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Self-Healing Concrete by Microorganisms in the Altered Pozzolan of Madagascar with Calcium from Crab Wastes

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ABSTRACT

Concrete is at a high risk of cracking which threats its durability. Self-healing bacterial concrete has been developed in recent years to tackle this issue and its effectiveness has been massively studied. However, bacteria need carriers and expanded clay is the most used as such, but it reduces concrete strength. Moreover, bacterial culture and immobilization processes are highly expensive; calcium salts also contribute to the high cost of this type of concrete. This work therefore aims to overcome these limitations. Calcium salts were collected from the chitosan production process from crab waste. In addition, we noticed that Betafo pozzolan contains an abundant number of microorganisms, and for the first time, we discovered that those microorganisms have polyextremophilic characters, are resistant to various sterilization methods, and allow the self-healing concrete process. We concluded that calcium salts from the crab, with those microorganisms are able to heal crack up to 350 µm wide, reducing the expenses related to nutrients, and eliminating those related to the growth and the immobilization of bacteria on carrier. Moreover, we pointed out that pozzolan significantly increases compressive strength by 90.04%. However, the behavior of those microorganisms and the pozzolanic reactions need to be further investigated.

INTRODUCTION

Concrete is today the most consumed manufactured material in the world with a global production of cement, an essential component of concrete, of 4.4 billion tons in 2021 (U.S. Geological Survey, 2022). However, the microcracks apparition in it is almost inevitable, altering its functionality, accelerating its degradation and reducing its lifespan, deterioration can begin in less than 10 years (Moriconi, 2009). Additionally, recycling the concrete is difficult and expensive, reduces its strength, and can catalyze chemical reactions that accelerate decomposition (Meyer, 2002). As huge expenses are incurred for the maintenance and repair of structures, it is more profitable to demolish and build a new one. Indeed, in the United States, the annual economic impact associated with the maintenance, repair or replacement of deteriorating structures is estimated at 18-21 billion dollars (SDC, 2004). Moreover, emissions from the manufacture of cement amount to 1.5×10^9 tones of CO_2 (Andrew, 2019). Concrete also makes up the largest proportion of construction and demolition waste, and accounts for about a third of all landfill waste (Meyer, 2002). It is therefore necessary to stop cracking in its initial phase.

The idea of self-healing bacterial concrete was introduced at the end of the 2000s. In contact with water, the spores of certain species are activated and precipitate calcium carbonate (Jonkers and Schlangen, 2007). Bacterial spores have low metabolic activity and an extremely long lifespan of up to 200 years (Schlegel, 1993).

The main problem with bacterial concrete is that the spores need carriers to survive in the harsh environment of concrete and the clay granules, usually used as a carrier for the self-healing agent, lead to a considerable reduction in the compressive strength (Tziviloglou *et al.*, 2016). This type of concrete is also more expensive than the usual concrete. Silva *et al.* (2015) even reported that the production of 1 m^3 of self-repairing concrete would be 5760 €/m^3 due to the processes for obtaining the spores and their encapsulation in a carrier. The organic calcium salts, such as calcium lactate, calcium acetate, etc., also contribute to these exorbitant costs.

On the other hand, the seafood industry generates annually about 106 tons of waste (Schmitz *et al.*, 2019). Not only do the wastes of these industries cause a significant part of the contamination of the environment, but they also give off unpleasant odors (Nguyen and Wang, 2019). Chitosan is an obtained from shrimp and crab shells (Munoz *et al.*, 2018) by several stages, including demineralization with an acid to remove minerals, mainly calcium.

On the other hand, volcanic rocks can harbor a remarkable microbial diversity despite their hostile environment to microorganisms (Byloos *et al.*, 2018).

In all this context, we therefore stated the following hypotheses: that the salts from crabs would allow the precipitation of $CaCO_3$ as much as the usually used salts does; that the microorganisms naturally present in pozzolan, a volcanic rock, could precipitate $CaCO_3$; and that the pozzolan bacterial concrete would be stronger than the usual bacterial concretes.

This study was carried out to develop a self-healing concrete where the carriers of bacteria do not lead to a reduction or improve its compressive strength, and where the expenses related to calcium salts and bacteria are reduced.

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MATERIALS AND METHODS

Bacterial strain

In order to evaluate the efficiency of the repairs by the microorganisms present in the pozzolan, *Bacillus pseudofirmus* was chosen as a control. It is an ideal candidate since it has shown promising results in mortar specimens through the metabolic conversion of organic acids (Jonkers *et al*., 2010). It is also non-pathogenic. *Bacillus pseudofirmus* strain DSM 9718 was purchased at the Leibniz Institute DSMZ.

