EFFECTS OF BACILLUS SUBTILIS ON THE COMPRESSIVE STRENGTH, POROSITY AND RAPID CHLORIDE PERMEABILITY OF CONCRETE

Thanh Ha Nguyen, Elhem Ghorbel*
University of Cergy Pontoise, L2MGC, 5 Mail Gay Lussac, Neuville/Oise 95031, France
*Corresponding author; e-mail: elhem.ghorbel@u-cergy.fr

Abstract

Durability enhancement of concrete structures is an interesting issue which is fascinating many scientists in all fields. The application of bacteria in concrete has become an important area and a promising solution for sustainable concrete. This study investigates the effects of nutrients and bacteria *Bacillus Subtilis* on compressive strength, porosity, water absorption and rapid chloride permeability at different ages. Test results indicate that the addition of *Bacillus Subtilis* enhances the compressive strength and reduces the porosity as well as the chloride permeability of concrete. The main production of this processing is the microbial precipitation ($CaCO_3$) induced by the metabolism of bacteria. This precipitation, by filling up the pores, reduces the porosity and makes the concrete denser. This phenomenon enhances the resistance to chloride ingress and increases the compressive strength. The finding of this investigation indicates that bacterium with nutrients could act as a promising admixture and could improve the durability of cementitious materials.

Keywords:
Bacteria, Compressive strength, Porosity, Rapid chloride permeability, SEM.

1 INTRODUCTION

Many researchers studied the possibility of using supplementary cementitious materials such as silica fume, fly ash, metakaolin, etc. in the concrete to enhance mechanical and durability properties. However, the application of bacteria is an alternative promising sustainable solution which can improve the durability of concrete structures.

Different microorganisms have been used to increase the durability of concrete. Numerous studies in the literature showed that bacteria such as genus Bacillus is able to precipitate $CaCO_3$ by the production of a urease enzyme. This enzyme catalyzes the hydrolysis of urea to CO_2 and ammonia, resulting in an increase in the pH and the carbonate concentration in the bacterial environment. Microbially induced calcium carbonate precipitation via urea hydrolysis shows an interesting ability to precipitate, relatively rapidly, large quantities of calcite.

The quality of microbial precipitation ($CaCO_3$) is determined by many factors such as: the concentration of dissolved inorganic carbon, the pH, the concentration of calcium ions and the presence of nucleation sites. The biochemical process can be summarized as follows (Eq.1-5) [Castanier 1999][W. De Muynck 2010]:

$$\mathrm{CO(NH_2)_2 + H_2O \xrightarrow{\text{Bacterial urease}} NH_2COOH + NH_3} \tag{1}$$

$$\mathrm{NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3} \tag{2}$$

$$\mathrm{2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^-} \tag{3}$$

$$\mathrm{2OH^- + H_2CO_3 \leftrightarrow 2CO_3^{2-} + 2H_2O} \tag{4}$$

Ureolysis results in a pH increase creating an alkaline environment around the bacterial cell. The precipitation occurs in form of calcium carbonate with the presence of calcium ions in the surrounding of bacterial cell wall according to the Eq.5:

$$\mathrm{Ca^{2+} + {CO_3}^{2-} \rightarrow CaCO_3} \tag{5}$$

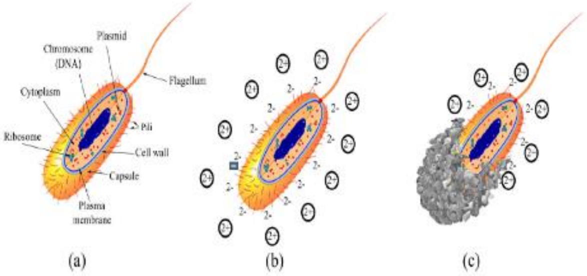

Fig. 1: Precipitation of $CaCO_3$ by bacterial cells.

Fig.1 illustrates the microbially induced carbonate precipitation processes.

(a) The bacterial cell is negatively charged.
(b) The precipitation can be seen on the surface of the bacterial cell.
(c) The cells are seen encapsulated by calcium carbonate.

Two methods, named direct and indirect, exist for the incorporation of different types of bacteria in concrete.

