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Microbial Participation in the Formation of Calcium Silicate Hydrated (CSH) from *Bacillus subtilis*

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Abstract

Primary hydration of cement induces the formation of calcium silicate hydrated (C-S-H) gel and the latter contributes towards the strength development of concrete. The secondary hydration derived from pozzolanic materials such as silica fume is dependent upon the formation of C-S-H gel of the primary cement hydration reaction. The additional formation of C-S-H gel as a result of second hydration process, densities the cement microstructures producing low permeability concrete. However, the silica fume is considered expensive material and its availability is limited. Therefore, it is essential to utilise living elements as an alternative agent to form the C-S-H gel. In the present study, the untreated *Bacillus Subtilis* and chemically modified *Bacillus subtilis* (CMBS) were prepared. CMBS was prepared by reacting with ethylenediamine to modify its cell wall to become electropositive facilitating the binding of the silicate during the incubation process. The cell was then incubated in the Si solution ($Na_2SiO_3.5H_2O$) for 10 days which enables the SiO_3^{2-} (silica ion) from the solution to be bonded with the cell wall. The C-S-H gel is expected to be formed from the bonded silica of the cell wall when mixed with saturated calcium hydroxide solution which the latter simulates the concrete environment. The presence of C-S-H gel was then substantiated using X-Ray Diffraction (XRD) analysis. In another series of study, the difference concentration of *Bacillus subtilis* were incorporated into the grade 30 concrete specimens and the compressive strength up to 60 days of age were tested. The results showed that the silicate was adsorbed by *Bacillus subtilis* and there is no difference in the amount of Si adsorbed between untreated *Bacillus subtilis* and CMBS. The incorporation of *Bacillus subtilis* into the concrete enhanced the compressive strength and the concentration of 10^6 cell/ml was found tSo be the optimum concentration.

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Keywords : *Bacillus subtilis; C-S-H gel;* microbial; silica precipitation, bio concrete ;

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1. Introduction

Commonly, cement induces calcium silicate hydrated (C-S-H) gel and calcium hydroxide in aqueous form after reacting with water during the primary reaction. Secondary hydration will proceed with the presence of pozzolanic material inside the concrete and produces additional C-S-H gel that accelerate and enhance the strength of final concrete. However, the pozzolanic materials such as silica fume are expensive and limited in Malaysia. Besides, the cement production released about a tonne of green house gases (GHG) for every tonne of cement [7]. Therefore, living elements such as microorganisms would be used as an alternative method to produce a precipitation that possesses characteristics similar to that of the pozzolans. This living element produces a precipitation pozzolan which is derived naturally from the process of biomineralisation from microorganism. Recently, new interest research of biomineralisation induced from microorganism area has been found [11, 13, 15].

Biomineralisation process forms precipitation and fills the pores that enhanced the mechanical properties in concrete materials [1 2 3 4] such as strength and durability. It is reported that some specific microorganisms manage to make a transformation for most elements in the periodic table. For example, *Shewanella sp.* is able to produce calcium carbonate (calcite) [5] that binds to the wall of the bacteria. Similar precipitation induced by *Bacillus sphaericus* was also revealed by [11, 12, 16, 4]. Meanwhile, silica precipitation is another precipitation induced by the microorganism. Inagaki *et al.* [8] and Iwai *et al.* [9] have found that *Thermus Sp* has the capability to induce silica precipitation under alkaline conditions.

Bacillus subtilis, a common soil bacteria has also been found able to induce silica precipitation (Mera and Beveridge, 1993) and other metals [19, 17,18,15] by binding it to the cell wall of the bacteria. The cell wall would be modified using ethylenediamine to become electropositive to bind SiO_3^{2-} ion from the silicic acid [10]. Urrutia and Beveridge [17] proved that *Bacillus subtilis* is able to produce the fine grain silicate after the binding process. However, the previous study has not further confirmed the formation of the silica precipitation in the alkaline environment and concrete to form C-S-H.