Immobilization carrier

As mentioned earlier, the spores need carriers to survive when the concrete sets. However, as expanded clay and perlite, the most used mineral carriers for bacterial concretes (Tziviloglou *et al*., 2016) (Wiktor and Jonkers, 2011) (Alazhari *et al*., 2018) are very less resistant (Magaji *et al*., 2019), pozzolan was chosen. Its porous nature allows it to serve as a carrier. The natural pozzolan is a volcanic rock, and was collected from the Iavoko-Betafo mountain deposit, Madagascar (19°51'50"S 46°53'52"E 1556 m).

Calcium source

Crab wastes are potential source of calcium (as carbonate). They can be valorized, by extracting chitin and obtaining the chitosan, by processes of demineralization, deproteinization and deacetylation (Santos *et al*, 2020). It is the calcium salts from the first step that we collected in this study. Crab wastes were provided by the ANTARTICA society (Antananarivo, Madagascar) and contain 16.70% of calcium.

Demineralizing acids

As calcium lactate is the reference calcium source in the field of bacterial self-healing concrete (Jonkers and E. Schlangen, 2007) (Tziviloglou *et al*., 2016) (Jonkers and E. Schlangen, 2008), lactic acid (RESEARCH-LAB, Assay 88,0-92,2%, d=1,20-1,21) was used for demineralization. As acetic acid is one of the most used organic acids for the demineralization of crustaceans for the extraction of chitin (Gbenebor *et al*., 2018) (Baron *et al*., 2015), it was also used in this study (Acetic acid: MERCK, Assay 99,8%, d=1,05).

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Culture

The *Bacillus pseudofirmus* spores were obtained according to (Jonkers and E. Schlangen, 2008). The concentration was adjusted to 10^7 cells/ml.

Pozzolan Sterilization

The pozzolan was initially autoclaved before incorporating the *Bacillus pseudofirmus* strain. However, during the counting of the incorporated spores in the pozzolan, we noticed that the latter harbors an abundant quantity of microorganisms. Various sterilization methods were therefore carried out: in an autoclave at 121°C for 20 or 40 min, or in an oven at 180°C or at 30°C or at 60°C or at 120°C, or with 70% ethanol for 15 h, or with 12°Cl commercial bleach for 15 h, or in a muffle furnace (Thermo Heraesus) at 600°C for 3 h or 6 h, or in a charcoal flame for 10 or 20 min. The concentration of microorganisms in pozzolan was determined by bringing 1 g to 10 ml with sterile distilled water in a centrifuge tube, then mixed by vortex (CYAN CL001) for 60 s and the liquid was passed to the Thoma counting chamber for the enumeration. 100 µl of the liquid was injected into TSA medium (Trypton Soy Agar: ROTH) for a sterility test, and incubated for 48 h. Viability was calculated according to Eq. 1.

$$\vartheta = \frac{C_i}{C_0} \qquad (Eq.\ 1)$$

where

ϑ: Viability; C_i: Concentration of microorganisms in the sterilized pozzolan; C_0: Concentration of microorganisms in unsterilized pozzolan

In order to ensure that there were no other contamination sources, the sterile distilled water used was treated with the post-sterilization procedure.

Immobilization of spores

The pozzolan was sieved to have two series of grain sizes: 1-2 mm and 2-4 mm. In order to incorporate the spores into the pozzolan, the latter was mixed with the spore suspension until it was completely immersed. The whole was placed under vacuum for 5 min to eliminate air pockets and thus facilitate the penetration of the suspension, then filtered. Pozzolan was dried at room temperature for 6 days.

Preparation of calcium salts

Calcium carbonate (ROTH, Assay≥98.5%) and lactic acid were mixed in stoichiometric proportions. The acid concentration in the solution was 1 M. The same procedure was applied for acetic acid. The crab wastes were treated with the two acids in the same ways, replacing the mass of $CaCO_3$ with the equivalent mass of $CaCO_3$ present in the previously sieved crab wastes (0.2-1 mm). The solutions, brought to 100 ml, were stirred at 100 rpm for 24 h at 30° C, then centrifuged at 3000 rpm for 10 min. The calcium concentration was measured by atomic absorption spectrometry (VARIAN SpectrAA.20) with a wavelength of 424 nm. The solutions were dried at 60°C, the salts were collected in the oven and immediately hermetically packaged. Indeed, in contact with air, the salts from crabs became pasty and sticky. Their purities were evaluated by drying 20 ml of the solution resulting from the dissolution of crude $CaCO_3$ or from the demineralization of crab wastes, according to Eq. 2.

$$p = \frac{c \times V_{Sol} \times M_{Salt}}{m_{exp} \times M_{Ca}} \quad (Eq.\ 2)$$

where

p: Purity of the salt obtained (calcium lactate or calcium acetate); c: Measured calcium concentration V_{Sol}: Volume of the solution to be dried; M_{Salt}: Molar mass of the salt obtained; m_{exp}: Measured salt mass; M_{Ca}: Molar mass of calcium.