In the first method, the bacteria and the nutrients are introduced directly during the mixing process [Xu 2014][Chahal 2012]. In the second method, the bacteria and nutrients are protected by another material such as ceramsite [Chen 2016], polyurethane and glass tubes, lightweight aggregate [Tziviloglou 2016] and graphite nano platelets [Khaliq 2016], hydrogel [J. Wang 2014], and zeolite [Bhaskar 2017]. From these studies, it can be concluded that a concentration of 10^5 cells/ml leads to the best ability to improve the strength of concrete. In the report of N.Chahal and R.Siddique [Chahal 2012], it was found that a replacement of cement by 10% of fly ash accompanied by the inclusions of 10^5 cells/ml B. *Sparcious pasteurii* led to 22% improvement in compressive strength. In another study, the bacteria *B. Halodurans* [Wiktor 2011], *B. Aerius* [Siddique 2016] *and Shewanella* [Ghosh 2009] improved the strength by 7.15 %, 12% and 26%, respectively.

This bio-technology also improves the durability of structures, especially in marine environments where chlorides are able to penetrate easily. The concrete resistance to chloride ions diffusion was the subject of the standard NT Build 492 [D. Muynck 2007] and ASTM C1202 standards [Chahal 2012][Nosouhian 2016).

In this study, *Bacillus Subtilis,* member of Bacillus genus, is used because it is able to form spores under unfavorable conditions [K. Todar 2005]. The peptone and yeast extract were also used because they promote the urease-catalyzed ureolysis as shown in K. Wang et al. [K. Wang 2018].

The influence of bacteria (10^5 cells/ml) and nutrients on compressive strength, water absorption, rapid chloride permeability has been studied at the age of 28 and 90 days. The study was completed by the micro-structure analysis using Scanning Electron Micrographs (SEM).

2 MATERIALS AND METHODS

2.1 Materials and mixtures

A Portland cement (CEM II/A-L42.5) and a Betocarb HP-OG limestone fillers with densities of 3.09 and 2.7 g/cm^3, respectively, were used. For all designed mixtures, 0-4mm natural sand and two size fractions of coarse calcareous aggregates (4-10 mm and 6.3-20 mm) were also used. Finally, *MC PowerFlow 3140 superplasticizer* (SP) was employed to ensure a high workability of all mixes.

Concrete mixture has been designed to achieve C35/40 class of compressive strength and S4 class of workability according to the standard NF 206-1. From a control material, mixed with pure water, two other mixtures have been elaborated:

- The first one, called Nutrient: the raw materials were mixed with water containing 10g/l Peptone and 5g/l Yeast extract.
- The second one, named Bacteria: nutrients with bacterium *Bacillus Subtilis 5265T* with a concentration of 10^5 cells/ml were directly incorporated in the mixing water.

The components were introduced into the mixer by starting with coarse gravels, followed by sands, cement, fillers, mixing water. The mixing procedure is summarized below:

- 0 - 1’: Mixing of all dry materials.
- 1’ to 1’30’’: Introduction water + superplasticizer/ water + superplasticizer + nutrients/ water + superplasticizer + nutrients + bacteria.
- 1’ 30’’ to 4’: Mixing of all components.

15x30 cm and 10x20 cm cylindrical specimens were prepared for mechanical and durability tests. The specimens were removed from the molds after 24h and conserved in water at room temperature.

2.2 Compressive strength test

Compressive strength tests were carried out on 15x30 cm cylinders using a servo-hydraulic INSTRON machine with a loading rate of 0.5 MPa/s (Fig. 2). For each mixture, three specimens have been tested at 28, 90 days and the giving results are the averages of obtained values.

Fig. 2 : INSTRON machine for compressive strength.

2.3 Water absorption and porosity, capillary water absorption

Water absorption and porosity tests were conducted according to the standard NF P 18-459. The capillary water absorption test was carried out according to ASTM C1585-04 standard, in order to compare the penetration resistance between specimens with and without bacteria. 10x5 cm cylindrical discs were dried in an oven at 105±5°C until mass stabilization. After drying, all specimens were coated with epoxy on the sides to ensure unidirectional absorption through the treated surface. The specimens were immersed in water to a depth of 10±1 mm. At regular time intervals (5 min, 30 min, 1h, 2h, 4h, 6h, 24h and 2, 3, 6, 7 days), the specimens were taken out of water and weighed after drying the surfaces with a wet towel. The sorptivity coefficient, k (g.cm^{-2}.s$^{-1/2}$) were determined by the following expression:

$$\frac{Q}{A} = \mathrm{k}\sqrt{t} \tag{6}$$

Where Q is the mass of absorbed water [g];

A is the cross section in contact with water [cm^2];

t is the time [s];

2.4 Rapid chloride permeability

The rapid chloride permeability test was performed on 10x5 cm discs by means of the CTH rapid test according to the NT Build 492 Nordtest method [NT Build 492 1999] at the ages of 28 and 90 days. After 24h, the specimen was split and sprayed with silver nitrate solution. The non-steady-state migration coefficient is obtained as follows:

$$D_{nssm} = \frac{0.0239(273+T)L}{(U-2)t}\left[X_d - 0.0238\sqrt{\frac{(273+T)LX_d}{U-2}}\right] \tag{7}$$

Tab. 1: Effect of Bacillus Subtilis addition on concrete strengths.