Therefore, in the present study, the *Bacillus subtilis* was incorporated into the aqueous solution of calcium hydroxide and into the concrete. The evidence of the presence of silicate by reacting the untreated and CMBS with specimens aqueous calcium hydroxide to form C-S-H and enhancement made to the compressive strength development in concrete constitutes a significant finding of the present work.

2. Materials and Method

2.1 Growth and harvesting of Bacillus subtilis

Bacillus subtilis was grown in tryptic soy broth with a 0.5% yeast extract. As the culture of *Bacillus subtilis* reached the optical density at 600nm (OD_{600}) approximately 0.4, the cell of the *Bacillus subtilis* were pelleted by centrifuging at 10 000rpm for 3 minutes. The pellets then were washed two times using ultrapure deionized water (udw).

2.2 Chemical modification of the cell wall of Bacillus subtilis

For the purpose of chemically modifying *Bacillus subtilis*, the cell walls of the *Bacillus subtilis* were modified to become electropositive. The modification was made by reacting chemically the *Bacillus subtilis* with ethylenediamine solution. Prior to that, the ethylenediamine was activated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride solution [10]. The pelleted cell of the *Bacillus subtilis*

were mixed up with 0.5M ethylenenediamine and 0.2M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride to become aqueous solution. Then, the mix was continuously stirred under room temperature for 6 hours. After stirring the solution which contains the pelleted cell, the mix was centrifuged at 10 000 rpm for 3 minutes. Then, the centrifuged cells were washed four times with udw and recentrifuged to pellet the cell. At this stage, the *Bacillus subtilis* cell was completely and chemically modified (CMBS).

2.3 Silica (Si) incubation

Firstly, the 50mM H_4SiO_4 was prepared by dissolving $Na_2SiO_3.5H_2O$ in distilled water. Then, the two *Bacillus subtilis* cells which were untreated *Bacillus subtilis* and CMBS were incubated in H_4SiO_4 solution in two different containers at room temperature. Both containers were continuously shaken at 150 rpm for 10 days. After 10 days, the incubated cell for untreated *Bacillus subtilis* and CMBS were pelleted by centrifuging at 10 000 rpm for 3 minutes. The pelleted cells were expected to have adsorbed silica onto it. The pH of the silicate solution during the incubation process was kept constant 5.5 throughout the process.

2.4 Calcium hydroxide simulation

The pelleted cells were vacuumed to dry out all the water until the pelleted cell turned into powder. The cell powder was then reacted with aqueous calcium hydroxide for 2 days to form the C-S-H gel. The product once again was vacuumed to dry out excessive water from the reaction to produce in the form of powder and was ready for XRD analysis.

2.5 XRD analysis

The powder was tested on XRD. The presence of silica adsorb in the *Bacillus subtilis* cell wall was detected by the appearance of the peak of the quartz representing silica oxide (SiO_2).

2.6 Preparation of concrete specimen

Six series of the concrete specimens made of grade 30 concrete size of 100mm x 100mm x 100mm were prepared. The materials to cast concrete are cement, sand, aggregate and distilled water. The mix proportion for the grade 30 concrete specimens is shown in Table 1. Six series designated for concrete specimen with six different concentration of *Bacillus subtilis* cell which are 10^3, 10^4, 10^5, 10^6 and 10^7 cell/ml and control (without *Bacillus subtilis*) were cast. After 24 hours, the moulded concrete specimen were demoulded and cured in distilled water until the day of testing. The distilled water was used to preserve *Bacillus subtilis* from the presence of any contamination.

Table 1. Mix proportion for grade 30 in kg/m^3

Water	Cement	Sand	Aggregate
190	302	845	1033

2.7 Compressive strength

The concrete specimens incorporating without and with *Bacillus subtilis* were tested for its compressive strength. The compressive strength was tested at the ages of 3, 7, 14, 21, 28 and 60 days. The compressive strength test was performed in accordance with BS EN 12390-3:2000.

