, as expected, in contrast to non-biological-based cement paste. In general, the CM 2.25% mixture provided a good balance of fluidity and temperature, thereby being regarded as the most stable dosage requiring workability without greatly affecting the thermal behaviour of concrete.

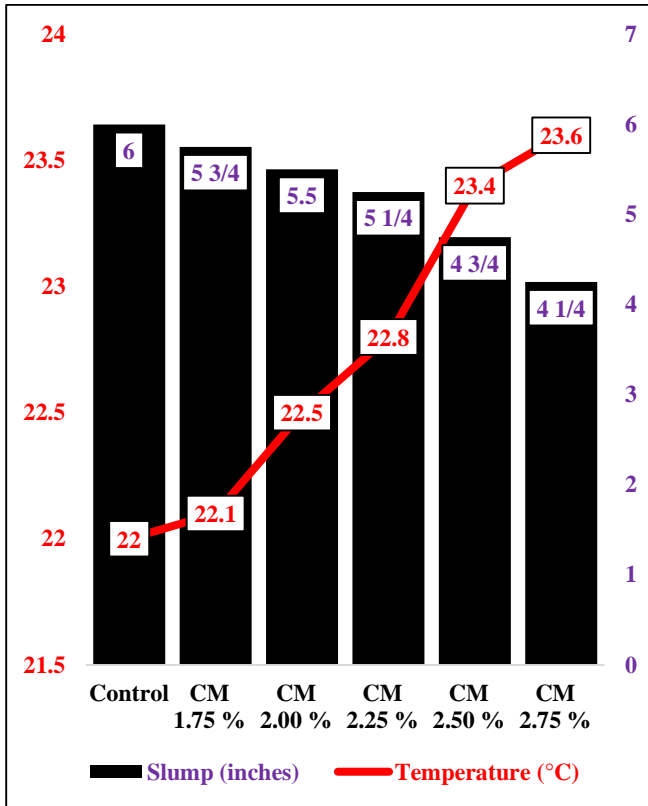


Fig. 13 Slump and temperature

4.2. Compressive Strength Test

As supported by Table 5, all mixtures increased their compressive strength gradually with time at each curing age, reaching their peak value at 28 days. The control mixture achieved compressive strengths of 134.56, 175.56, and 219.51 kg/cm² at the ages of 7, 14, and 28 days, respectively. Compared to this behaviour of biphasic mixtures for the composition containing microencapsulated cushuro (CM), it exhibited different behaviour depending on the dosage.

Despite just outshining the standard mix, the CM 1.75% blend gave the greatest qualities at all ages (136.21, 178.41, and 221.35 kg/cm²), though higher dosages hinted at step-by-step diminishes. The 1.75% improvement found for our composite plant set reflects the inhibition of viable cyanobacterial biomass. Polysaccharide matrix probably helped it in improving internal water retention, to increase cement hydration time, and provide a denser microstructure.

Furthermore, the interaction between Ca²⁺ ions released during hydration with carboxyl groups from Nostoc sphaericum polysaccharide molecules likely also facilitated Calcium Carbonate (CaCO₃) nucleation and matrix densification. In contrast, a cumulative loss of strength was observed during the 2.25%.

This behaviour might be associated with the additional regions of biopolymer and bound water that potentially generated lower cohesion regions or microvoids in the cementitious matrix. It also indicates that the microcapsules disturbed the integrity of the mineral skeleton by partially obstructing the formation of C-S-H gels in hydration. In summary, the results showed that moderate dosages of cushuro content (around 1.75%) provide an optimum between moisture retention and densification of their structure, while higher dosages reduced strength due to excess organic components.

Table 3. Compressive strength

Description	Strength at 7 days (kg/cm ²)	Strength at 14 days (kg/cm ²)	Strength at 28 days (kg/cm ²)
Control	134.56	175.56	219.51
CM 1.75%	136.21	178.41	221.35
CM 2.00%	133.25	173.94	217.97
CM 2.25%	132.98	172.32	214.11
CM 2.50%	131.29	167.51	212.46
CM 2.75%	128.44	161.96	209.33

As illustrated in Figure 14, all mixtures showed an increasing strength trend upon curing progress, but that was with visible appreciable differences from the standard mix. Our results demonstrated a marginal increase in CM 1.75% at 7 days (1.2%), compared with a reduction seen from the addition of CM concentrations beyond 1.75%, with the following decreases: CM 2.00% (-0.97%), CM 2.25% (-1.17%), and CM 2.50% (-4.02%). After 14 days, the same pattern was observed: mixtures containing 1.75% CM increased by 1.6%, whilst the other mixtures decreased steadily to reach 7.2% (mixtures containing 2.75% CM), as shown in Figure 14.

