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Contribution of Lysinibacillus sphaericus to crack repairing and compressive strength in durable concrete

Contribución de Lysinibacillus sphaericuus en la reparación de fisuras y resistencia a la compresión en hormigón durable

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Abstract
Concrete is a material of massive use due to its strength and durability. However, it presents post-concreting anomalies that cause damage to structures, partially or totally, interrupting their service and reducing their designed useful life. The corrective maintenance is an anthropogenic intervention, which demands the use of epoxy additives, resins, tools, equipment and techniques that represent unforeseen costs, labor risks and environmental impact. In response is an anthropogenic, $CaCO_3$ precipitating bacteria into concrete by biomineralization. This article seeks to determine the influence of Lysinibacillus sphaericus on the Compressive strength and self-repair of concrete f'c=350kg/cm^2, previously carrying out tests for aggregates and culture media. The preparation of ABC concrete (Bacterial additive for concrete) in doses of 0, 15, 20 and 25ml/m^3 of mix, the design of standard concrete with an A/C ratio of 0.43. At 28 days of setting, it reached a Compressive strength of 105% for the standard concrete, and the best strength obtained was by incorporating 25ml/m3, improving by 16% compared to the standard concrete. After 14 days of curing, SEM images showed 69% self-repair of the cracks.

Keywords: *Concrete self-repair; calcite precipitation; bacterial concrete; Lysinibacillus sphaericus; bacterial culture.*

Resumen
El hormigón es un material de uso masivo por su resistencia y durabilidad. Sin embargo, presenta anomalías posteriores al hormigonado que provocan daños en las estructuras, parcial o totalmente, interrumpiendo su servicio y reduciendo su vida útil diseñada. El mantenimiento correctivo es una intervención antropogénica, que demanda el uso de aditivos epoxi, resinas, herramientas, equipos y técnicas que representan costos imprevistos, riesgos laborales e impacto ambiental. En respuesta, hay una bacteria antropogénica que precipita $CaCO_3$ en el concreto mediante biomineralización. Este artículo busca determinar la influencia de Lysinibacillus sphaericus en la Resistencia a la compresión y autoreparación del concreto f'c=350kg/cm^2, realizando previamente ensayos para agregados y medios de cultivo. La preparación de hormigones ABC (Aditivo bacteriano para hormigones) en dosis de 0, 15, 20 y 25ml/m^3 de mezcla, el diseño de hormigones estándar con una relación A/C de 0,43. A los 28 días de fraguado alcanzó una Resistencia a la Compresión del 105% para el hormigón estándar, y la mejor resistencia obtenida fue al incorporar 25ml/m^3, mejorando en un 16% respecto al hormigón estándar. Después de 14 días de curado, las imágenes SEM mostraron un 69 % de autorreparación de las grietas.

Palabras Claves: Concreto; autoreparacion; Lysinibacillus sphaericus, cultura bacterial

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1. Introduction

The use of concrete is inevitable in construction due to its hardness, strength, and durability. This resource is important for developing reliable, resilient, sustainable, and quality infrastructure, which enables the achievement of human well-being, economic development, and other purposes aligned with Goal 9: industry, innovation, and infrastructure of the United Nations 2030 agenda.

Concrete degradation is commonly due to cracks or microcracks that cause the deterioration of the reinforcing bars and are an entry point for external agents. These anomalies represent a risk that compromises its useful life and generates environmental problems. This has aroused the interest of many researchers, who seek to apply new techniques to design bioconcretes with greater efficiency and with less effect on the ecosystem.

Microcracking is unavoidable and detrimental in concrete due to exposure or connection with external agents; this mainly occurs due to the action of external loads (Algaifi et al., 2018). The incorporation of biological agents significantly reduces environmental threats (Shanmuga et al., 2019).

The alkaliphilic L. sphaericus bacterium of the SKC/VA1 strain, using calcium lactate pentahydrate as culture medium, contributes favorably on the physical properties and crack curing of mortar (Chaerun et al., 2020). With the use of advanced technology at the University of Novi Sad in Serbia, SEM analysis was performed comparing samples with and without bacterial additive; differentiated by calcite precipitation in the microcracks, identifying ettringite and calcium silicate. Likewise, by means of X-ray tomography and Hg porosimetry, the shape, porosity and sealing of induced cracks in concrete can be observed (Sidiq et al., 2020).

