INVESTIGATION ON THE POTENTIAL IMPROVEMENT OF COMPRESSIVE STRENGTH OF PORTLAND CEMENT MIXTURES BY APPLICATION OF BACTERIA – '*Sporosarcina pasteurii*'

Samson Walelign Aregawi[1],Abiy Shimeles Goshu[2],Bisrat Eshetu Alemu[2],Dagmay Gebeyehu Woldaregay[2],Natnael Deres Abdulkadir[2], Yonatan Birhane Mesfin[2],

[1]MSc, BSc, P.E., Lecturer, Member of Concrete Materials and Structures Chair, School of Civil and Environmental Engineering, AAiT, [2]BSc graduates of the academic year 2014/15, SCEE, AAiT

Abstract

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, a common soil bacterium which can induce the precipitation of calcite has been used. This research has tried to show the effect of introducing Sporosarcina pasteurii into concrete as well as mortar with a particular focus on the compressive strength.

Different set of experiments were carried out to investigate the effect of the bacterial medium for different water-cement ratio, the effect of bacterial concentration as well as the effect of the bacterial nutrient. This experiment tried to show the effect of introducing these components on the compressive strength of concrete which was conducted at 7^{th}, 28^{th} and 90^{th} day, to indirectly prove the possibility of calcite precipitation due to the inclusion of the bacteria. 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 compared with samples with only the nutrient.

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 so that with only calcite precipitation, one will be able to achieve a higher performance for mortar and concrete.

Keywords: -Compressive Strength, Sporosacina pasteurii, Portland Cement Mixes, Bacterial-Concrete, Microbiologically Induced Calcite Precipitation

Corresponding author's email: samson.walelign@aait.edu.et

Introduction

With an annual production of about 10 km^3/year Portland cement concrete is the most consumed construction material on Earth [1]. Economy, product-ability, convenience to cast and ease of availability of constituent materials are some of the reasons. On the contrary, resistance to crack is the major problem in concrete. Crack in concrete affects its performance behaviour. In the past two decades researchers have been working towards improving the performance behaviour 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) [2]. 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. [2, 3]

Microbiologically induced calcite precipitation is one of the more promising techniques which have been used in geotechnical engineering for soil and sand cementation [4]. 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. S. pasteurii, microbial concrete as concrete healer, Bacillus Sphaericus, B. Subtilis, for surface treatment and Bacillus Subtilis, Sphaericus Thiobacillius sp, microbial concrete as water purifier are among the different bacteria which have the potential for calcite precipitation [2, 3, 4, 5, 6]. These bacteria are able to influence the precipitation of calcium carbonate by the production of urease enzyme. This enzyme catalyzes the hydrolysis of urea to CO2 and ammonia, resulting in an increase of the pH and carbonate concentration in the bacterial environment. [2, 5]

Jagadeesha Kumar, et al. (2013) investigated the effect of bacterial calcite precipitation on compressive strength of mortar cubes. In this research a bacteria named Bacillus Pasteurii and Bacillus Sphaericus were used. The media composition for grow of culture was peptone, beef extract and sodium chloride. Test results showed that the aforementioned bacteria improved the compressive strength of mortar specimens. This improved compressive strength can be indirect evidence for occurrence of microbial calcite precipitation [2]. One drawback of this paper is that, the research didn't consider the effect of bacterial concentration and the aforementioned medium, which was used for grow of culture and precipitation. On the other hand, other researchers showed that bacterial concentration and type of medium are among the different factors which affect the efficiency of calcite precipitation [7]. Another research was also conducted to study the application of bio mineralization in crack remediation. On this research Bacillus flexux, Bacillus Pasteurii and Bascillus Sphaericus were used. Scanning Electron Micrograph Image and XRD results showed that there was calcite precipitation by the bacteria which was observed through reduced capillary pores compared with the control specimens. [8]

The concept of microbial concrete is barely exercised in the country, Ethiopia, as it is also relatively new to the more advanced, developed world. This particular topic is currently under extensive researches and has a vast room for improvement, so the idea of innovation is not yet a finished article.

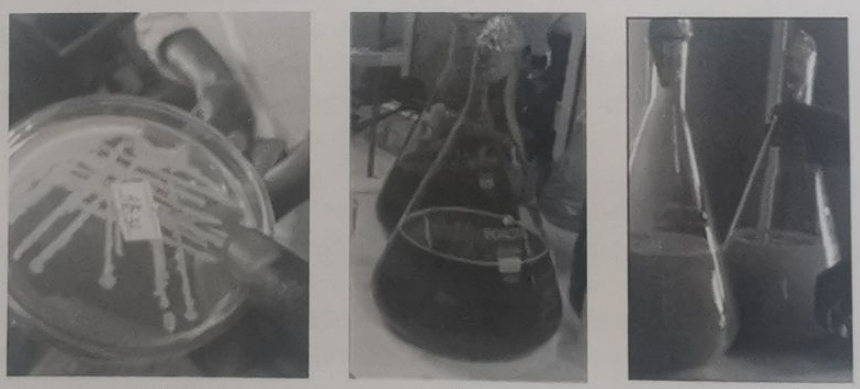
The main objective of this research is to identify and highlight the possibility of microbiologically induced calcite precipitation by the bacteria named Sporosarcina pasteurii by indirectly observing its effect on compressive strength of Portland cement mixes.