Formulation of the concrete

The mix designs are given in Table 1. As the roughness of the specimens did not allow the observation of cracks, superplasticizer was added to deflocculate the cement particles. A more or less smooth surface was achieved at 3% superplasticizer relative to the cement mass. The calcium salts were dosed in the same molar concentrations. Specimens were cured at 20±2°C and 95% humidity.

Concrete mix designs without pozzolan (denoted B) and with unincorporated pozzolan (denoted P) were carried. Concrete mix designs with *Bacillus pseudofirmus* incorporated pozzolan were denoted Bp. For the concrete mix designs without pozzolan, the volume occupied by the pozzolan was replaced by the equivalent volume in sand and the water was replaced by the suspension of spores. Specific gravities of pozzolan and sand were measured according to (ASTM, 2019).

Table 1: Concrete mix designs per liter

Specimen		Mortar control	B	Concrete control	P	Bp
2-4 mm Pozzolan	[g]	-	-	330	330	-
1-2 mm Pozzolan	[g]	-	-	113	113	-
2-4 mm incorporated Pozzolan	[g]	-	-	-	-	330
1-2 mm incorporated Pozzolan	[g]	-	-	-	-	113
0,500-1,000 mm Sand	[g]	943	943	483	483	483
0,200-0.500 mm Sand	[g]	712	712	364	364	364
0,125-0,200 mm Sand	[g]	74	74	38	38	38
Cement CEM I 42,5	[g]	463	463	463	463	463
Water	[g]	231,5*	231,5*	231,5	231,5	231,5
Calcium salts	[mol]	-	0,03	-	0,03	0,03

** spore solution*

Concrete Repair

For the creation of cracks, concrete was cast in molds of dimensions 160 mm x 40 mm x 40 mm reinforced with two steel wires of 1 mm in diameter to avoid total fracture during the three-point bend test. A three-point bend test was used for crack introduction on the 28-day specimens. For specimens without pozzolan, this test took place after 7 days of curing. Indeed, without immobilization carrier, the viability of the spores decreases drastically after 28 days (Jonkers *et al*., 2010). The specimens were placed horizontally in a container containing tap water for crack healing, the specimens were immersed leaving 2 to 3 mm in height of emerged part to ensure an exchange of O_2 and CO_2 with the air. The container was kept open to atmosphere at standard room temperature (20±2°C) and specimens were allowed to repair for 56 days, additional water was added to maintain a constant water level.

Adopted experimental procedure for measuring the water absorption rate of repaired concrete

As there is not yet any established method to determine the ability of repaired concrete to resist water absorption (Alazhari *et al*., 2018), the rate of absorption before and after repair were evaluated to determine the reduction of absorption rate. The initial absorption rate was evaluated by immersing specimens in tap water for 24 h. The absorption rates before and after self-healing were measured and the reduction of the absorption rate is given in Eq. 3. In order to evaluate their dry weight, specimens were dried at 60°C in an oven for 24 hours.

$$\eta = \frac{\tau_0 - \tau_{56}}{\tau_0} \text{ ; where } \tau_n = \frac{w_{wn} - w_{dn}}{w_{dn}} \quad Eq.\ 3$$

η: Reduction of absorption; τ_i: Absorption rate at time i days; w_{wn}: Wet weight of the specimens after n days; w_{dn}: Dry weight of the specimens after n days

for measurement of initial absorption rate, n = 1 day

for measurement of absorption rate after 56 days of healing, n = 56 days

Repair follow-up

Weekly measurements of maximum healed fissure width were measured. The images of the cracks are taken by a stereomicroscope (LEICA EZ4E) and analyzed by the ImajeJ 1.52 software (Rasband, 2018).

Effect on strength of calcium salts and pozzolan added to concrete

The influence of calcium salts (calcium lactate and acetate resulting from the respective treatments of $CaCO_3$ and crab wastes with lactic and acetic acid) as well as the influence of pozzolan were evaluated. Compressive tests were thus carried out after 28 days of curing and according to (Sierra-Beltran *et al*., 2014).

Data Analysis

The ANOVA followed by a Bonferroni post-hoc test was used to identify significant differences. All data were processed by ANOVA after the conditions of normality (Shapiro-Wilk test (α=0.05)) and homogeneity (Levene's test (α=0.05)) were met. Data were statistically analyzed using XLSTAT software (Addinsoft Corporation) (Addinsoft, 2014).

differences of viabilities of microorganisms between pozzolan sterilization methods; differences from 56-days repair of each salt to identify their respective stagnation times; between the effects of salts on compressive strength; between the compressive strengths of specimens with and without pozzolan: 1-way ANOVA;

differences between the absorption rates according to the salts and according to the concrete compositions;

between the reductions of the absorption rate: 2-way ANOVA;
differences between the repairs of the concrete at each instant regarding the composition; between the repairs of the concrete at each moment regarding the salt: repeated measures ANOVA.