The 1.75% CM again yielded the best results at 28 days, with an increase greater than that of the standard mixture (0.8%), whilst other concrete mixtures recorded decreases ranging from 0.7% (2.00% CM) to 4.4% (2.80% CM). The results confirmed, under these conditions, that the optimal dosage of microencapsulated Nostoc sphaericum was 1.75% by weight of the cement, in that, at this level, the residual biological activity and the polymeric structure favoured a more stable and less porous matrix.

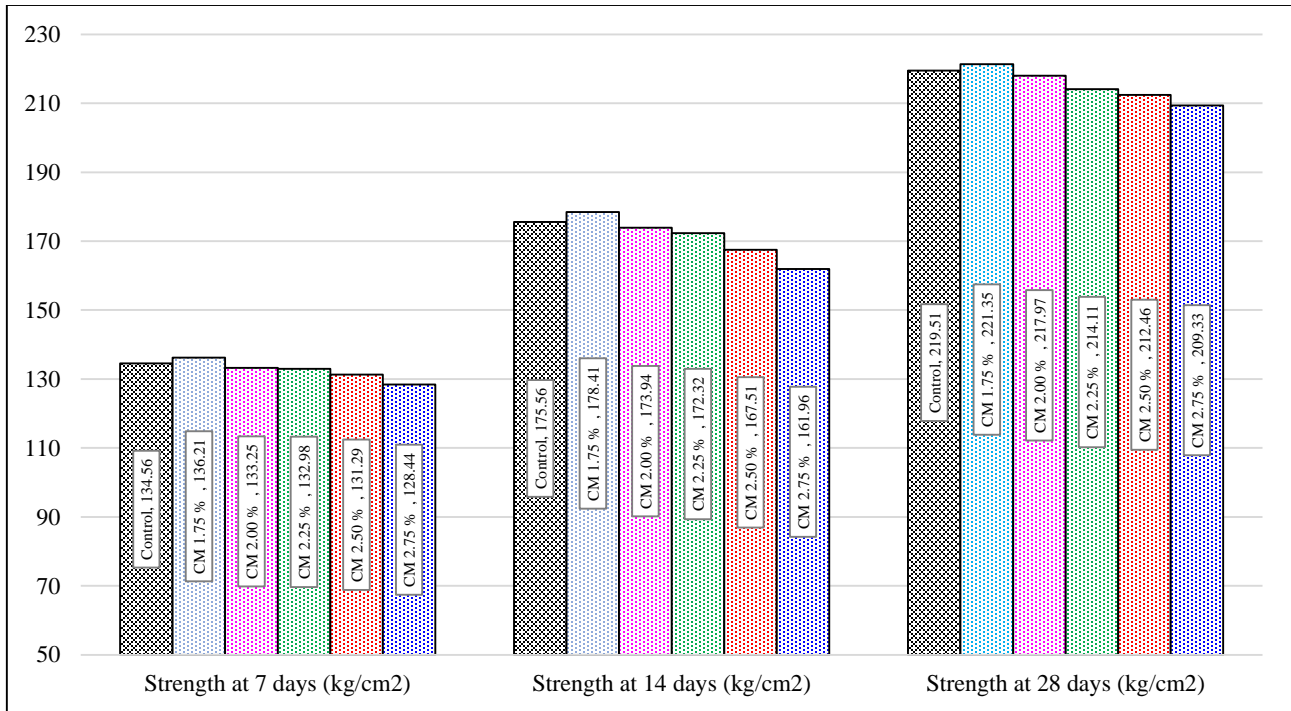


Fig. 14 Compressive strength test

4.3. Indirect Tensile Strength Test

As seen in Table 6, all the mixtures showed a continuous increase in indirect tensile strength with time over the test period, and the CM 1.75% mixture achieved consistently higher values as compared to others at all ages of tests carried out. This combination, at 7 days, yielded 16.02 kg/cm², just above that of the control mix (15.56 kg/cm²), whereas the rest of the mixtures exhibited a steady decline as microencapsulated cushuro was added. The mix achieved a tensile strength of 24.86 kg/cm² by 28 days, while CM 1.75% reached an impressive figure of 25.38 kg/cm², suggesting that a small increase in the performance of the biocementitious concrete was obtained. This behaviour may be due to the positive impact of polysaccharides from *Nostoc sphaericum* on interfacial cohesiveness of cementitious matrix, which in turn is possibly contributing to forming an intact bond between paste and fine aggregates, effectively resulting in the proper resistance against cracking under tensile stress.

Microencapsulation also aided cell viability and, at low to moderate concentrations, calcification of CaCO₃ within pores and cross-links that coincided with improved transfer of post-operative stresses on a small scale. However, as the dosage increased to more than 2.25%, the tensile strength decreased continuously, probably due to excessive organic content and moisture trapping, which possibly created raw density zones that lowered the load-bearing behavior of the material. In summary, results suggest that approximately 1.75% cushuro at an optimal content provides a good interplay between biological activity, internal structure cohesion, and densification.