Sporosarcia 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. Bacillus species are known for their ubiquity in nature and their resistance to chemical and physical agents, which enable their use in open environments in the field [4, 9]. 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. MICP is highly desirable because the calcite precipitation induced as a result of microbial activities, is pollution free and natural. [3]

Experimental Program

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.

Due to the fact that Sporosarcina pasteurii is an aerobic bacteria, 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. In the case of mixtures with high water cement-ratio, there is relatively higher void space while specimens with low water cement ratio has lower initial voids which may affect the effectiveness of calcite precipitation.



(a) (b) (c)

Fig 1 – Sporosacina pasteurii on (a) nutrient, (b) Clear vs. (c) turbid growth media

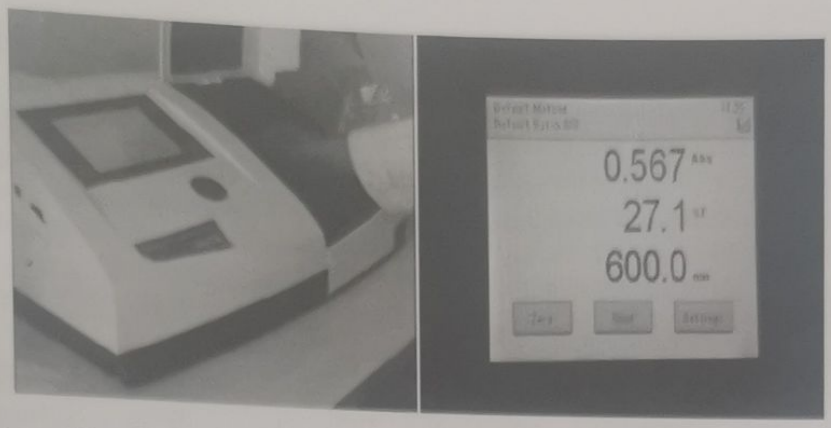


Fig 2 –Optimum Density (Bacteria Concentration) measurement using Spectrophotometer

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 summarizes 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%	

Mix Proportion

The ACI mix design manual, ACI 211.1-91 for concrete with w/c of 0.575 and ACI 211.4R-08 for w/c of 0.35 has been used. A sum of 5 mortar mixes of W/C ratio 0.485 was prepared. The variables are bacterial concentration, 0.5 OD and 1.5 OD, medium only, which is Urea - $CaCl_2$, complete medium constituents and the fifth one is the control mix. A sum of 6 different concrete mixes with varying W/C ratio, 0.35 and 0.575, and varying bacterial concentration, 0.5 OD and 1.0 OD, as well as the control mix were also prepared for testing. Table 2 and 3, summarizes the quantity of each constituent materials required for the mortar and concrete respectively.

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

Mix Code	Cement [kg]	W/C ratio	Fine aggregate [kg]	Bacterial Concentration	Other
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

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

Mix Designation	Cement [kg]	W/C ratio	Coarse aggregate [kg]	Fine aggregate [kg]	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 $CaCl_2$

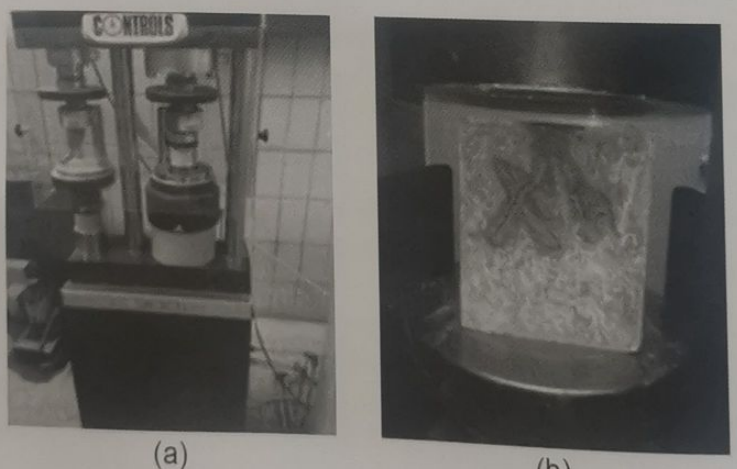
***13 g/l nutrient broth, 5 g/l peptone, 8.5 g/l yeast extract, 4.5 g/l beef extract, 25 mM $CaCl_2$ and 20 g/l Urea

Once the amount of water required for mixing is determined, the amount equal to the mixing water is used for bacterial growth in the laboratory. The total amount of water used for mixing contains the required concentration of bacteria which was measured in terms of Optical Density.