Bacteria are classified according to their structure as Gram + and Gram -. The cell wall defines their coloration, morphology and association (Gebremariam et al., 2021); (Corrales and Caycedo, 2020). The addition of bacteria in concrete is mainly done in two ways; direct application or encapsulating; this is going to depend on the precipitation of $CaCO_3$ by CO^2 metabolism performed by bacteria as concrete contains portlandite (Gupta et al., 2017). Studies conducted at the Magnel Laboratory of Ghent University - Belgium analyzed samples with cracks of 0.35mm; observed separately in test tubes, one based on polyurethane (PU) and the other on silica gel; the latter having higher $CaCO^3$ precipitation activity (Wang et al., 2012). On the other hand, research conducted by the University of Khon Kaen in Thailand indicates that isolation is developed with the use of calcium-based products that make bacterial culture possible, considering a pH of 7.0 for the culture, incubating at 30°C with 150rpm in a period of 7 days (Ekprasert et al., 2020). Concrete samples can be treated in two ways: by immersing the samples in bacterial culture or in diluted urea with added calcium (Ca2+) for 72 hours in order to observe the precipitation of $CaCO_3$. L. sphaericus survived in pH 12-13 without being able to reproduce and showed 75% pore efficiency due to its high pH; it improved at pH 9,10 and 15% in crack resistance and even total sealing of 0.4mm cracks was observed at 70 days of curing (Jang et al., 2020). Studies conducted at Universiti Teknologi Malaysia (UTM) show the importance of the use of urea in the hydrolysis and precipitation of $CaCO_3$ in bioconcrete, since it improves its compressive strength by approximately 15% and seals cracks cracks up to 0.4 mm during 70 days of setting (Algaifi et al., 2020).

In view of the above, this article seeks to determine the influence of Lysinibacillus sphaericus on the Compressive strength and self-repair of concrete f'c=350kg/cm^2, previously carrying out tests for aggregates and culture media.

2. Materials and methods

2.1 Materials

2.1.1 Aggregates, cement and plasticizer

The mixture design contemplates the use of coarse aggregate from the "Portachuelo" quarry and fine aggregates extracted from the "Teófila" quarry, both located in the department of Piura, Peru. The main

properties of the aggregates are shown in (Table 1) and their corresponding particle size distribution (Figure1) and (Figure 2).

Table 1. *Properties of the aggregates used*

Type of aggregate	Fine Aggregate	Coarse Aggregate
Fineness module	2.92	6.63
Maximum size	3/8"	3/4"
Humidity (%)	1.01	1.70
Loose unit weight (kg/m3)	1583	1475
Compacted unit weight (kg/m3)	1750	1630

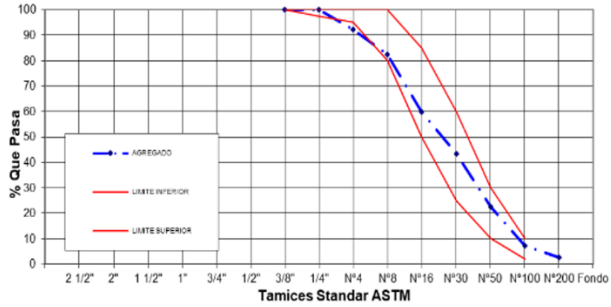


Figure 1. *Granulometric Curve of Fine Aggregate*

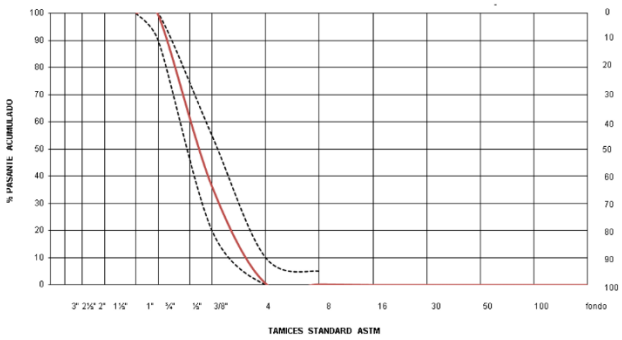


Figure 2. *Granulometric curve of coarse aggregate*

The cement used is Portland type V that complies with ASTM C 150, with high resistance to salt contamination, since the aggregates come from the coastal zone of Peru. Likewise, to meet the standards of workability and pouring of the mixture, a plasticizer composed of biopolymers is added in compliance with ASTM C494M type A and D to be compatible with the ABC additive in its different dosages.

2.1.2 Bacterial additive

A. Obtaining the bacterial additive for concrete (ABC)

The Lysinibacillus sphaericus 4525 strain is acquired from the American Type Culture Collection - ATCC (USA) in lyophilized presentations in plastic bacteriologic asas (loops). Its conservation should be refrigerated between 2-8°C, and for inoculation the supplier's manual should be reviewed and taken into account.