Table 6. Indirect tensile strength

Description	Strength at 7 days (kg/cm²)	Strength at 14 days (kg/cm²)	Strength at 28 days (kg/cm²)
Control	15.56	19.71	24.86
CM 1.75%	16.02	20.13	25.38
CM 2.00%	15.04	19.04	23.94
CM 2.25%	14.63	18.54	23.31
CM 2.50%	14.25	18.12	22.68
CM 2.75%	13.42	17.18	21.35

All mixtures show an upward trend in tensile strength overtime similar to the Control (with significantly different results) as seen in Figure 15. The CM 1.75% mixture was 2.96% more desirable than the Control at 7 days, while the mixtures with 2.00%, 2.25%, 2.50% and 2.75% were reduced by levels of acceptability corresponding to being -3.3%-6.0%-8.4%-13.8%. The control average strength at 14 days was 19.71 kg/cm², and CM 1.75% exceeded the standard by (2.1%), while the other treatments ranged from a reduction of (3.4%) to (12.8%). At 28 days, CM 1.75% with the best performance showed a strength increase of +2.1% in comparison to the control mix and dose-dependent reductions up to -14.1% were maintained for higher dosages (CM 2.75%). This behaviour indicates that the lower encapsulated cushuro fraction leads to a more homogeneous microstructure with higher ability of tensile stress dissipation and slower fatigue crack propagation.

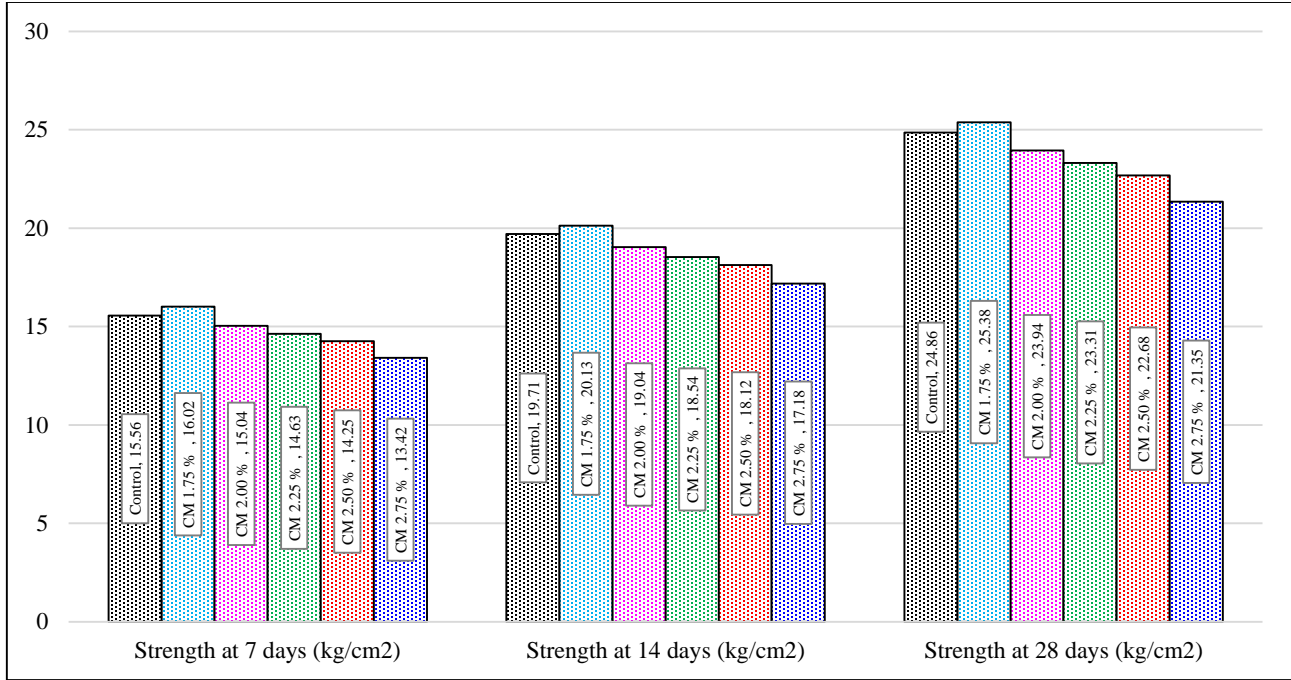


Fig. 15 Indirect tensile strength

4.4. Crack Control

4.4.1. Crack Control in Mortar

As summarized in Table 7, none of the controls showed a double significant difference; fissure width was found constantly around 0.31 mm from day 0–30, while in all other mixed, including microencapsulated cushuro (CM), fissure width was decreased progressively. At 30 days, the fissure width decreased from 0.35 mm to 0.03 mm, was found highest closure at CM 1.75% with self-repair efficiency of up to (91.4%). CM 2.00% also showed a decline from 0.36 mm–0.04 mm (88.9%), whilst CM 2.25%, CM 2.50%, and CM 2.75% efficiencies were recorded at, respectively, (66,7%), and (57-8%). This is because this behaviour occurs due to the photosynthetic activation of *Nostoc sphaericum*