3. Results and discussions

3.1 Silica precipitation by the Bacillus subtilis in the calcium hydroxide

The quantification of the silica oxide of untreated *Bacillus subtilis* and chemically modified (CMBS) after incubation with silicate solution for 10 days at pH 5.5 are shown in Figure 1 and Figure 2 respectively. The two figures show that there is no significant difference in the amount of silica oxide adsorbed by *Bacillus subtilis* and that of CMBS. This result contradicts to the findings of the study conducted by [10] of which the finding revealed that the CMBS adsorbed significantly greater amount of silica than for untreated *Bacillus subtilis.* In the present investigation, it appears that the amount of silica oxide that was adsorbed onto the cell wall of untreated *Bacillus subtilis* equals to those of CMBS.

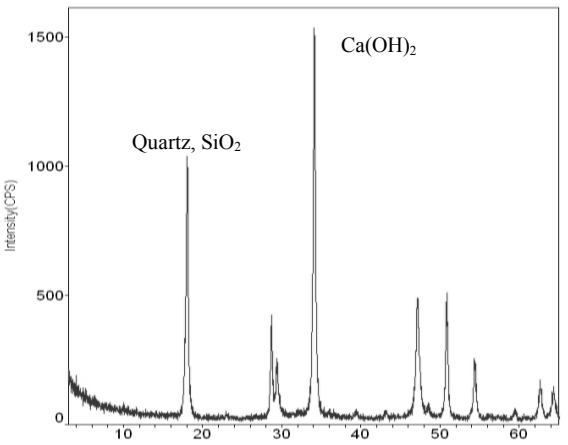

Fig. 1. XRD peaks of the untreated *Bacillus subtilis*

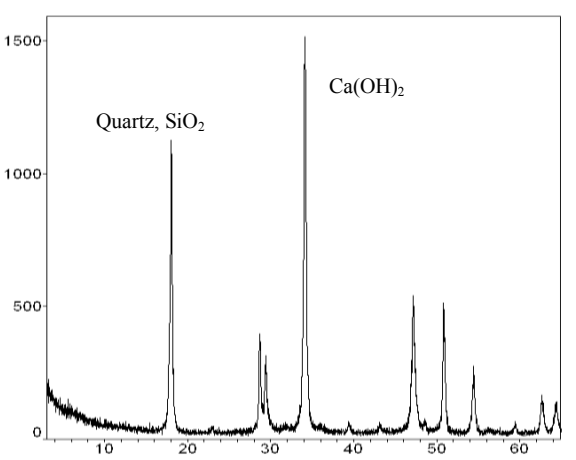

Fig. 2. XRD peaks of the CMBS

3.2 Compressive strength of concrete specimen with Bacillus subtilis

Figure 3 depicts the compressive strength of concrete specimens incorporating without and with *Bacillus subtilis* cells up to 60 days of age. The concrete specimens which contained 10^6 cell/ml attained higher compressive strength as compared to the control specimen. The increase of the compressive strength for those of 10^6 cell/ml over the control are about 17%, 22%, 27%, 17%, 22% and 28% at 3, 7, 14, 21, 28 and 60 days respectively were noted. The improvement of the strength is attributed to the precipitation formed by the *Bacillus subtilis* within the cement matrix. The deposition of the precipitation on the cell surface as well as within the cement matrix plugged the smaller pores in the cement matrix.

It is noted that there was no marked improvement in compressive strength of the concrete specimens made of 10^5 cell/ml and with lower concentration. The concrete specimens with the inclusion of 10^3 cell/ml concentration was found to attain significantly higher compressive strength up to 28 days of age as compared to the control. However, the drop in the compressive strength when reaching the age of 60 days was observed. At this point the phenomenon remains unknown.

The previous researcher, Ghosh *et al.* [6] reported that the optimum increment of cement mortar strength of about 25% was noticed when incorporated with *Shewanella sp.* at concentration of 10^5 cell/ml. Meanwhile, [1] found the optimum concentration of *Bacillus pasteruii* is 10^9 cell/ml with the increment 12% at the age of 7 days of concrete.

