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Improvement of tensile strength and durability of mortar through bio cementation

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تحسين قوة الشد وقوة تحمل الملاط من خلال التضمين الأحيائي

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Abstract

Throughout the field of construction, intrinsic brittleness of the mortar that lead to cracking, especially after plastering, has been frequently observed, which makes it a common hurdle in most of the constructed structures, due to the significant loss of water tightness and durability. Cementous structures are subject to damage continuously by harassment conditions. Therefore, special biological systems can be exploited to overcome such problems. Many bacterial species have been employed to precipitate calcium carbonate through the oxidation of organic carbon, changing the properties of the construction material. The oxidation process would fill the pores between cement matrices, which shows significant decrease in the porosity, permeability and capillary water penetration. Deposition of calcium carbonate by bacteria is a phenomenon known as microbiologically induced calcite precipitation (MICP), biomineralization, or bio cementation, which considered as natural and eco-friendly process. Such deposition is a promising practice for integrating and protecting various building materials. The strength refinement is due to growth of filler material inside the pores of the cement matrix. In addition, a modification in pores' size, distribution, and total volume are also noted, that could enhance durability properties. Due to the relative dryness and shortage of nutrients required for bacterial growth in concrete, those common bacteria unable to thrive. Some extremophiles spore forming species are able to survive in such hostile environment and boost the durability, compressive strength, and self-healing and repairing of micro-cracks in the cement concrete. *Bacillus spizizenii* 6633 was added to cement mortar, and found that the tensile strength was increased up to 12.6%, and the deterioration was decreased through the reduction of the microcracks by self-healing process.

Key words: Mortar, biomineralization, bio cement, *Bacillus spizizenii*, microcracks.

الملخص

في جميع مجالات البناء، لوحظ بشكل متكرر هشاشة الملاط التي تؤدي إلى التشقق، خاصة بعد التجصيص، مما يجعل من ذلك عقبة مشتركة في معظم الهياكل المشيدة، بسبب الفقد الكبير في المسامية والمتانة. تتعرض الهياكل الإسمنتية للضرر بشكل مستمر بسبب الظروف الغير ملائمة. لذلك، يمكن استغلال أنظمة بيولوجية خاصة للتغلب على مثل هذه المشاكل. تم استخدام العديد من الأنواع البكتيرية لترسيب كربونات الكالسيوم من خلال أكسدة الكربون العضوي، مما يؤدي إلى تغيير خصائص مواد البناء. سوف تملأ عملية الأكسدة المسام بين مصفوفات الإسمنت، مما يظهر انخفاضًا كبيرًا في المسامية والنفاذية والخاصية الشعرية. يُعد ترسب كربونات الكالسيوم بواسطة البكتيريا ظاهرة تُعرف باسم ترسيب الكالسيت الناجم عن الميكروبات، أو التمعدن الحيوي، أو التضمين الحيوي، والتي تعتبر عملية طبيعية وصديقة للبيئة. يعتبر هذا الترسيب ممارسة واعدة لدمج وحماية مواد البناء المختلفة. تحسين قوة الملاط ناتج عن نمو مادة الحشو داخل مسامات الإسمنت. بالإضافة إلى ذلك، لوحظ أيضًا تعديل حجم المسامات وتوزيعها والحجم الإجمالي، مما قد يعزز خصائص المتانة. بسبب الجفاف النسبي ونقص العناصر الغذائية اللازمة لنمو البكتيريا في الخرسانة، فإن تلك البكتيريا الشائعة غير قادرة على النمو. بعض الأنواع التي تكون الأبواغ القادرة على البقاء على قيد الحياة في مثل هذه البيئة المتطرفة وتعزيز المتانة وقوة الانضغاط والشفاء الذاتي وإصلاح الشقوق الدقيقة في الخرسانة الإسمنتية. تمت إضافة بكتريا من نوع Bacillus spizizenii 6633 إلى ملاط الإسمنت، ووجد أن مقاومة الشد زادت حتى 12.6٪، وانخفض التدهور من خلال تقليل التشققات الدقيقة عن طريق عملية الشفاء الذاتي.

الكلمات المفتاحية: الملاط، التمعدن الحيوي، الإسمنت الحيوي، Bacillus spizizenii، الشقوق الدقيقة.

