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INVESTIGATION OF THE COMPRESSIVE STRENGTH OF PORTLAND CEMENT MIXTURES BY APPLICATION OF BACTERIA – '*SPOROSARCINA PASTEURII*'

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The concept of microbial concrete is one of the recent advances in concrete technology. In the past two decades, concrete technologies are working towards developing high performance concrete. Researches over the globe are being carried out in the wake of promising results found on the improvement of cementitious mix performances due to the application of live microorganisms. In this research live microorganism named *Sporosarcina pasteurii*, soil bacterium, has been used. Different set of experiments were carried out to investigate the effect of the bacterial medium, bacterial nutrient and bacterial concentration. From the test results it was found out that the bacterial medium had little effect, while the bacterial nutrient, whose main constituent is yeast extract, significantly reduced the compressive strength and increased the flow table as well as the slump in both mortar and concrete. Those samples with aforementioned bacteria together with the bacterial nutrient showed an improved compressive strength. The micro behaviors observed in terms of compressive strength indicate that this gain of strength was due to the calcite precipitation induced by the bacteria. The paper concludes by stating the need of further investigation, especially with regards to finding a better substitute for yeast extract of the bacterial nutrient.

Keywords: Sporosarcina Pasteurii, Bacterial-concrete, Microbiologically induced calcite precipitation, Portland cement mixes, Compressive strength.

1 INTRODUCTION

In the past two decades researchers have been working towards improving the performance behavior of concrete. And recently a novel technique is adopted in re-mediating cracks and fissures in concrete by utilizing microbiologically induced calcite or carbonate (CaCO3) precipitation (MICP) (Kumar *et al.* 2013a). Microbiologically induced calcite precipitation (MICP) is a technique that comes under a broader category of science called *bio-mineralization*. It is a process by which living organisms form inorganic solids. Bio-mineralization is a promising technology for producing crack resistance/highly self-healing concrete (Kumar *et al.* 2013a, Verma *et al.* 2015).

Microbiologically induced calcite precipitation is one of the more promising techniques which have been used in geotechnical engineering for soil and sand cementation (Martinez *et al.* 2013). The idea of microbiologically induced calcite precipitation has also been investigated for its application in Portland cement mixtures. Considerable researches have been conducted on different bacteria, which are considered to have a potential for calcite precipitation (Kumar *et al.* 2013a, Verma *et al*. 2015, Martinez *et al*. 2013, Chahal *et al.* 2011, Gupta *et al.* 2013).

The main objective of this research is to identify and highlight the possibility of microbiologically induced calcite precipitation by indirectly observing its effect on compressive strength of Portland cement mixes.

*Sporosarcina pasteurii (*formerly known as *Bacillus pasteurii)* is a bacterial species that is well known for producing urease and hydrolyzing urea to form ammonia and bicarbonate ions, and was thus used in this study (Martinez *et al*. 2013, ATCC 2013). As a microbial sealant, CaCO3 exhibited its positive potential in selectively consolidating simulated fractures and surface fissures in granites and in the consolidation of sand.

1 EXPERIMENTAL PROGRAM

1.1 General

Bacteria species called *Sporosarcina pasteurii* (previously known as *Bacillus pasteurii*), obtained from Ethiopian Institute of Biodiversity – Microbiology Department, was used in this study. One potential reason for selecting *S. Pasteurii* for its application in concrete for calcite precipitation is that *S. pasteurii* is a common bacterium naturally occurring in the subsurface soil containing CaCO3. Finding these bacteria in CaCO3 reach soil leads us to the assumption that presence of CaCO3 in the soil might be due to microbiologically induced calcite precipitation by the aforementioned bacteria. *Sporosarcina pasteurii* is an aerobic microorganism that uses oxygen for propagation. 13 g/l nutrient broth, 5 g/l peptone, 8.5 g/l yeast extract, 4.5 g/l beef extract, 25 mM $CaCl_2$ and 20 g/l Urea solution medium were used to prepare the media mixture needed to cultivate *S. Pasteurii*. The pH of the medium was then adjusted before sterilization to 6.5 by 1 N HCl. Growth was measured using Optical Density (OD) measurements which were carried out at different intervals using a Spectrophotometer Equipment.

(a) (b) (c)

Figure 1. *Sporosarcina pasteurii* on (a) nutrient, (b) Clear vs. (c) turbid growth media.

Due to the fact that *Sporosarcina pasteurii* is an aerobic bacterium, it was hypothesized that the effectiveness of calcite precipitation can be affected by the available voids in the specimen. As a result, concrete mixture with low and high water cement ratio was prepared during the experimental investigation.