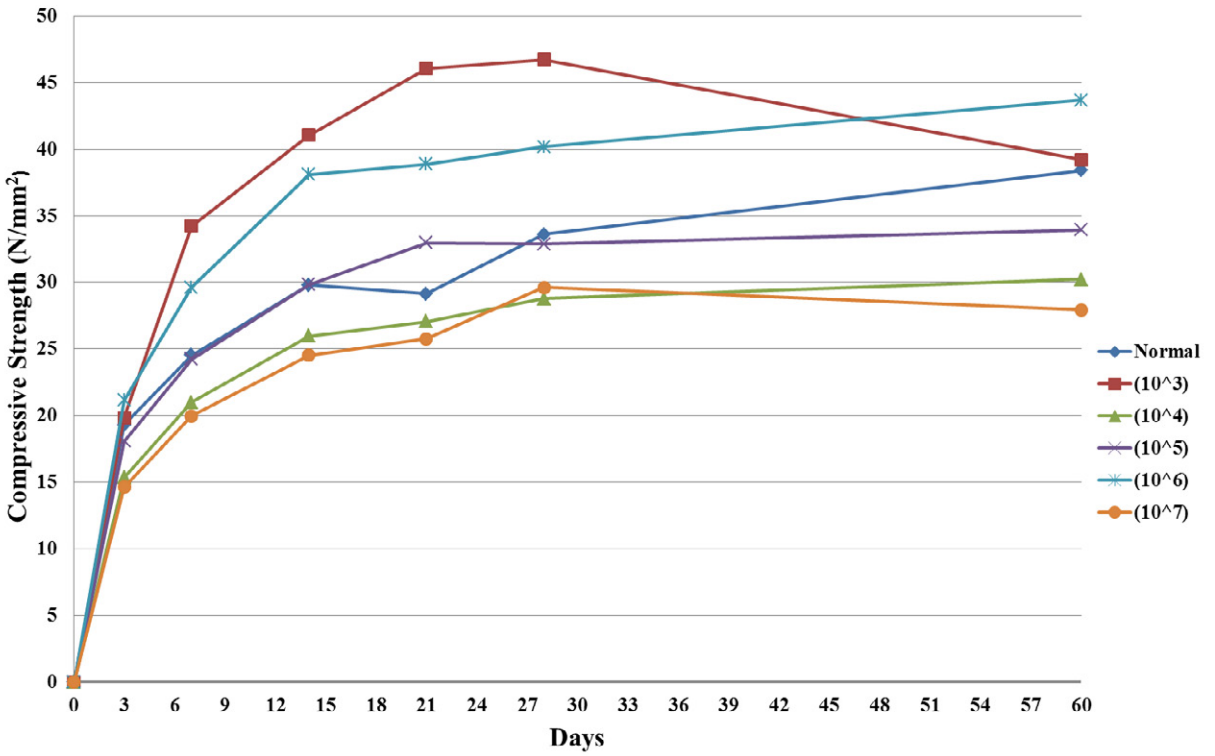

Fig. 3. Compressive strength of six (6) series of concrete specimens incorporated with six concentration of Bacillus subtilis

4. Conclusions

From the results, conclusions can be drawn as follows:

a. It is revealed that the silicate precipitation was formed when incubating the untreated *Bacillus subtilis* in the silica solution for 10 days.
b. It appeared that there is no difference in the amount of Si adsorbed between the untreated *Bacillus subtilis* and that of chemically modified *Bacillus subtilis* (CMBS).

c. It is found that the incorporation of the *Bacillus subtilis* into the concrete has a positive effect on the compressive strength. The enhancement was found to be obvious for those made of 10^6 cell/ml. The increase in compressive strength which is about 28% as compared to that without inclusion of *Bacillus subtilis* was found to be maximum when the concentration of 10^6 cell/ml of *Bacillus subtilis* was included.

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