when exposed to light, air, and environmental humidity. In this context, enzymes including urease and carbonic anhydrase become reactivated, catalysing the production of Carbonate ions (CO₃²⁻). These ions subsequently react with the Ca²⁺ present in the mortar matrix to precipitate Calcium Carbonate (CaCO₃) in the cracks. At 1.75% and 2.00%, the ideal mix between a high cell density (containing viable microorganisms) and low mortar permeability promoted the diffusion of CO₂ and Ca²⁺ for a better crack sealing process. On the contrary, an excess biopolymer phase due to higher dosages possibly reduced light and air access to the cracks, restricting photosynthesis and subsequently CaCO₃ biomineralization. In summary, the findings show that mid-level doses facilitate biogenic self-repair.

Table 7. Crack control with mortar

Description	Cracks Day 0 (mm)	Cracks Day 7 (mm)	Cracks Day 14 (mm)	Cracks Day 21 (mm)	Cracks Day 28 (mm)	Cracks Day 30 (mm)
Control	0.31	0.31	0.31	0.31	0.31	0.32
CM 1.75%	0.35	0.24	0.12	0.07	0.04	0.03
CM 2.00%	0.36	0.22	0.14	0.09	0.05	0.04
CM 2.25%	0.39	0.27	0.21	0.15	0.11	0.09
CM 2.50%	0.42	0.31	0.25	0.22	0.15	0.14
CM 2.75%	0.45	0.35	0.31	0.27	0.24	0.19

Figure 16 graphically shows the sustained reduction in crack opening throughout the observation period (30 days) for all mixtures with cushuro, while the Control remained unchanged. The fastest closure rate corresponded to the CM 1.75% mixture, which reduced the initial width by 31% during the first week and reached 80% closure at 21 days, completing

the process almost entirely by day 30. The CM 2.00% mixture followed a similar trend, although with a slightly slower repair rate. In contrast, the mixtures with contents of 2.25%, 2.50% and 2.75% showed slower behaviour, with gradual reductions ranging from 60% to 40% at the end of the period. This behaviour confirmed that the microencapsulated *Nostoc*

sphaericum system maintained its viability and CO₂ fixation capacity within the mortar, provided that humidity conditions were above 60% and light exposure was around 5000 lux. In general terms, the figure showed that the biological mortar

with 1.75% microencapsulated cushuro achieved almost total repair, demonstrating the effectiveness of the biocementing mechanism and its potential application as a surface self-repair technology in cementitious materials.