Introduction:

In the scope of construction industries, mortar is a mainly utilized material. It is a combination of cement, water and sand, which primarily exploited for bonding stones or bricks collectively. Usually, in most of the building structures, cracks are created subsequent to plastering, which is generally applied at the final phase of the work. These cracks are created because of different factors, which include shrinkage effect, temperature alteration, and because of pressure applied on it etc. Thus, cracks formed readily, which in turn conducts to minimization in durability. Therefore, restoration of cracks is mandatory practice and also preserving very often is much necessary (Poornima *et al*., 2020). However, restoration of cracks is expensive and it needs high labor. The suitable substitutional comes in the concept of cracks self-healing, which can be achieved using bacteria (Zhang *et al*., 2019).

The precipitation of $CaCO_3$ in natural environments joins biological operations, both of which often happen with each other or one after the other. Bacteria living in soil and aqueous environments are usually noted to enhance calcium carbonate precipitation in mineral phases (Lian *et al*., 2006). Former surveys have revealed that the incorporation of particular microorganisms to sand-cement mortar or inorganic materials for concrete deposits, which can be brought to service as fillers to treat cracks within the structure (Ramakrishnan *et al*., 1999).

Microbial mineral deposition techniques have already been used to standardize sand and improve brick strength (Dhami *et al*., 2012). The predecessor minerals are transformed into $CaCO_3$ by bacteria. Self-healing mortars are utilized to treat cracks that are shown on the plaster by biologically producing limestone (Poornima *et al*., 2020). Thus, the biologically enhanced cement-based materials also presented better performance in crack repair and durability than regular concrete materials (Ghosh *et al*., 2008).

Among the bacteria utilized in studies about bio cementation, the Gram-positive, motile, spore-forming soil bacterium *Bacillus spizizenii* ATCC 6633. These bacteria are able to produce a crystalline form of calcium carbonate (calcite), which precipitates when grown in a medium with a calcium origin (Marvasi *et al*., 2013). This species is capable of precipitating calcium carbonate on their cellular components and in their microenvironment by converting urea into ammonium and carbonate (Wang *et al*., 2010)

The aim of the study:

This study helps to investigate the influence of microbial stimulate calcite precipitation to evolve the physico-mechanical features of cement mortar and the repairing of the cracks formed on the surface of construction materials.

Materials and methods:

Preparation of bacterial suspension:

Bacillus subtilis subsp. *spizizenii* ATCC 6633 was purchased from American Type Culture Collection (ATCC). The lyophilized cells were suspended in brain heart infusion broth for enrichment. After obtaining pure culture of bacteria on nutrient agar, they are maintained on slants of nutrient agar and preserved in refrigeration until use. Sub-culturing was carried out monthly,

and checked periodically for contamination by other bacteria through streaking on nutrient agar plates.

Luria-Bertani (LB) medium was used for growing bacterial cells to perform experiments. The constitution of this medium for each 1 liter as follows:

Tryptone	10 g
Yeast Extract	5 g
NaCl	10 g

These components were dissolved in 1 liter of distilled water, sterilized by autoclave at 121°C and 15 psi for 15 minutes. After cooling down, $CaCl_2$ was added using cold sterilization through 0.22 filter units, to deny chemical decomposition in high temperature in the autoclave (Abo-El-Enein *et al*., 2013).

Before implementing the tests, few colonies taken by loop and inoculated into 225 ml Luria-Bertani (LB) medium-containing flasks, placed in 150 rpm orbital shaker incubator set for 37°C for 24-48 h.

After incubation, the bacterial-containing medium was poured into polystyrene tubes (15 mL), centrifuged at 5000 rpm for 10 minutes, so as to discrete the cells from medium. The supernatant was dismissed and the pellet of cells was re-suspended in a phosphate buffer, mixed for homogenization and centrifuged again. This was repeated four times and the final pellet was re-suspended again in phosphate buffer solution, stored in refrigerator for 48 hours for spores' formation (Schwantes-Cezario *et al*., 2019). The number of bacterial cells was measured by a spectrophotometer that quantifies optical density.

Preparation of mortar was achieved through the weight of sand: cement ratio 3:1. After mixing, eggshell powder and limestone was added to the mixture, and then the needed amount of water was added at a W/C ratio of 0.46. Different specimens were prepared through the addition of phosphate buffer alone instead of water, and phosphate buffer solution containing bacterial spores. measured by spectrophotometer at wavelength of 600 nm. The pastes of fresh mortar were casted into the mold,

left for compaction by a vibration machine, then restored for 24 h in a humidity chamber with relative humidity of 100%. Control specimen were cured with tap water and all samples are tested after 7, 14, and 28 days (AL-Ridha *et al*., 2020).