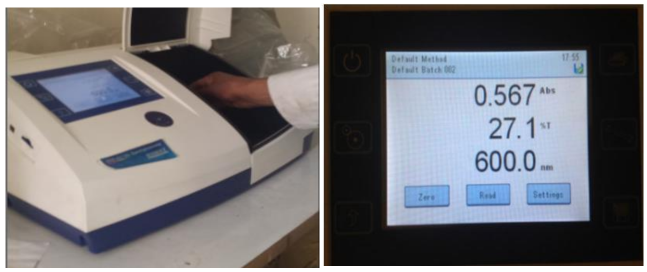


Figure 2. Optimum Density (Bacteria Concentration) measurement using Spectrophotometer.

1.2 Materials

While conducting this research, Ordinary Portland Cement of grade 42.5, which was available in the market, crushed basaltic coarse aggregate, Natural River sand and commercially available high range water reducer was used. Table 1 summarize physical properties of the fine and coarse aggregate.

Table 1. Physical properties of constituent materials.

Properties	Fine aggregate	Coarse aggregate
Specific Gravity (SSD)	2.5	2.76
Absorption	4.8%	0.82%
Unit Weight	1534	1553
Silt Content	3.45%	

1.3 Mix Proportion

The ACI mix design manual, ACI 211.1-91 for concrete with w/c of 0.575 and ACI 211.4R-08for w/c of 0.35 has been used. Table 2 and 3, summarize the quantity of each constituent material required for the mortar and concrete respectively. The description for the mix codes is as shown in result and discussion part.

Table 2. Mix Proportion for Mortar (kg/m^3).

Mix Code	Cement [kg]	W/C ratio	Fine aggregate [kg]	Bacterial Concentration	Othe r
MW	525	0.485	1505	0	None
MM	525	0.485	1505	0	**
MN	525	0.485	1505	0	***
MB5	525	0.485	1505	0.5 OD	None
MB15	525	0.485	1505	1.5 OD	None

Mixing and casting was done in accordance with ASTM C305-99 for the mortar and ASTM C 192-99 for the concrete. The control specimens were cured in tap water while the rest of the mixes were cured in a solution containing Urea (20gm/l) and $CaCl_2$ (2.75gm/l).

Finally, Compressive strength test on both mortar and concrete cubes was conducted using load - gauged type test machine with a capacity of 300kN and 3000kN respectively. The testing was in accordance with ASTM C39-99 for concrete and ASTM C109-99 for mortar cubes.

Table 3. Mix Proportion for Concrete [kg/m^3].

Mix Designation	Cement [kg]	W/C ratio	Coarse aggregate	Fine aggregate	Bacteria Concentration	Other	Super plasticizer
YW	330	0.575	938	894	0	None	0
YB0	330	0.575	938	894	0	**	0
YB5	330	0.575	938	894	0.5 OD	None	0
XW	545	0.35	938	720	0	None	1lit/100kg of cement
XB0	545	0.35	938	720	0	**	
XB10	545	0.35	938	720	1.0 OD	None	

**20gm/l Urea and 2.75gm/l $CaCl_2$; ***13 g/l nutrient broth, 5 g/l peptone, 8.5 g/l yeast extract, 4.5 g/l beef extract, 25 mM $CaCl_2$ and 20 g/l Urea

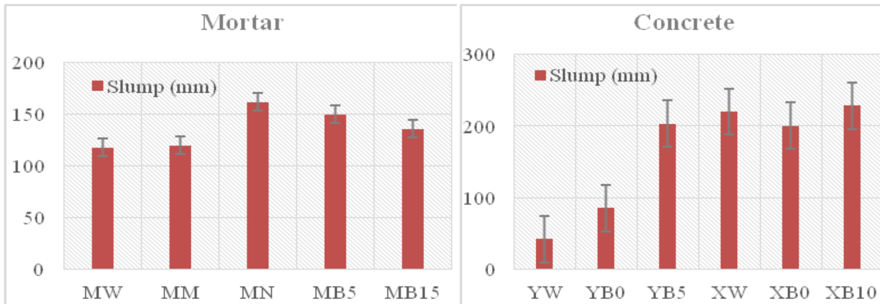
2 RESULTS AND DISCUSSIONS

This research commenced with identifying and highlighting the potential of bio-mineralizing bacteria for calcite precipitation by indirectly testing the compressive strength of concrete and mortar. The bacteria, *Sporosarcina pasteurii*, were preserved until all other mixing conditions were fulfilled. Once everything was ready, the bacteria were checked for contamination before inoculating in their growth medium. The fresh and ready bacteria underwent turbidity measurements using a Spectrophotometer before their application into the mix. Figure 3-7summarize results of both fresh and hardened properties of mortar and concrete.