RESULTS AND DISCUSSION

Pozzolan Sterilization Methods

After counting the microorganisms naturally present in pozzolan, their number is $5.21 \times 10^8 \pm 0.23 \times 10^8$ cells/g. The growth of microorganisms after the sterility test on the TSA medium revealed the ineffectiveness of the various sterilization methods, without even taking into consideration viable but non-cultivable microorganisms.
Since the number of microorganisms in the pozzolan which have undergone sterilization methods is of the order of 10^8/g, which is equivalent to the concentration per milliliter of a rich and bacteria-saturated inoculum, the hypothesis of external contamination is discarded.

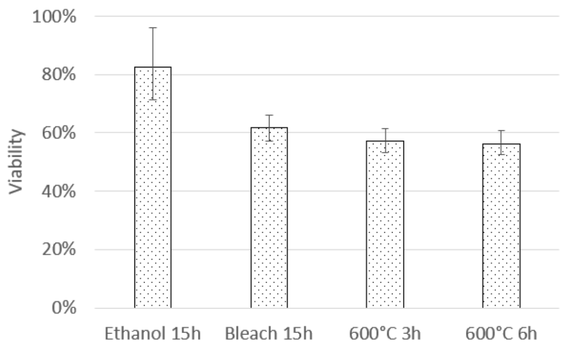

Figure 1: Viability of microorganisms after different sterilization methods

Figure. 1 shows the resistance of the microorganisms naturally present in the pozzolan toward the different sterilization methods, thus showing that these microorganisms are polyextremophiles.
Exposures to 600°C for 3h and 6h show no significant difference $(p=0.819)^{\alpha=0.008}$. Herrera *et al.* (2008) found a total number less than 104 cells/gram in obsidian, a volcanic rock, which is lower than the number found in our pozzolan. The honeycomb structure of pozzolan would allow it to harbor more microorganisms while protecting them from the hostile external environment. Byloos *et al.* (2018) reported 6.80×10^7 to 7.54×10^8 cells/g of basalt, in Iceland, of which 33% to 67% of the operational taxonomic units (OTUs) are unclassifiable.
The viability of these microorganisms with heat would be explained on the one hand by the low thermal conductivity of pozzolan (Rocher, 1992) which would play a protective role.
Since many phylotypes in volcanic rocks cannot be attributed to known bacterial phyla, thus showing that these environments harbor new bacterial diversities (Herrera *et al.*, 2009), it is difficult to deeply explain their behaviors towards hostile environments. Some authors (Ciera *et al.*, 2014), (Beladjal *et al.*, 2018) reported microorganisms with some extremophile characters and concluded that these microorganisms could contain a very versatile and efficient DNA repair mechanism capable of reversing heat-induced damage (Ciera *et al.*, 2014),. However, this is the first time we discover that some microorganisms are still able to live after long period of exposition to heat, to bleach and to ethanol. The high viability in ethanol could be explained by the fact that ethanol could serve as carbon source, allowing the growth of some microorganisms.

Concrete Repair

In order to evaluate the repair of pre-cracked concrete, the reduction of the absorption rate after 56 days of immersion in water, as well as the evolution of the crack repair were monitored.

Absorption Rate

Since the precipitation of $CaCO_3$ by the microorganisms fills the voids within the concrete, the reduction in the absorption rate was evaluated.

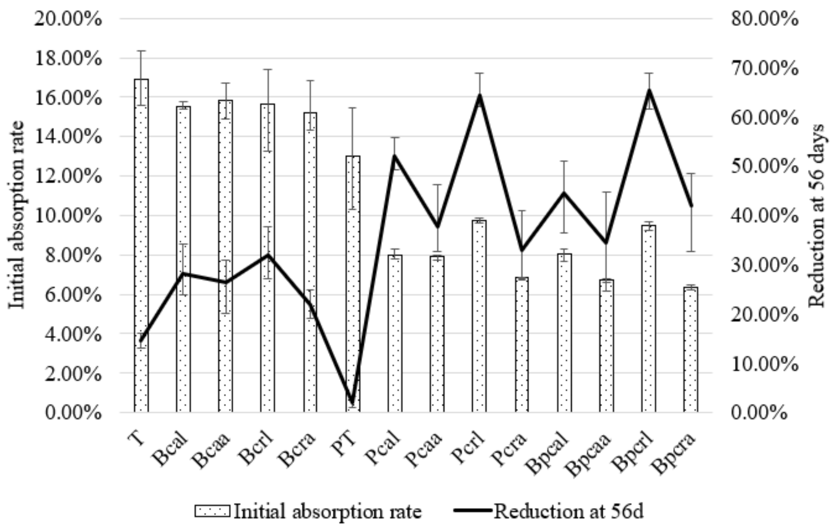

Figure 2: Initial absorption rate of concrete specimen and reduction of this rate after repair