The pastes of fresh mortar were casted into the mold, left for compaction by a vibration machine,

then restored for 24 h in a humidity chamber with relative humidity of 100%. Control specimen were cured with tap water and all samples are tested after 7, 14, and 28 days (Smitha *et al*., 2022).

The cylinders and cubes were prepared for concrete mixtures for all experiments. Cubes were 10 cm^3 while the cylinders diameter and height were 10 and 20 cm, respectively. These specimens were demolded after 24 h then cured in water bath for 7, 14, and 28 days (Gavimath *et al*., 2012).

The process of self-healing was visually observed using an inverted microscope to investigate healing because of bacterial precipitation of calcium carbonate at the top of the crack in each specimen (Chaerun *et al*., 2020).

Results and discussion:

Compressive strength of mortar is considered as one of the most efficient properties of hardened concrete, by which the durability and strength of concrete is affected. Other properties for solidifying concrete rely on several factors, like water to cement ratio, cement to sand ratio, curing period, and other various factors (Sandip and Aparna, 2018). The development in the tensile power of the cement-sand combination relies on the pores structural shape. Because of bacterial growth, a process of calcium precipitation occurs constantly, obstructing the pores with calcium deposits (Seshagiri *et al*., 2017)

The key point of the current study is to incorporate the bacterial cells unto cement mortar and left for the efficient activation. The potential biochemical reaction taken place in medium is catalysis the hydrolysis of urea,

by urease, to precipitate $CaCO_3$ at the surface of the cell (Senthilkumar *et al*., 2014). It was found that tensile intensity of mortar was increased in the existence of calcium carbonate precipitation bacterium, *Bacillus subtilis* subsp.

spizizenii ATCC 6633, increased up to 12.6% after 28 days of demolding (table 1), which indicates a powerful technique in the increasing durability of mortar by the process bio cementation.

Calcium carbonate can remove pores and compact cement-established materials, leading to enhanced intensity. Although the sediment may not completely block the void, the calcium carbonate sediment was necessary enough to increase the properties of the mortar (Ameri *et al*., 2019). The bacterial spores fixed in the concrete mold will turn metabolically active when they are revived by water and calcium medium in the concrete. The empty space will then be quickly blocked and closed by metabolic-mediated microbial $CaCO_3$ precipitation, blocking the entry of more water and other chemicals (Gavimath *et al*., 2012).

Table (1): Tensile strength of different specimens of mortar throughout the experiment:

average Tensile Strength (MPa)	After7 days	After14 days	After28 days
Mortar with water	**27.5**	**30.1**	**39.5**
Mortar with PBS (control)	**27.4**	**29.8**	**39.1**
Mortar with bacteria	**30.2**	**33.5**	**44.5**
Percentage of increasing	**9.8%**	**11.2%**	**12.6%**

The addition of bacterial cells can increase compressive strength during concrete construction, or curing, or even following curing process as a spray (Sumit *et al*., 2018).

The differences in the compressive strength of the mortar in the different studied periods, compared to the control one, can be clarified that spores have to sneak into the samples to reach the hydration products for reaction and calcium carbonate precipitation. Furthermore, spores may find it hard to penetrate the samples due to deposition. Perhaps, given enough time, this spore addition procedure could have been more effective. It can be indicated that the development in tensile strength is a result of improved compressive strength (Schwantes-Cezario *et al*., 2019)

Throughout the beginning of processing period, the microbial cells received enough nutrition, because the matrices of the cement was much porous; but their growth may not be appropriate because of the entirely new conditions for bacteria. It may also be potential that because the cement pH remained high, the cells were in arid conditions, and as raising of curing period, they began to grow quietly. Upon cell growth, calcite may precipitate on the surface as well as inside cement matrix. Once most of pores are blocked, the availability of oxygen and nutrients for bacterial cells hinders, and ultimately the cells either die or converted into endospores. This is related to an improve in the compressive intensity of the slurry cubes. This clarifies the behavior of the higher compressive intensity value after 28 days in the case of slurry cubes prepared with microbial cells (Bang *et al*.,2002; Abo-El-Enein *et al*., 2013).

Self-healing process:

The substantial standard for healing the small cracks and micro-cracks of concrete is self-healing, which also increases the strength and durability of structures. Under certain conditions, small cracks and micro-cracks can be healed. The metabolism of *Bacillus subtilis* subsp. *spizizenii* ATCC 6633 can also heal surface cracks, known as self-healing concrete. It was found that precipitation of Ca CO_3 is the most influential factor for self-healing of concrete (Tziviloglou *et al*.,2016) as shown in Figure 1.