The bacterium under study is naturally present in many environments and even in our digestive system and it is not pathogenic. Therefore, it does not represent any danger to humans (Gupta et al., 2017) since it coexists with them. The culture can only be developed in laboratory under unique aseptic conditions according to INS-018 and INACAL 2020.

B. Morphology and bacterial identification

The morphological characterization of the bacteria is essential even though they are isolated and certified strains. It is recommended to perform at least the Gram staining test. For the identification of the bacteria, a sample of the breeding broth was taken for the Gram staining procedure, resulting to be Gram positive with flagella and bluish color. (Figure 3)

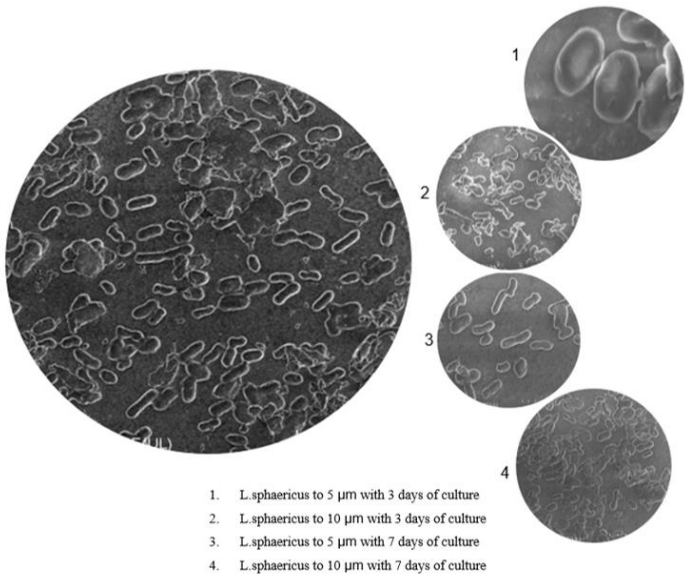


Figure 3. *Morphology of Lysinibacillus sphaericus 4525 by SEM.*

C. Inoculation process

The bacteriologic asa (loop) to be used is left at room temperature to avoid shock when it comes into contact with the agar, since this nutrient is commercialized in glass bottles. Immersion in bain marie for a period of 5-7 minutes is required to dilute it. Then, it is expected to cool down to room temperature for the inoculation process. This sowing is done by rubbing the loop (asa) in zigzag over the nutrient with total asepsis in the laboratory. Once the sowing is done, the plates are closed and taken to the incubator for a period of 24 to 30 hours at a temperature range of 30 to 35ºC, until growth and colony formation is observed.

D. Sowing process in nutrient broth

The nutrient broth is a nutrient substance that comes in powder form. In this project, 250g diluted in 500ml of sterile water was used. Once colony formation is observed on the agar culture medium inside the Petri dishes, proceed with the sowing by removing these colonies with the help of a sterile spatula and depositing

them in the culture medium, which is loaded into the incubator at 30 to 35ºC for 72 hours. In this study, on the third day the measurement was made thanks to the spectrophotometer with a result of 0.585 OD at 600nm, which was not favorable for the application to the mixture, having to wait for the growth of the culture, which was achieved after 10 days. For bacterial additive application it should be at 1.0 in optical density (Shanmuga et al., 2019). (Figure 4)

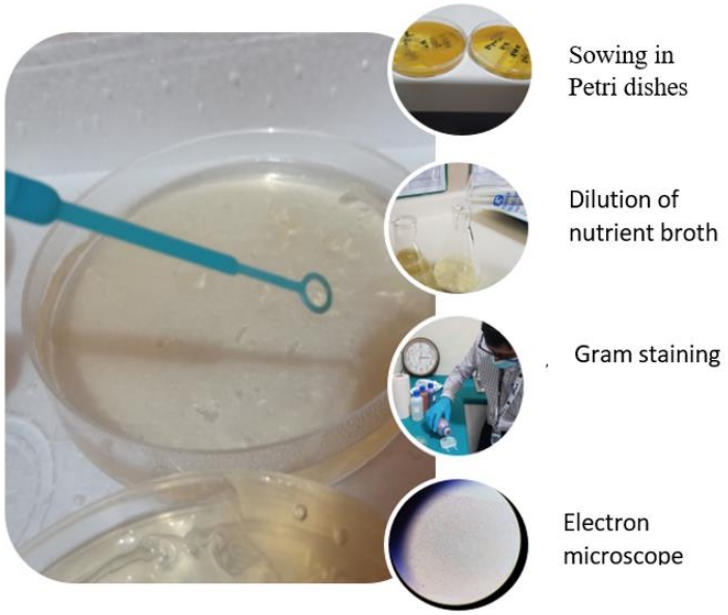


Figure 4. *Inoculation process and identification of L. sphaericus.*

2.2 Methodology

The mixture design was prepared for hydraulic works with a f'c=350kg/cm^2, in compliance with the provisions of the ACI 211 committee. (Table 2)