Mix. No	Average concrete compressive strength in MPa			
	28 days		90 days	
	Strength ± S.D	% increases to Control	Strength ± S.D	% increases to Control
Control	36.94 ± 1.10	-	40.26 ± 0.29	-
Nutrient	39.39 ± 0.64	6.63	42.62 ± 0.58	5.86
Bacteria	42.68 ± 0.73	15.54	44.25 ± 0.24	9.91

Where D_{nssm} is the non-steady-state migration coefficient ($x10^{-12}$ m^2/s), U is the absolute value of the applied voltage (V), T is the average value of the initial and final temperatures in the anolyte solution (°C), L is the sample thickness (mm), X_d is the average value of the chloride penetration depth (mm) and t is the test duration (h).

To evaluate the effect of *bacteria* on the durability, the pH (before and after tests) in the anolyte (NaOH) and catholyte (NaCl) solutions has been measured.

3 RESULTS AND DISCUSSIONS

3.1 Compressive strengths

Tab. 1 summarizes the compressive strength of different concrete specimens. The maximum increase in compressive strength was achieved for the specimen prepared with *bacteria*. The increase was 15.54% and 9.91% at 28 and 90 days respectively as compared to the control concrete. This improvement is due to the precipitation of $CaCO_3$ in the pores, which exhibit denser microstructure and reduced porosity (Tab. 2). Similar results were reported by R.Siddique et al. [Siddique 2016] who observed that the addition of *Bacillus aerius* results in an increase in the compressive strength of 9% and 11.8% at the age of 28 and 56 days, respectively, compared to control sample. The results from this study shows that due to the inclusion of bacteria in concrete, the compressive strength is improved which can enhance the overall durability performance of the material. The analysis of SEM picture confirms the presence of $CaCO_3$ in the porosity (Fig. 7).

3.2 Water absorption and porosity, capillary water absorption

Tab. 2: Water absorption and porosity of concretes at 28 and 90 days.

	Porosity (%)		
Mix.no	Control	Nutrient	Bacteria
28 days	14.40±0.21	14.05±0.35	12.35±0.29
90 days	12.83±0.33	12.18±0.13	10.30±0.64
	Water absorption (%)		
28 days	6.35±0.14	6.19±0.18	5.39±0.23
90 days	5.64±0.17	5.35±0.06	4.48±0.26

Water absorption and porosity of different concretes are given in Tab. 2. It can be seen that with the incorporation of nutrients and bacteria, both water absorption and porosity decrease at all ages.

At 28 days, the presence of nutrients and bacteria induces a reduction in the porosity of 2.45% and 14.24% in both Nutrient and Bacteria concretes, respectively. At 90 days, a porosity decrease of 5.05% (Nutrient) and 19.69% (Bacteria) compared to the control material (Fig. 3). This reduction can be attributed to the filling of pores with calcite produced by bacteria [W. De Muynck 2008]. This result is in agreement with the results of the literature [Chahal 2012b][Chahal 2012a][Siddique 2014].

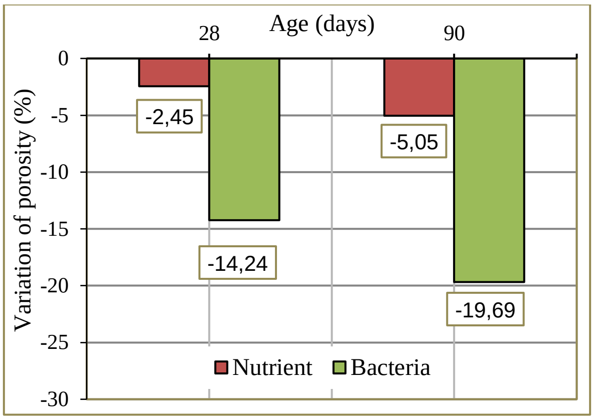

Fig. 3: Variation of porosity at different ages.