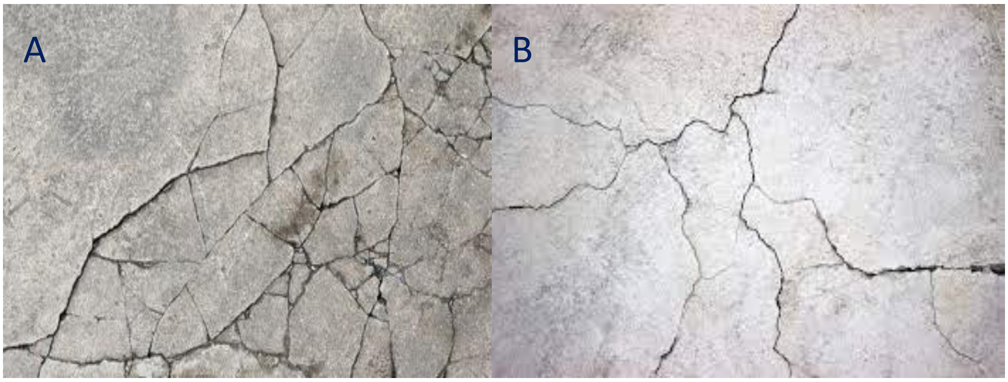

Figure (1): Small and micro-cracks of concrete under microscope; A: Before healing, B: after the addition of bacterial spores for healing activity.

The efficiency of self-healing process requires not only enough quantity of healing compounds, but also the least number of bacterial spores are needed to secure that during the healing procedure, there are appropriate number of cells taking part (Alazhari *et al*., 2018). Increasing cracking period and width will decrease the self-healing capacity of concrete (Luo *et al*., 2015).

In general, there is a better existence of calcium when incorporating spores, possibly because spores may have utilized calcium in cement products and in lime to precipitate $CaCO_3$ (Schwantes-Cezario *et al*., 2019).

Bacterial treatment of concrete could heal the excessive load on concrete that may lead to cracks (Bundura, 2015). Protecting the bacteria with silica-gel have revealed a preferable self-healing competence than non-protected bacterial cells (Tittelboom *et al*., 2010). If the spores were encapsulated by hydro-gel, the efficiency of self-healing process will be improved (Wang *et al*., 2014).

Conclusions:

This study revealed the ability of bacteria incorporated in the mortar mixture to promote its features and self-heal any formed cracks. The combination of bacterial cells, limestone, and eggshell could be suggested for a novel concrete, showing optimum improvement of the properties, despite the fact that the self-healing took place in most optimum condition, while reducing the repairing cost. This approach can be utilized as a candidate for innovative technology to convert the construction to more environmentally friendly and sustainable.

References:

- Abo-El-Enein, S.A., Ali, A.H., Talkhan, F.N. and Abdel-Gawwad. H.A. (2013). Application of microbial biocementation to improve the physico-mechanical properties of cement mortar. HBRC Journal. 9:36–40.
- Alazhari, M., Trupti, S., Andrew, H., Richard, C. and Kevin, P. (2018). Application of expanded perlite encapsulated bacteria and growth media for self-healing concrete. Construction and Building Materials. 160: 610–619.
- AL-Ridha, A.S.D., Atshan, A.F., Hussein, H.H., Abbood, A.A., Dheyab, L.S., Alshaikh Faqri, A.M. (2020). Evaluation of Tensile Strength and Durability of Microbial Cement Mortar. In: Shehata, M., Anastasopoulos, G., Norma, M. (eds) Recent Technologies in Sustainable Materials Engineering. GeoMEast 2019. Sustainable Civil Infrastructures. Springer, Cham.
- Ameri, F., Shoaei, P., Bahrami, N., Vaezi, M. and Ozbakkaloglu, T. (2019). Optimum rice husk ash content

and bacterial concentration in self-compacting concrete. Constr. Build. Mater. 222:796–813.