(T) control (PT) control with pozzolan (B…) specimens without pozzolan (P…) specimens with pozzolan (Bp…) specimens with *Bacillus pseudofirmus* incorporated pozzolan (.ca.) specimens with salts from $CaCO_3$ (.cr.) specimens with salts from crab (…l) specimens with salts from lactic acid (…a) specimens with salts from acetic acid

Figure 2 shows the absorption rate of the concrete specimens before the self-healing process and the reduction of this rate after 56 days of self-healing. Before self-repair, the lactic acid salts CaL (calcium lactate from $CaCO_3$) and CrL (calcium lactate from crab) have no significant influence (p=0.010 and p=0.058 respectively) $^{\alpha=0.005}$ on the concrete. The acetic acid salts CaA (calcium acetate from $CaCO_3$) and CrA (calcium acetate from crab) significantly reduces (p=0.005 and p=0.001 respectively) $^{\alpha=0.005}$ the rate of absorption.

The low initial absorption rate of specimens with acetic salts would be due to the fact that the process of calcium carbonate precipitation is faster during the concrete setting. As calcium acetate is composed of a simpler structure (2 chains with 2 carbons) than calcium lactate (2 chains with 3 carbons with an additional alcohol function on each chain), it would be more easily transformed by microorganisms and the precipitated $CaCO_3$ improves the cohesion of the elements of the concretes and reduces the available volume for water.

The compositions without pozzolan B show a significantly $(p<0.0001)^{\alpha=0.017}$ high initial absorption rate compared to the other compositions.

The high absorption rate of compositions B is due, on the one hand, to the fact that they are only 7 days old before having undergone immersion, which is insufficient to complete all the chemical reactions in itself. On the other hand, pozzolan chemically reacts by pozzolanic reactions which defines the ability of mineral substances to react with calcium hydroxide in the presence of water. The silicate and aluminate ions produced in contact with the Ca^{2+} ions form hydrated silicates of the CSH type, calcium aluminate C_4AH_{13}, hydrated gehlenite C_2ASH_8, and $C_3A.CaCO_3.12H_2O$ (Navrátilová and Rovnaníková , 2014). A denser microstructure is thus created, reducing porosity. However, these reactions usually take place when the pozzolan is reduced to a fine powder form (ASTM, 2007), which is not the case in this work. It would be therefore difficult to comment on the effect of the pozzolanic activity.

After 56 days of repair, all salts provided significant reductions in absorption rate (p<0.0001 for all four salts) $^{\alpha=0.005}$.

Reductions in absorption rates after 56 days of repair are caused by $CaCO_3$ precipitation.

The CrL salt has the most influence (p<0.0001 with the other salts)$^{\alpha=0.005}$ with reductions of 32.25±5.38%, 64.29±3.83% and 65.35±3.60 % respectively for compositions B, P and Bp. The CaL salt has less influence $(p<0.0001)^{\alpha=0.005}$ than the CrL salt but has more influence than the acetic acid salts (p=0.002 with CaA, p=0.001 with CrA)[5]. No significant difference $(p=0.833)^{\alpha=0.005}$ was observed between CaA and CrA acetic acid salts.

An insoluble colorless gel, probably resulting from the demineralization of the crab by lactic acid, forms on the specimens with the CrL salt, which would provide additional impermeability. Indeed, some myofibrillar proteins play a key role in the flesh gelation (An *et al.*, 1996), they would have been solubilized by lactic acid during demineralization.

The great reduction of lactic salts is explained by the fact that even if the acetic acid salts are easily transformed by microorganisms, the CO_2 produced which will react with the portlandite $Ca(OH)_2$ is greater with the transformation of the salts of lactic acid and the yield of $CaCO_3$ is higher.

calcium lactate:

$(CH_3\text{-}CHOH\text{-}COO)_2Ca + 6O_2 \rightleftarrows CaCO_3 + 5CO_2 + 5H_2O$ Eq. 4

calcium acetate:

$(CH_3\text{-}COO)_2Ca + 4O_2 \rightleftarrows CaCO_3 + 3CO_2 + 3H_2O$ Eq. 5

$CO_2 + Ca(OH)_2 \rightleftarrows CaCO_3 + H_2O$ Eq. 6

The reductions are significantly (p<0.0001 with P and $Bp)^{\alpha=0.017}$ lower for specimens without pozzolan. No significant difference was observed between the P and Bp specimens $(p=0.958)^{\alpha=0.017}$. However, even if no significant difference $(p=0.010)^{\alpha=0.0004}$ was observed, the reduction is higher (14.52±1.57%) with the control of compositions B compared to that of the control of specimens with pozzolan (3.08±2.08%).