The reduction of porosity leads consequently to a decrease in the water absorption (Fig. 4). The reduction is 2.57% and 5.18% for Nutrient concrete at 28 and 90 days while it is 15.08% and 20.58% for Bacteria concrete. The maximum reduction of water absorption was also observed with bacterial specimens.

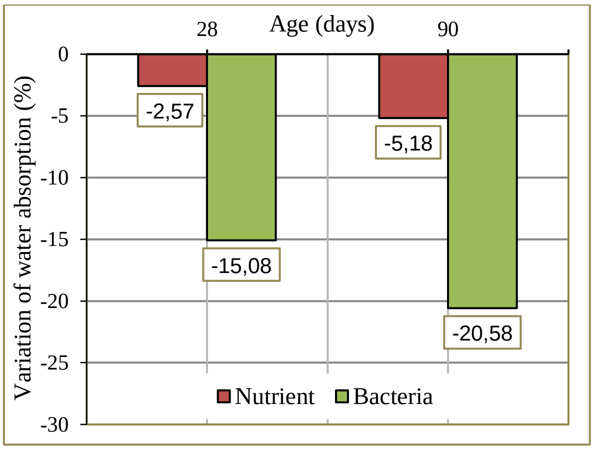

Fig. 4: Variation of water absorption at different ages.

The presence of bacteria results in a significant decrease in the water uptake compared to control specimens. Fig. 5 illustrates the effect of bacteria on the water absorption rate. When the nutrients and bacteria are added to the material, a significant decrease of the capillarity coefficient is obtained. Over a period of 24h, Bacteria and Nutrient specimens absorbed respectively about 17% and 26% less water than control specimen. These results are consistent with those of the porosity.

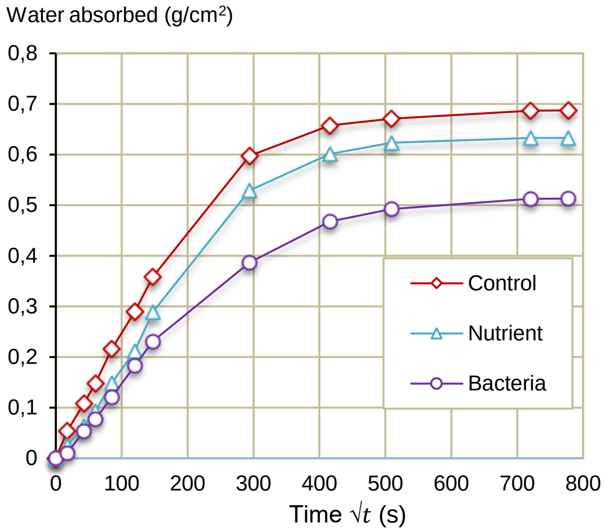

Fig. 5: The influence of bacteria on the rate of water absorption versus time.

3.3 Rapid chloride permeability test

Tab. 3: The rapid chloride permeability of concretes at 28 and 90 days.

	Dnssm [x10^$^{-12}$ m^2/s]		
Mix.no	Control	Nutrient	Bacteria
28 days	23.42±0.59	18.52±0.18	14.90±0.89
90 days	17.04±0.88	14.06±0.77	11.67±0.49

Corrosion of reinforcing steel due to chloride ingress is one of the most prevalent environmental attacks that lead to the deterioration of structures. The durability of concrete structures exposed to salts and marine environments was evaluated by means of chloride diffusion test. The effect of bacteria on the chloride migration coefficient (D_{nssm}) at different ages is given in Tab. 3 where it can be observed that nutrients and bacteria reduce D_{nssm}.

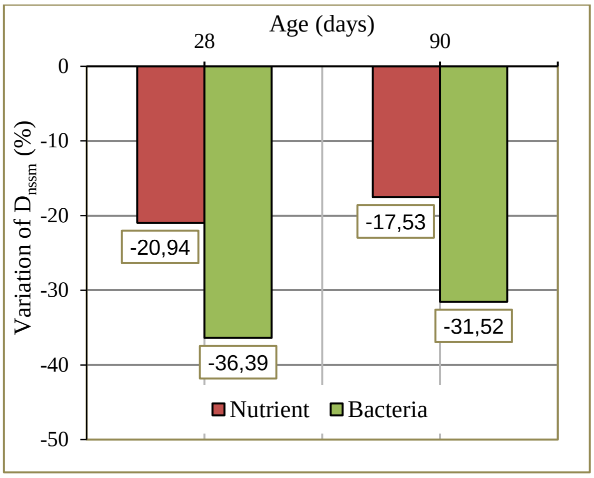

Fig. 6: Variation of chloride permeability at different ages.