The different mortar and concrete mixes are described as follows: (M: Mortar, Y & X for Concrete)

- MW, YW, XW – Control sample group for mortar, concrete with w/c of 0.575 and concrete with w/c of 0.35 respectively,
- MM, YB0, XB0 – The medium mix group: the water used had 20gm/l Urea and 2.75gm/l $CaCl_2$,
- MN – The nutrient mix group: the water containing all the nutrients; yeast, beef extract, nutrient bronze, peptone, urea, $CaCl_2$
- MB5, YB5 – The bacterial mix group: the mix proportion with bacteria of 0.5 OD for mortar and concrete respectively
- MB15 – The bacterial mix group: the mix proportion with bacteria of 1.5 OD
- XB10 – The bacterial mix group: the water with the solution having OD 1.0

As it can be seen from figure 3(a), the highest slump was recorded on mortar containing all nutrients; yeast is the main constituent of the nutrients. As the bacterial concentration increases, reduction in slump is also observed; the reason can be due the fact that the bacteria consumed the yeast and lessening its effect in creating voids. The other slump test conducted was on the concrete mixture. From figure 3(b), the first three charts, the Y series, are of concrete without admixture and the rest three, which is the X-series, are concrete mixtures with super plasticizer. The mix, YB5, which consists of yeast, beef extracts and bacteria, showed the highest slump from the Y-series mixes. During the slump test it is also observed that the mix is like foamed concrete (figure 4(a) &4(b), side and front view respectively). This might be the effect of the yeast.



(a) (b)

Figure 3. Summary of Slump Results.


(a) (b)

Figure 4. "Foam-Like" Slump (Bacterial Concrete).

The other result observed, in addition to the slump, was compressive strength in both mortar and concrete. Figure 5 and 6, summarize these results.

From the test results, it was observed that cement-mortar cubes containing all the nutrients (MN mix group) showed a significant reduction in compressive strength up to 70% at 28th day, compared with the control group. On the other hand, mortar cubes with only medium showed a significant increase in compressive strength up to 20%. However, with the inclusion of bacteria (*Sporosarcina pasteurii*), the reduced compressive strength due to the nutrient effect, which was 70% reduction, has improved. The reduction in strength is only 18% for bacterial concentration of 0.5 OD and 11% for 1.5 OD when it is compared with the control group. This improved compressive strength can be due to microbiologically induced calcite precipitation. And when we see results of compressive strength test of concrete cubes (figure 6), the effect of medium is not significant as it was observed in mortar cubes. There is a slight increase in compressive strength up to 2% which was 20% in the case of mortar. However, a significant reduction in compressive strength was observed on concrete cubes with w/c of 0.575 and bacterial concentration of 0.5 OD. As it was observed during the mixing process and slump test result, the yeast extract created numerous void spaces and increases the volume during mixing.

However, as the bacterial concentration increased (1.0 OD), in the case of water-cement ratio of 0.35, the compressive strength was improved compared with the control group. The higher bacterial concentrations resulted in increased compressive strength compared with control specimen.

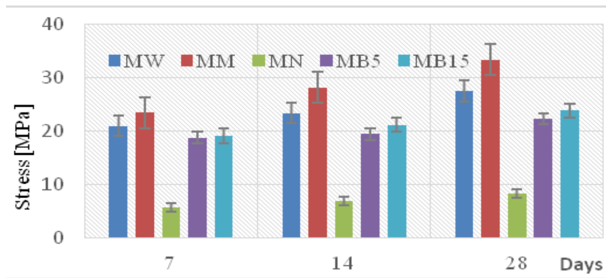


Figure 5. Summary of Compressive Strength of mortar cubes.

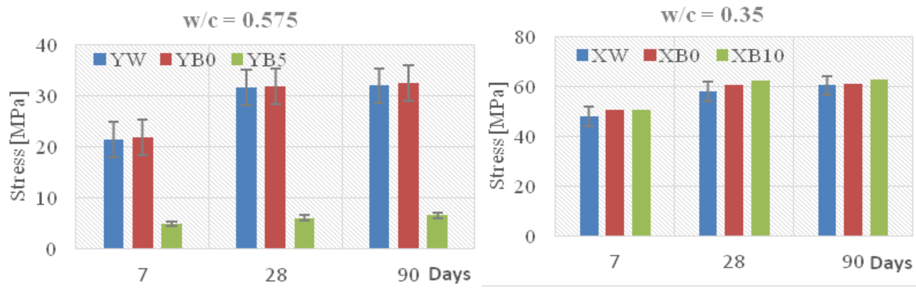


Figure 6. Summary of Compressive Strength of Concrete.

3 CONCLUSION

From the test results, it can be concluded that, bio-mineralization has potentially occurred by *Sporosarcina pasteurii*; but this statement is only backed by results obtained from compressive strength tests. This can be strongly proven with the employment of direct tests on the microstructure of crushed samples. For the bio- mineralization to be efficient the yeast in the nutrient should be optimized or an alternative nutrient has to be used.

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