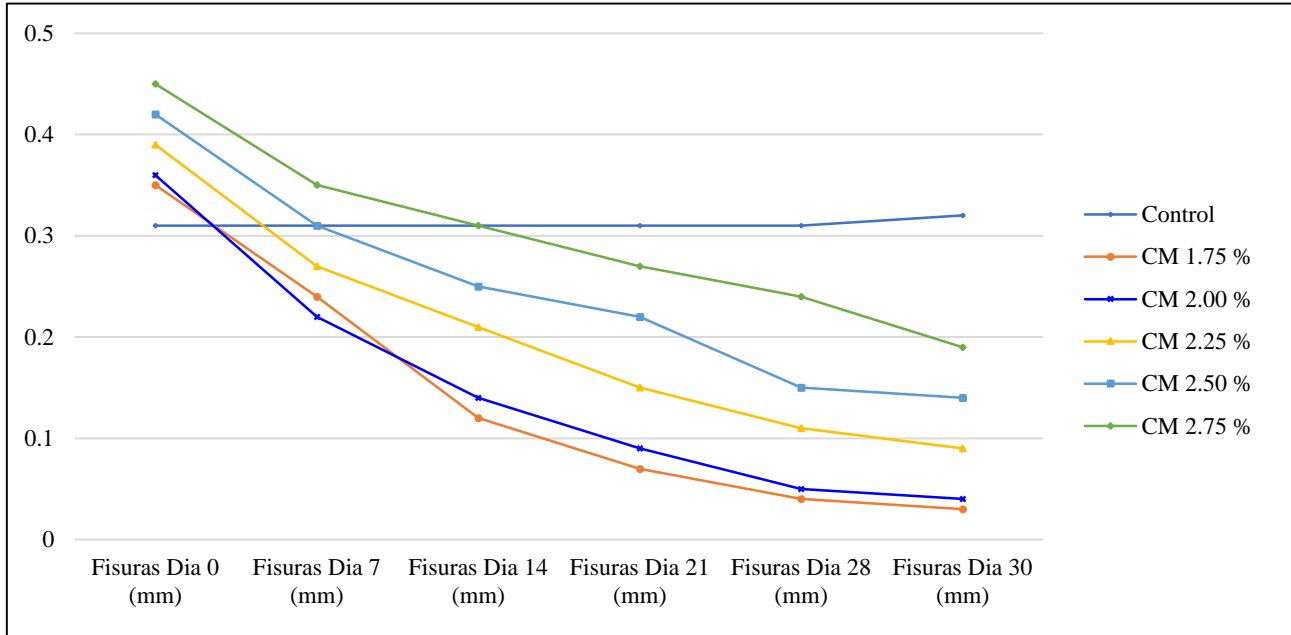


Fig. 16 Crack width reduction in mortar with microencapsulated nostoc sphaericum

4.4.2. Crack Control in Concrete

Table 8 shows that all concrete mixtures with direct incorporation of microencapsulated cushuro (CM) experienced a progressive reduction in crack width over the 30 days of evaluation, while the Control remained virtually unchanged, with average values of 0.28 to 0.29 mm. Among the biocement mixtures, the CM 1.75% sample performed best, reducing the crack width from 0.34 mm to 0.05 mm, representing a sealing efficiency of 85.3%. The CM 2.00% mixture achieved a reduction from 0.36 mm to 0.07 mm (80.6%), while CM 2.25%, CM 2.50%, and CM 2.75% achieved decreasing efficiencies of 70.7%, 60.9%, and 57.1%, respectively. The behaviour showed that self-repair was more effective at low dosages (1.75% and 2.00%), where the amount of microcapsules allowed for a homogeneous distribution of Nostoc sphaericum without interfering with the

concrete structure. Over this range, both the reactivation of cyanobacteria during the light and moisture exposure phases and the internal precipitation of Calcium Carbonate (CaCO₃) were used to fill in the mechanically fractured crack zone. This process was associated with the activity of urease and carbonic anhydrase, which convert dissolved CO₂ into Carbonate ions (CO₃²⁻), later reacted with Ca²⁺ provided by the cementitious medium, filling discontinuities over time. On the other hand, high doses (≥2.25%), which were supported by low-density organic microzones and lower permeability through limited diffusion of air and light to the microcapsules, negatively influenced both the biological activation rate and crystalline growth. These results also confirmed that when incorporated at moderate amounts, cushuro could be implemented within the concrete matrix.

Table 8. Concrete crack control

Description	Cracks Day 0 (mm)	Cracks Day 7 (mm)	Cracks Day 14 (mm)	Cracks Day 21 (mm)	Cracks Day 28 (mm)	Cracks Day 30 (mm)
Control	0.28	0.28	0.28	0.28	0.29	0.29
CM 1.75%	0.34	0.25	0.17	0.11	0.06	0.05
CM 2.00%	0.36	0.27	0.21	0.13	0.08	0.07
CM 2.25%	0.41	0.32	0.25	0.18	0.13	0.12
CM 2.50%	0.46	0.37	0.32	0.24	0.19	0.18
CM 2.75%	0.49	0.41	0.34	0.31	0.24	0.21

Figure 17 clearly shows the decreasing trend in crack width in the biocement mixtures, in contrast to the stability of the Control, which showed no evidence of sealing processes. From day 7 onwards, the CM 1.75% and CM 2.00% mixtures began to show significant reductions of 26% and 25%, respectively, compared to their initial opening, reaching cumulative decreases of 50% and 41% on day 14. On day 21, the cracks were reduced to 0.11 mm (67.6% closure) in CM 1.75% and 0.13 mm (63.9%) in CM 2.00%, while the mixtures with higher doses maintained slower reductions. Finally, on day 30, total closure reached 85.3% for CM 1.75%, 80.6% for CM 2.00%, 70.7% for CM 2.25%, 60.9% for CM 2.50%, and

57.1% for CM 2.75%. These results indicated that biogenic repair was slower than in mortar, but with greater structural stability of the seal, which was attributed to the density and lower porosity of the concrete. The general trend showed that, even in a denser cementitious medium, the biological system was able to remain active, provided that favorable environmental conditions of lighting (5000 lux) and humidity (>60%) were present. Therefore, the use of microencapsulated cushuro incorporated into the mixture allowed for effective and self-sustaining internal self-repair, with a significant reduction in crack opening and a potential increase in the durability of biocementitious concrete.