Table 2. *Dosage by weight and volumen*

Materials	Weight (Kg/m3)	Volume (foot3)
Cement	468	1
Fine aggregate	837	1.7
Coarse aggregate	893	1.9
Water	177	16.1
Plasticizer	4.7	0.355

A total of 36 cylindrical and 12 prismatic samples were cast with various dosages of bacterial additive (0, 15, 20, 25 ml/m^3), which have been evaluated for axial compressive strength (ASTM C 39) and concrete self-repair by SEM (scanning electron microscopy) analysis. (Figure 5)



Figure 5. *Prismatic samples for SEM analysis*

3. Results and discussion

3.1 *Compressive strength*

For the tests, three samples were made for each type of ABC additive dosage. These were subjected to cracking at 7, 14 and 28 days of curing, in compliance with ASTM C39 and NTP 339.034. The results obtained are shown below (Table 3):

Table 3. *Axial compressive strength*

Addition of ABC	**Resistance to axial compression (Kg/cm²)**		
	07 days	14 days	28 days
0 ml.	261.33	317.33	366.67
15 ml.	275.00	328.33	393.00
20 ml.	288.67	345.00	409.33
25 ml.	291.67	348.67	422.33

The results obtained at 28 days were very favorable for designs ABC00, ABC15, ABC20 and ABC25, reaching strengths of 367, 393, 409 and 422 kg/cm² respectively for each concrete design.

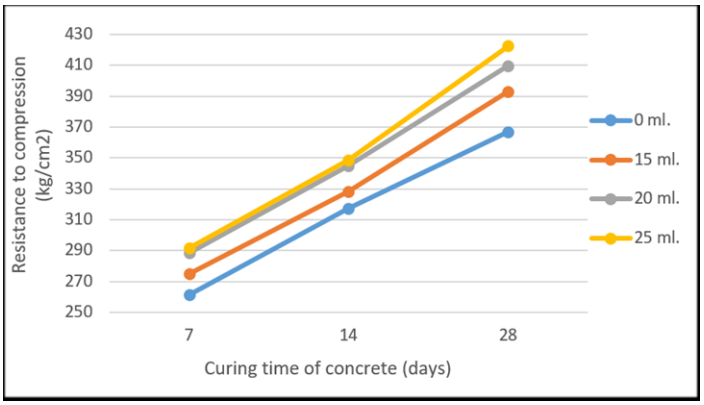


Figure 6. *Compartaive strength and curing time*

It is important to indicate that in the research by (Shanmuga et al., 2019), the design that resulted in the best contribution to compressive loads was when using 20.0 ml/m^3, reaching up to a 16.0% increase over the standard. In our case, a 16% improvement was obtained when using 25.0 ml. For this reason, it is agreed that the ABC additive does have a positive influence on this property. Additionally, the above is confirmed by the work of (Vashis et al., 2018), who used the culture of Lysinibacillus sphaericus strain I13, which had a good contribution to Compressive strength giving values higher than 34.60 % in comparison to the standard. (Figure 6)

3.2 Concrete Self-Repairing

The process of visualizing and capturing images of the sealing of the microcracks at ages 7 and 14 days was carried out using SEM images captured by the SU8200-CFE-SEM-HTD-E210 equipment of the UNI-LIMA science laboratory, as well as in the specialized equipment laboratory of the Faculty of Biological Sciences of the UNMSM-LIMA (Table 4).

Table 4. *Results of SEM analysis on concrete samples*

Prismatic sample	Code	Width of crack (fissure)	Width of crack	
			7 days	14 days
Standard concrete	ABC00-1	825microns	NV	NV
	ABC00-2	128 microns	NV	NV
	ABC00-3	265 microns	NV	NV
Concrete with 15ml ABC additive	ABC15-1	632 microns	4%	36%
	ABC15-2	325 microns	6%	40%
	ABC15-3	456 microns	5%	38%
Concrete with 20ml ABC additive	ABC20-1	349 microns	8%	50%
	ABC20-2	332 microns	6%	42%
	ABC20-3	726 microns	6%	44%
Concrete with 15ml ABC additive	ABC25-1	243 microns	11%	71%
	ABC25-2	129 microns	18%	68%
	ABC25-3	376 microns	10%	69%