The low reductions caused by the B specimens are due to the loss of viability of the spores over time since they are not protected by a carrier (Jonkers *et al.*, 2010), the amount of $CaCO_3$ produced in the specimens without pozzolan is then limited. On the other hand, over time, the reactions within them continue during the first 21 days of immersion, increasingly reducing its porosity as shown with the controls of each composition. The incorporation of *Bacillus pseudofirmus* does not bring more influence on the absorption rate, giving similar absorption rates to the P and Bp compositions. Han *et al.* (2019) reported that only 1% per milliliter of spores in the inoculum is actually incorporated per gram of carrier, which is equivalent to 10^5 spores/g of pozzolan, which is negligible compared to the number of microorganisms present per gram of pozzolan.

Evolution of the Repair

Crack widths are reduced during immersion by the precipitation of $CaCO_3$. The changes in this reduction were evaluated according to the composition of the concrete and the nature of the salts introduced.

Figure. 3 shows the evolution of the maximum widths crack repaired with the CrL salt for the compositions without pozzolan B, with pozzolan P and with incorporated pozzolan of *Bacillus pseudofirmus* Bp. On the 7th day, all the compositions show significant repairs (p=0.004 for B; p<0.0001, for P and $Bp)^{\alpha=0.005}$ compared to their respective controls. They do not show significant differences between them (p>0.030 between them)$^{\alpha=0.005}$. On the 14th day, B composition shows a significantly high repair compared to P ($p<0.0001$)$^{\alpha=0.005}$ and Bp ($p=0.0001$)$^{\alpha=0.005}$ compositions, this trend is the same for the 21st day. After 28 days, the repairs for each composition are significantly ($p>0.024$ between them) $^{\alpha=0.005}$ similar. The absence of difference between the three compositions is noted until the 49th day and on the 56th day, P composition presents a significantly ($p=0.003$)$^{\alpha=0.005}$ high repair compared to B composition. The absence of difference between P and Bp is observed from 7 to 56 days ($p>0.020$ for each day)$^{\alpha=0.005}$.

The high efficacy of B composition at 14 and 21 days would be due to its high porosity, as illustrated in Figure. 2 and explained previously. This porosity would facilitate the diffusion of microorganisms and chemical species within it. However, over time, the hydration reactions of the concrete reduce the porosity, leading to a loss of spore viability without protection (Jonkers *et al*., 2010).

For the control specimens, the one without pozzolan shows a repair of 96.7±7.5 µm at 56 days, the one with pozzolan amounts to 29.0±2.0 µm. The difference between the two controls is significant ($p=0.002$)$^{\alpha=0.005}$.

Concrete is known to have autogenous self-healing properties: the cement particle hydration process would continue thanks to the incomplete hydration of young specimens (7 days) and the precipitation of $CaCO_3$ crystals due to the carbonation of the calcium hydroxide. Reported maximum crack widths range from 100 to 300 µm depending on exposure conditions (Wiktor and Jonkers, 2011). As B composition had not completed the hydration process, the proportion of unhydrated cement was relatively considerable, and as the compositions with pozzolan were 28 days old before the repair process the proportion of unhydrated cement is reduced.

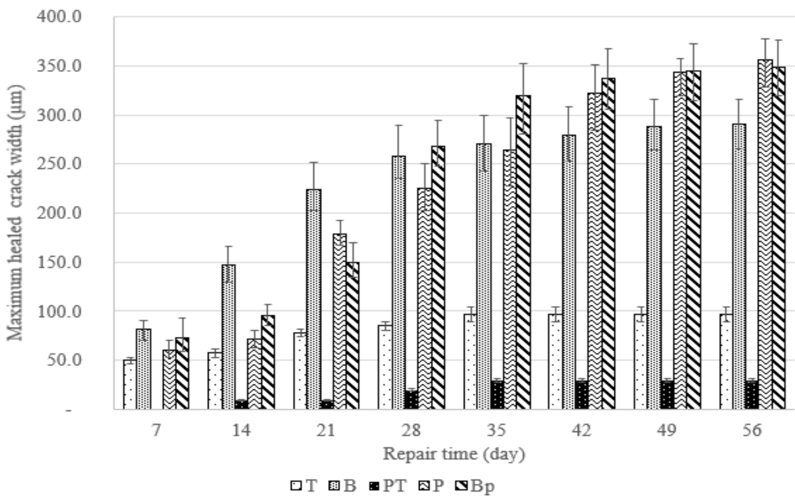

Figure 3: Evolution of the repair according to the composition of the concrete

(T) control (PT) control with pozzolan (B) specimens without pozzolan (P) specimens with pozzolan (Bp) specimens with *Bacillus pseudofirmus* incorporated pozzolan