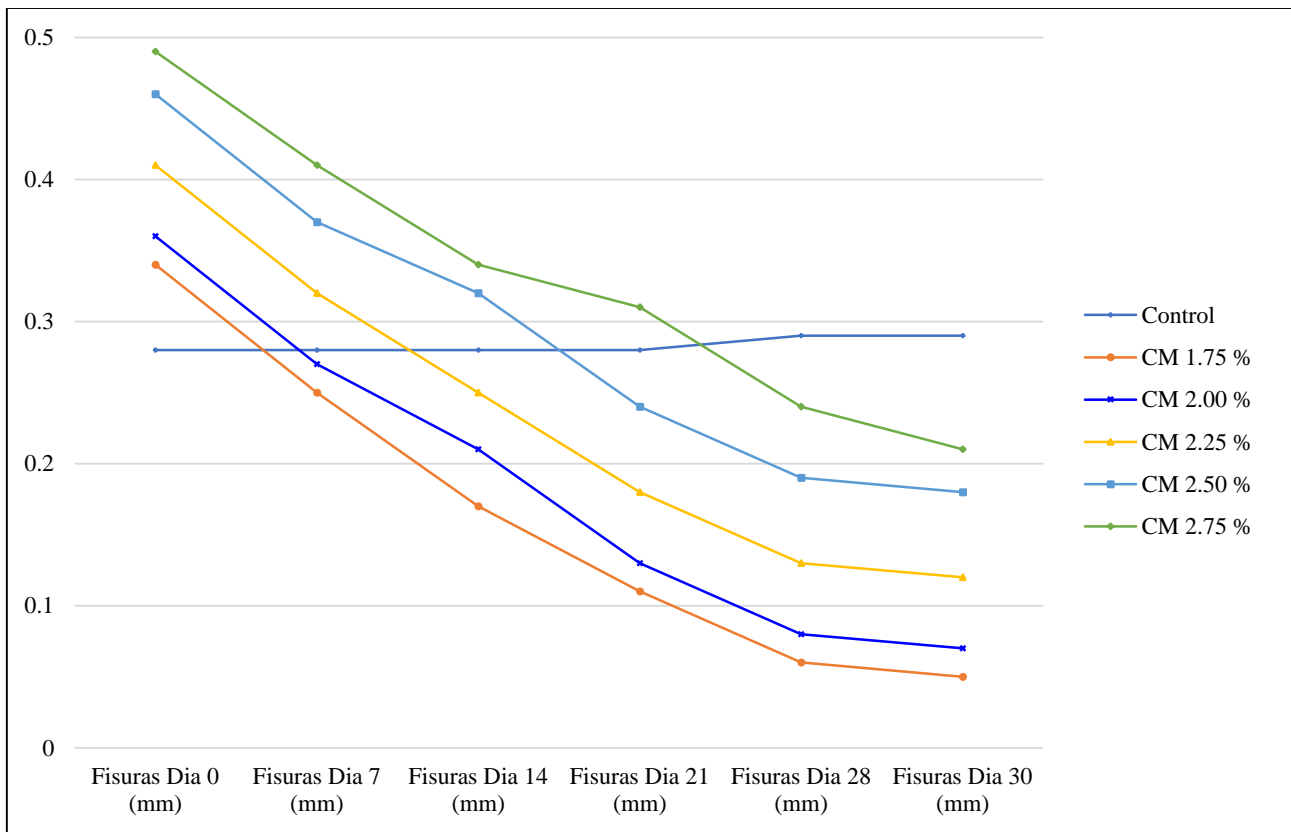


Fig. 17 Crack width reduction in concrete with microencapsulated nostoc sphaericum

4.5. CO2 Absorption Control

Table 9 shows that the incorporation of microencapsulated cushuro significantly increased the mass of Calcium Carbonate (mCaCO₃) and, consequently, the amount of carbon dioxide fixed (mCO₂ and total CO₂ in grams) compared to the standard. The mixture with 1.75% had the highest concentration of CaCO₃ with 5.87 mg/cm³, equivalent to an absorption of 2.583 mg CO₂/cm³ and a total fixation of 4.057 g of CO₂. This result indicated that at low dosages, the biological system maintained high photosynthetic activity, favoring the biogenic precipitation of Calcite (CaCO₃) through the metabolism of Nostoc sphaericum. As the dosage increased, a decreasing trend in CO₂ absorption was observed: the CM 2.00%, CM 2.25%, CM 2.50%, and CM 2.75%

mixtures reached values of 3,670 g, 3,221 g, 2,709 g, and 2,405 g, respectively. This decrease was related to microbial packing and the increase in viscosity of the cementitious medium, which restricted CO₂ diffusion and light penetration into the microcapsules, thus reducing the photosynthetic efficiency of cushuro. Similarly, the high excess in biomass and polysaccharides may have contributed to an increase in closed porosity with less effective gas exposure to trigger CaCO₃ formation inside open pores of the material. In general, the results indicated a threshold range of bioreactivity: between 1.75% and 2.00% supplementation produced better conditions for more CO₂ to be fixed via biocatalysis due to an increase in the availability of nutrients overall, with an appropriate balance of permeability and light.

Table 9. CO₂ absorption and CaCO₃ formation in concrete specimens

Description	mCaCO ₃ (mg/cm ³)	mCO ₂ (mg CO ₂ /cm ³)	V (cm ³)	CO ₂ (g)
Control	0.92	0.405	1570.8	0.636
CM 1.75%	5.87	2.583	1570.8	4.057
CM 2.00%	5.31	2.336	1570.8	3.670
CM 2.25%	4.66	2.050	1570.8	3.221
CM 2.50%	3.92	1.725	1570.8	2.709
CM 2.75%	3.48	1.531	1570.8	2.405

As illustrated in Figure 18, the absorption capacity of all mixtures containing cushuro far exceeded that of the standard (0.636 g of CO₂). The CM 1.75% mixture increased by 537%, CM 2.00% increased by 477%, CM 2.25% by 406%, CM 2.50% by 326%, and finally, the CBM of the mixtures at a range of CM % values (dry) was found to be 278%. This downward trend was directly proportional to the increase in biological content, thus corroborating that the main peak of capture efficiency occurred at low cushuro concentrations due to photosynthetic activity being more efficient with better oxygenation and less intracellular competition. Therefore, this finding indicates that the biocement system could achieve CO₂ fixation capabilities six times higher than conventional concrete and demonstrates the potential of cushuro as a bioactive agent for carbon capture and mineralization.