- Bang, S.S., Galinat, J.K. and Ramakrishnan, V. (2001). Calcite precipitation induced by polyurethane-immobilized Bacillus pasteurii, Enzyme Microb. Technol. 28:404–409.
- Bundura, Z. B., Amiria, A., Ersanb, Y. C., Boonc, N. and Belie, N. D. (2017). Impact of air entraining admixtures on biogenic calcium carbonate precipitation and bacterial viability. Cement and Concrete Research. 98:44–49.
- Chaerun, S.K., Syarif, R. and Wattimena, R.K. (2020). Bacteria incorporated with calcium lactate pentahydrate to improve the mortar properties and self-healing occurrence. Sci Rep. 10:17873.
- Dhami, N.K., Reddy, M.S. and Mukherjee, A. (2012). Improvement in strength properties of ash bricks by bacterial calcite, Ecol. Eng. 39:31–35.
- Gavimath, C.C., Mali, B.M., Hooli, V.R., Mallpur, J.D., Patil, A.B., Gaddi, D.P., Ternikar, C.R. and Ravishankera, B.E. (2012). Potential application of bacteria to improve the strength of cement concrete. International Journal of Advanced Biotechnology and Research. 3(1):541-544.
- Ghosh, S., Chattopadhyay, B.D. and Mandal, S. (2008). Use of hot spring bacteria for remediation of cracks and increment of durability of structures, Indian Concr. J. 82(9):11–16.
- Lian, B., Hu, Q., Chen, J., Ji, J. and Teng, H.H. (2006). Carbonate biomineralization induced by soil bacterium *Bacillus megaterium*, Geochim. Cosmochim. Acta. 70:5522–5535.

- Luo, M., Qian, C. and Li, R. (2015). Factors affecting crack repairing capacity of bacteria-based self-healing concrete. Construction and Building Materials. 87: 1–7.
- Marvasi, M., Davila-Varquez, Y.C. and Martinez, L.C. (2013). Supplemental Materials for Laboratory Activity to Effectively Teach Introductory Geomicrobiology Concepts to Non-Geology Majors. Journal of Microbiology Biology Education. 14(2):206-212.
- Poornima, V., Venkatasubramani, R., Sreevidya, V., Pavan, C. and Nourin, A. (2020) IOP Conf. Ser.: Mater. Sci. Eng. 872:012173.
- Ramakrishnan, V., Deo, K.S., Duke, E.F. and Bang, S.S. (1999). SEM investigation of microbial calcite precipitation in cement, in: Proceeding of the International Conference on Cement Microscopy, Las Vegas, Nevada. pp. 406–414.
- Sandip, M. and Aparna G. (2018). Investigation into the optimal bacterial concentration for compressive strength enhancement of microbial concrete. Construction and Building Materials. 183:202–214.
- Schwantes-Cezario, N., Porto, M.F., Sandoval, G.F.V., Nogueira, G.F.N., Couto, S.F. and Toralles, B.M. (2019). Effects of Bacillus subtilis biocementation on the mechanical properties of mortars. SciELO journals.
- Senthilkumar, V., Palanisamy, T. and Vijayakumar, V.N. (2014). Enrichment of compressive strength in microbial cement mortar. Advances in Cement Research. 26(6):353–360.
- Seshagiri, R.M.V., Srinivasa R.V. and Sasikala, Ch. (2017). Performance of microbial concrete developed using bacillus subtilus JC3. J. Inst. Eng. India Ser. A.

- Smitha, M.P., Suji, D., Shanthi, M. and Adesina, A. (2022). Application of bacterial biomass in biocementation process to enhance the mechanical and durability properties of concrete. Cleaner Materials. 3:100050.
- Sumit, J., Shweta, G. and Sudhakara, R.M. (2018). Influence of nutrient components of media on structural properties of concrete during biocementation. Construction and Building Materials. 158:601–613.
- Tittelboom, K.V., Belie, N.D., Muynck, W.D. and Verstraete, W. (2010). Use of bacteria to repair cracks in concrete. Cement and Concrete Research. 40:157–166.
- Tziviloglou, E., Wiktor, V., Jonkers, H.M. and Schlangen, E. (2016). Bacteria-based self-healing concrete to increase liquid tightness of cracks. Construction and Building Materials. 122: 118–125.
- Wang J.Y., Van Tittelboom K., De Belie N.M. and Verstraete W. (2010). Potential of applying Bacteria to heal cracks in concrete. Second International conference on sustainable construction materials and technologies.
- Wang, J.Y., Snoeck, D., Vlierberghe, S. Van, Verstraete, W. and De Belie, N. M. (2014). Application of hydrogel encapsulated carbonate precipitating bacteria for approaching a realistic self-healing in concrete. Construction and Building Materials. 68:110–119.
- Zhang, Z., Weng, Y., Ding, Y. and Qian, S. (2019). Use of Genetically Modified Bacteria to Repair Cracks in Concrete. Materials (Basel). 12(23):3912.