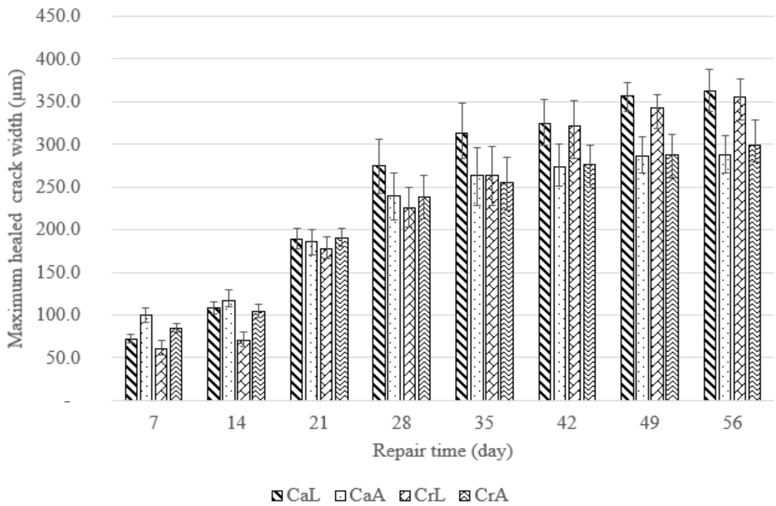

Figure 4: Evolution of the repair according to the salts

(CaL) Concrete specimens with salts from $CaCO_3$ and lactic acid (CaA) Concrete specimens with salts from $CaCO_3$ and acetic acid (CrL) Concrete specimens with salts from crab and acid lactic acid (CrA) Concrete specimens with salts from crab and acetic acid

Figure 4 illustrates the weekly evolution of the repairs according to salts with the P composition. On the 7th day, the repairs by CaL and CrL do not show significant differences (p=0.095) $^{\alpha=0.008}$, the same for those by CaA and CrA (p =0.030)$^{\alpha=0.008}$, repairs by CaA is significantly (p=0.001)$^{\alpha=0.008}$ higher than those by CaL, likewise repairs by CrA are higher than those by CrL (p=0.003)$^{\alpha=0.008}$. On the 14th day, the repairs by CrL are significantly lower (p<0.002 against the three others)$^{\alpha=0.008}$ compared to those by the other salts which are significantly the same (p>0.105 between them)$^{\alpha=0.008}$. On day 21, no significant difference was observed between the effects of the salts (p>0.265)$^{\alpha=0.008}$. This absence of significant difference is observed until the 42nd day (p>0.058)$^{\alpha=0.008}$ and on the 49th day, the repairs by the CaL salt are significantly higher compared to those by the acetic salts CaA (p=0.004) $^{\alpha=0.008}$, any significant difference was not observed with the repairs by CrL (p=0.448)$^{\alpha=0.008}$ nor between those by CaA and by CrA (p=0.898)$^{\alpha=0.008}$. This trend is the same for the 56th day.

The reason why repairs by salts from acetic acid are initially higher than those from lactic acid is explained by the fact that they would be easily transformed by microorganisms as explained previously. However, the final yield of the lactic salts is increased by the reaction with portlandite $Ca(OH)_2$ with the CO_2 produced during the transformation which is 5/3 times greater than the CO_2 produced by the transformation of the acetic salts, as explained previously. The final efficiencies between salts from the same acids are the same since they had the same initial concentration and would be equally transformed despite the purity difference between them. It was previously explained that the compositions containing the CrL salt showed a great reduction of the absorption rate with the formation of an insoluble gel, but this gel would slow down the diffusion of microorganisms and chemical species, which reduces its repair speed. The stagnation would be due to the fact that the microorganisms are enveloped by impermeable layer of $CaCO_3$, preventing them from further metabolizing. The addition of 10% hydrochloric acid confirmed, by effervescence, the presence of $CaCO_3$ in the specimens.

Effect of salts and pozzolan on compressive strength

Salts added to concrete might positively or negatively change the compressive strength. Moreover, the strength of the concrete specimen control with pozzolan was evaluated in order to observe whether it has any influences on the concrete.