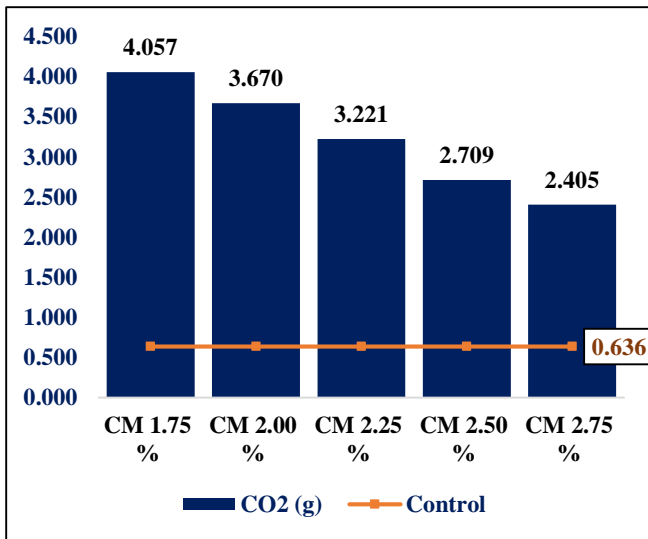


Fig. 18 CO₂ absorption control

5. Discussion

The inclusion of cushuro in concrete resulted in decreasing compressive strength when the amount of additive was increased, which ranged from 6.4%, 9.7%, and 19.7% for mixtures with 2.25%, 3.34%, and 4.46% content, respectively [22]. The strength of standard concrete in this study was 219.51 kg/cm², and the mixtures containing microencapsulated cushuro reached values of 221.35 kg/cm² (1.75%), 217.97 kg/cm² (2.00%), 214. 11 kg/cm² (2.25%),

212.46 kg/cm² (2.50%), and 209.33 kg/cm² (2.75%). From these results, moderate dosages (1.75%) are shown to slightly enhance strength by the mechanism of controlled moisture retention and biogenic activation of the material at lower concentrations and may generate excess organic phase loss on cohesion at higher concentrations. Overall, both studies agree that cushuro can be used as a sustainable, low-impact additive, provided that its proportion does not exceed 2.25% of the cement weight, thus ensuring a balance between mechanical performance and microstructural stability of biocement concrete.

According to research conducted by Rokaya et al., the development of a co-culture system between *Trichoderma reesei* and *Nostoc punctiforme* made it possible to obtain a synthetic lichen-like microbial community capable of fixing atmospheric CO₂ and N₂ and precipitating CaCO₃ using only air and light, without requiring external nutrients [23]. In the present study, *Nostoc sphaericum* (cushuro) was used independently, encapsulated in gelatin–alginate, and incorporated into concrete, achieving crack closure of up to 91.4% and maximum absorption of 4.05 g of CO₂ per test tube. Unlike Rokaya's symbiotic system, Cushuro proved to maintain its viability and photosynthesis in a solid cementitious medium, demonstrating that it can act as a self-sufficient biocatalyst. These variations can be attributed to the absence of the symbiotic fungus, the alkaline environment of the concrete, and the protective effect of microencapsulation, which enabled *Nostoc sphaericum* to adapt and promote CaCO₃ bioprecipitation without external support. Together, these findings highlight the strong potential of *Nostoc* as a biotechnological agent for developing living and sustainable concretes, capable of self-repair through natural processes such as photosynthesis and biogenic carbonation.

That this can be achieved, research completed in Egypt concluded that *Fragilaria* sp. CCAP1029 and *Synechocystis* sp. In this study, PCC 6803 was integrated into concrete compositions for the improvement of mechanical and long-term durability characteristics, showing an increase in compressive strength to 21.66% and a rise in durable ability up to 22.33%. It was also discovered that steel corrosion rates declined due to the precipitation of Calcite (CaCO₃) within the pores, which helped in crack sealing between 7 and 14 days of