From Fig. 6, it is observed that at 28 days the reduction in D_{nssm} is 20.94% for Nutrient and 36.39% for Bacteria while it becomes 17,53% and 31,52% at 90 days. This result can be explained by the lower porosity of the concrete with bateria as could be seen from the porosity results given in Tab. 2. Additionally, the results in the present case are in a good agreement with those of Chahal et al. [Chahal 2012a] et R. Siddique et al. [Siddique 2016]. The latter studies reported that the incorporation of bacteria in concrete leads to a decreased the chloride permeability.

The variation of pH in NaCl and NaOH solutions before and after tests is given in Tab. 4. The results show the pH of the NaCl solution increases while it remains constant for NaOH. The variation of pH is less important for Bacteria specimens. This smaller variation means that the material has not absorbed too much of Cl^- ions.

Tab. 4: Variation of pH (before and after tests) in the solutions NaCl and NaOH.

	Control		Nutrient		Bacteria	
	NaCl	NaOH	NaCl	NaOH	NaCl	NaOH
			28 days			
Before	7.94	13.21	8.31	13.22	8.75	13.2
After	12.9	13.02	12.65	13.1	12.53	13.11
Δ=After-Before	**4.96**	-0.19	**4.34**	-0.12	**3.78**	-0.09
			90 days			
Before	8.31	13.22	8.67	13.2	8.99	13.25
After	12.65	13.1	12.65	13.03	12.54	13.08
Δ=After-Before	**4.34**	-0.12	**3.98**	-0.17	**3.55**	-0.17

3.4 Scanning electron micrographs (SEM)

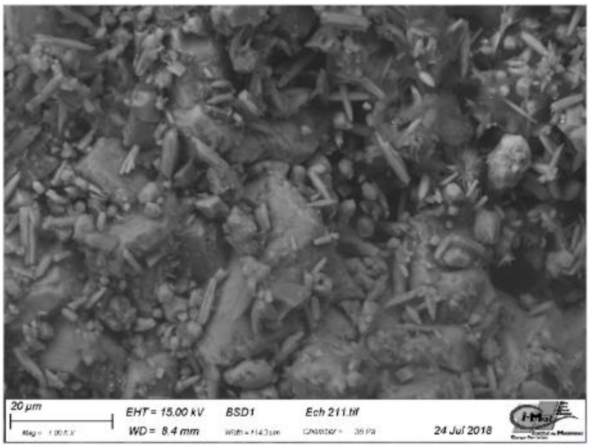

Fig. 7: SEM picture showing the surface of a specimen incorporated bacteria.

Fig. 7 shows an SEM picture of specimen incorporated *Bacillus Subtilis*. It can be observed that the complicated morphologies such as needle-like, bouquet-like, dumbbell-like and rhombohedral-shaped crystals have been successfully found through the decomposition of urea hydrolysis process. Two main morphologies (rhombohedral-shaped and needle-like) were observed with W. De Muynck et al [W. De Muynck 2008] and V. Wiktor and H. M. Jonkers [Wiktor and Jonkers 2011]. Following H. Cheng et al. [Cheng 2014] and H. Guo et al. [Guo 2011], when there are a lot of ammonia, the calcium carbonate tends to change the stable rhombohedral shape which depends on the concentration of $CO(NH)_2$, the bouquet-like crystals were formed through endlinkage of needle-like and become rhombohedral crystals. The result of SEM points out that the presence of $CaCO_3$ inside concrete is the main reason for the reduction in porosity, the resistance to chloride ingress and the enhancement of compressive strength.

4 CONCLUSIONS

Based on the obtained results, the following conclusions can be withdrawn:

- The bacteria and nutrients can be used as an admixture to enhance the durability of concrete.
- The compressive strength of the bacterial concrete increased at all of ages due to the calcium carbonate precipitation inside the pores.
- The presence of bacteria reduced significantly the rate of water uptake in bacterial concrete compared to the control material. The deposit of calcium carbonate on the surface and inside the pores of the microstructure resulted in a decrease of water absorption and porosity. Consequently, the chloride permeation is reduced.
- Microstructure analysis using SEM confirmed that calcite crystals morphologies such as needle-like, bouquet-like, dumbbell-like and rhombohedral-shaped were the main productions by the metabolism of bacteria which make the porous structure denser and reduce the porosity.
- The resistance to chloride ingress also increases. This reduction in chloride migration coefficient was confirmed by the decrease in the pH variation of NaCl in the bacterial specimens.

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