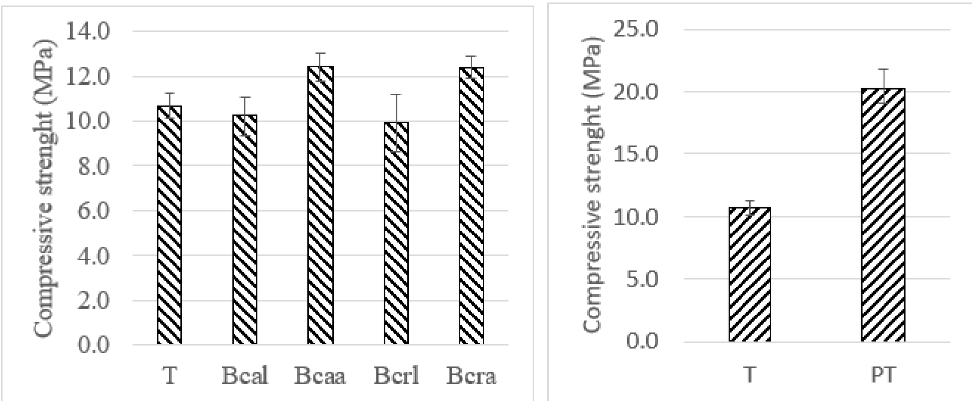

Figure 5: Compressive strength of the specimens

(T) control (PT) control with pozzolan (.ca.) specimens with salts from $CaCO_3$ (.cr.) specimens with salts from crab (…l) specimens with salts from lactic acid (…a) specimens with salts derived from acetic acid

Figure. 5 shows the effect of the four salts on B composition. Although lactic salts seem to reduce the compressive strength (by 3.66% and 6.70% respectively for CaL and CrL), these reductions are not significant (p=0.910 for CaL, p=0.599 for CrL compared to the control)$^{\alpha=0.005}$. The acetic salts also have no significant influence (p=0.022 for CaA, p=0.024 for CrA compared to the control)$^{\alpha=0.005}$ on the compressive strength. Whether from $CaCO_3$ or from crab, the influences on resistance do not differ (p=0.644 between CaL and CrL, p=0.938 between CaA and CrA)$^{\alpha=0.005}$.

These results contradict those of Jonkers *et al.* (2010) who observed a reduction in strength of about half by adding calcium acetate at a rate of 1% of cement weight. However, Cao *et al.* (2021) observed a 15.43% increase in compressive strength with a simple replacement of water with a 2% calcium acetate solution. They indeed concluded that this resistance varies according to the quantity introduced and concentrations of 1% and 3% reduce the resistance.

The compressive strengths of the P compositions are significantly higher than those of the B compositions (p=0.003)$^{\alpha=0.005}$, the P control has a resistance 90.04% higher than the B control.

Tzivilogiou *et al.* (2016) reported that replacing part of the sand with expanded clay leads to a considerable reduction in compressive strength. Pozzolanic reactions, if they take place, reduce porosity, leading to an increase in strength (Mindess *et al.*, 2003). Moreover, Othman *et al.* (2021) established that the compressive strength is directly proportional to the density of concrete. As the density of pozzolan is 2.459±0.062, whereas expanded clay's is 0.789±0.094 (Jonkers, 2011), specimens containing pozzolan are denser. Therefore, pozzolan offers a better option for the immobilization of microorganisms since it contributes to the increase in resistance.

Economic Outcome

Research on the self-healing concrete has been developed over the past 15 years, including the one by bacterial precipitation of $CaCO_3$. However, the biosourced additive, composed of encapsulated spores involves exorbitant prices. The additional costs added to the concrete amount to 5760 €/m^3 while the concrete itself costs 60 to 70 €/m^3 (Silva *et al*., 2015). These high prices are mainly due to the need for aseptic conditions to produce the spores, the expensive culture media, the methods of immobilizing the spores in the carrier, the amount of labor required and the sources of calcium.

Since the microorganisms used in this study are already present in pozzolan, the costs associated with the production of spores and the encapsulation processes are zero.

Since the calcium salts used are waste from chitosan production process and as they are usually thrown away, the costs associated with obtaining them are reduced.

With further evaluation, our approach is more and more interesting. The seafood industry generates about 10^6 tons of waste annually (Schmitz *et al*., 2019). About 2000 tons of chitosan are produced annually with a production cost of 8.39 $/kg and the market price is estimated at 58 $/kg (Riofrio *et al*., 2021).

CONCLUSION

The main objective of this study was to develop a self-healing concrete where the carriers of bacteria do not lead to a reduction or improve its compressive strength, and where the expenses related to calcium salts and bacteria are reduced. Results show that microorganisms in the altered pozzolan of Betafo present polyextremophilic features can convert organic calcium salts into calcium carbonate and thus, healing concrete cracks. Calcium from crab waste as well can be converted by those microorganisms. Therefore, the production cost is drastically reduced. Furthermore, pozzolan increases compressive strength of the mortar. Nevertheless, the effects of pozzolanic reactions on the self-healing process remains to be clarified. Moreover, as the microorganisms found in the pozzolan are polyextremophiles, the understanding of their behavior and origin might be useful for the life researches outside the Earth.

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