Cracks of 0.3 and 0.35 mm were induced in concrete samples which were cured with the bacterial additive and observed at 1-week intervals (Figure 7)

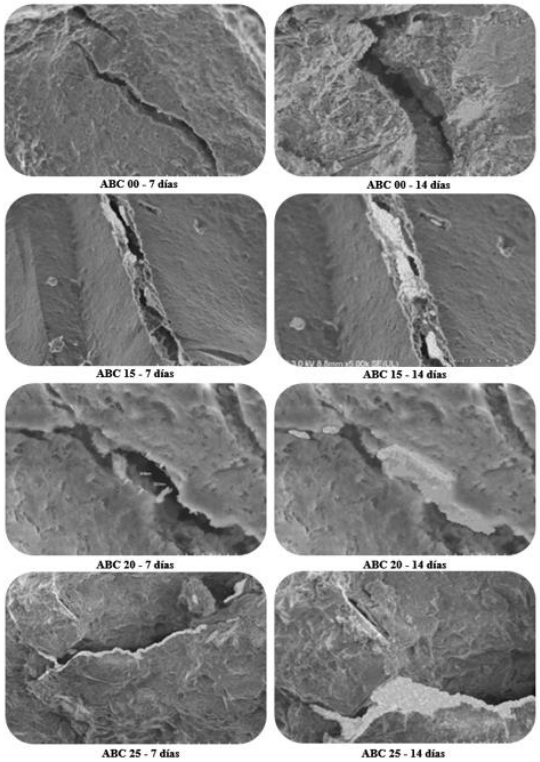


Figure 7. *Self-repair checking on microcracks in concrete samples*

When analyzing the obtained results in general, it can be indicated that the greater the amount of bacterial additive, the more favorable the behavior of the self-repair of cracks is. This is due to its property of precipitating calcite ($CaCO_3$) in these spaces where a crack is generated and where contaminating agents can enter, which in a matter of days will be repaired. (Figure 8).

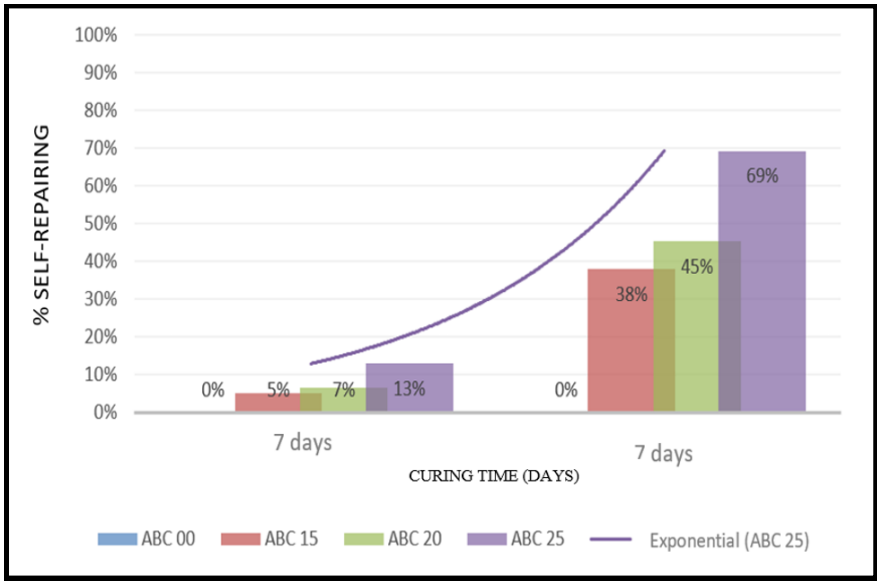


Figure 8. *Comparative self-repairing and curing time in prismatic samples*

4. Conclusions and recommendations

The most favorable culture medium for Lysinibacillus sphaericus is presented in nutrient broth, which is accessible to the construction sector, since it is very commercially available in powder form. In relation to the Compressive strength, an increase of 16% is evidenced when applying the dosage of 25ml/m^3 of ABC additive. In the same direction, the self-repairing of microcracks in the concrete is observed to have reached significant and important values in the second week of curing with values of up to 69.0% by SEM analysis.

With all this, it is of utmost importance that bioconcrete researchers continue in this Field of knowledge to increase the useful life of structures, reduce maintenance costs, reduce environmental risks and minimize risks due to exposure to chemical agents during repair and maintenance in constructions. All of this, in order to have a society with "sustainable" technology with a decrease in global warming, greenhouse effect and other phenomena resulting from conventional technological mismanagement.

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