exposure [17]. Thus, a similar behaviour has been recorded in Malaysia with *Synechococcus elongatus* from the incorporation of *Spirulina platensis* into cement mortars. Specimens with *S. platensis* achieved about 72% of the compressive strength of the Control, and after biological curing, they recovered 35% of the residual strength and reduced crack width by up to 95% in just 14 days [18]. In contrast, studies from the United Kingdom have shown that fast-growing highlight species such as *Synechococcus elongatus* UTEX 2973, PCC 11801, PCC 11901, and *Cyanobacterium aponinum* UTEX 3222 can achieve carbon fixation rates of approximately (1.83 kg of CO₂ per kg of biomass) [19]. In the same vein, soil with a microbial consortium of *Leptolyngbya* sp., using *A. oryzae* as an example (discussed in section 7 and shown in Figure 4), achieved CO₂ capture of up to 94% of the atmospheric level within only 2 to 4 days, evidencing its metabolic plasticity and strong potential for biomixture and pollutant removal applications [21]. Recently conducted the current study and microencapsulated *Nostoc sphaericum*(cushuro) in gelatin–alginate matrices, and these results are similar to, and in some cases surpass, those represent internationally. The mixture containing 1.75% cushuro reached a compressive strength of 221.35 kg/cm², which was higher than that of the standard mix (219.51 kg/cm²), while higher dosages did not change significantly, keeping within[BG1]changes lower than 5%, with respect to self-healing, biological mortar-treated specimens attained closed cracks of 91.4% at 30 days. Concrete samples containing ESM-enhanced *Nostoc sphaericum* also gradually recovered efficiently (up to 85.3%) under light (5000 lux) and relative humidity (>60%). The maximum carbon dioxide fixations per specimen (4.05 g, equivalent to 2,583 mg CO₂/cm³) was observed in the specimens with encapsulated bacteria that were released under low-sustained phosphonic acid concentration after 5 weeks of incubation which is much higher than the Control concrete value of 0.636 g; We could explain these differences using environmental aspects and microorganism type alone in regard with every present studies [1-3]. The species from many international studies have been grown on nutrient media and under tightly controlled laboratory conditions, so their stability and performance are limited in cementitious environments.

6. Conclusion

This study first demonstrates a novel biotechnological approach for sustainable transformation of conventional concrete into a living, self/heal repair and carbon capture material by embedding microencapsulated *Nostoc sphaericum* (cushuro) within gelatin–alginate matrices. The methodology studied from cultivation to mechanical evaluation included microencapsulation, photosynthetic activation, which ultimately facilitated the in situ assessment of the hypothesis concerned with maintaining living autotrophic microorganisms within cementitious environments and promoting internal biocarbonation processes. Second, in the

mixtures of 1.75% cushuro, were found a peak structural performance caused by moderate biological activity, taking advantage of promoting prolonged cement hydration and biogenic Calcium Carbonate (CaCO₃) precipitation without affecting the compaction of the system. This resulted in a dense and strong microstructure, which could balance the water retention ability with confined mechanical properties. Also, such trends indicated that the metabolic activity of the microorganism can be harvested without compromising its structural integrity at low biofiller contents. This behaviour, in general, indicates that cementitious matrix interaction with encapsulated biomass can favor the self-healing process in a controlled and effective manner.

This resulted in a gradual decline of mechanical and sealing performance whenever dosages exceeded 2.25%, specifically due to excessive biopolymer phase and lower light permeability in the matrix [43]. Such behavior is indicative of unregulated increases in the cushuro content that generate low-density areas with limited gas diffusion and a reduction in active photosynthetic regions. Therefore, it can be inferred that the performance of the system is directly related to the proportion of microorganisms with environmental conditions for activation, since only fanned water mist increased bioreactivity without activation in light and humidity. This interpretation only consolidates the fact that proper dosage standards as well as exposure conditions should be established in order to maintain biocement concrete functional sustainability.

Experimentally, the last two points found clarification in tests of self-healing and biological CO₂ fixation—both methods demonstrated that microencapsulated cushuro, surface, or directly incorporated into specimens, can develop CaCO₃ precipitation autonomously and sustainably. This indicated the presence of a functional bio-induced repair mechanism that does not require additional nutrients, demanding only photosynthesis and enzymatic reactions driven by urease and carbonic anhydrase. Such behaviour indicates that the system can prolong the service life of concrete structures with a dramatically low CO₂ footprint. Thus, it provides a new, additional integrated method for the enhancement of concrete resilience and sustainability in exposed environments. Finally, it is concluded that *Nostoc sphaericum* represents an indigenous biotechnological base with high potential for the development of regenerative, energy-efficient, and environmentally responsible concretes. The evidence obtained supports its application in surface repair systems, ecological prefabricated materials, or CO₂-capturing cementitious materials in high Andean areas. However, further evaluation of its long-term durability, the stability of the encapsulation under thermal cycles, and its performance under real service conditions is still required. Likewise, future research should address the scalability of the proposed system for practical construction applications. Overall, this study constitutes a pioneering contribution to

sustainable civil engineering in Peru, demonstrating that the integration of native microorganisms can redefine the construction paradigm toward truly living and adaptive materials.

Conflicts of Interest

We declare that there is no conflict of interest regarding the publication of this article.

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