After proportioning the constituent materials for the mortar and concrete, mixing and casting was done in accordance with ASTM C305-99 for the mortar and ASTM C 192-99 for the concrete. After the mixing was completed, flow table and slump test was conducted for the mortar and concrete respectively. Then the mortar and concrete is casted in cubic molds of size 50 mm and 150mm to conduct compressive strength test respectively.

After 24 hours from casting, the concrete and mortar cubes were de-molded and were cured in curing baths. 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.



(a) (b)

Fig 3 – Compressive strength test setup for (a) mortar and (b) concrete

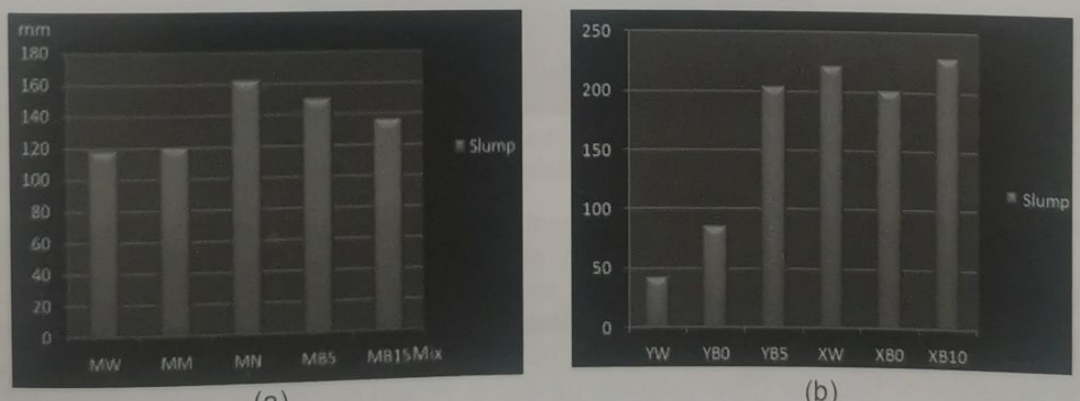
Results and Discussions

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. After successfully acquiring the required type of bacteria, *Sporosarcina pasteurii*, they 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. Different concentrations of bacteria were recorded and applied in the several mix proportions of the concrete and mortar.

Fresh and hardened properties were conducted on mortar as well as concrete mixes. Figure 4-7 summarizes results of both fresh and hardened properties of mortar and concrete. The mortar and concrete molds prepared included those containing only bacterial medium and those with bacterial nutrient in addition to others containing different concentrations of the bacteria.

The different mortar and concrete mixes are described as follows:

- MW – Control sample group, curing was done in tap water
- MM – The medium mix group: the mix proportion is similar to MW with the difference being the water used had 20gm/l Urea and 2.75gm/l $CaCl_2$, curing was done in tap water.
- MN – This particular mix consists of exactly the same mix proportions barring the water. Here, the water is replaced by the solution containing the nutrients, yeast, beef extract, nutrient bronze, peptone, urea, $CaCl_2$
- MB5 – The bacterial mix group: the mix proportion with bacteria of 0.5 OD
- MB15 – The bacterial mix group: the mix proportion with bacteria of 1.5 OD
- YW – Control sample group for w/c of o.575, curing was done in tap water.
- YB0 – The medium mix group: the mix done with the exact proportion similar to YW with the difference being the water used had 20gm/l Urea and 2.75gm/l $CaCl_2$, curing in tap water.
- YB5 – The bacterial mix group: the mix was done with similar proportion as of the previous two except the water was replaced by the solution having OD 0.5 bacterial concentrations.
- XW – Control sample group for w/c of 0.35, curing was done in tap water.
- XB0 – The medium mix group: the mix done with the exact proportion similar to XW with the difference being the water used had 20gm/l Urea and 2.75gm/l $CaCl_2$, curing in tap water.
- XB10 – The bacterial mix group: the mix was done with similar proportion as the previous two, except the water replaced by the solution having OD 1.0 bacterial concentration.



(a) (b)

Fig 4 – Summary of (a) Mortar and (b) Concrete Slump Results

As it can be seen from figure 4(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 4(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. YB5, the mix 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 5). This might be the effect of the yeast. Significant air voids are also observed in the hardened concrete.



Figure 5 – "Foam-Like" Slump (Bacterial Concrete)

The other result observed, in addition to the slump was compressive strength in both mortar and concrete. Figure 6 and 7, summarizes these results.

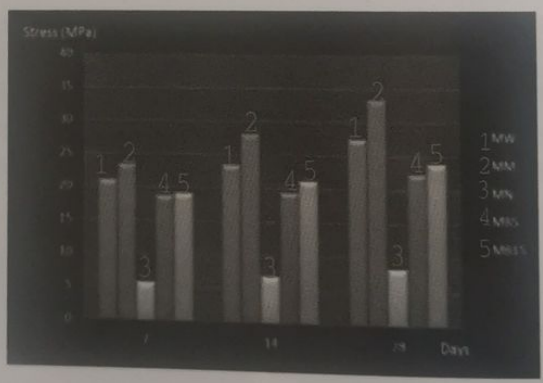
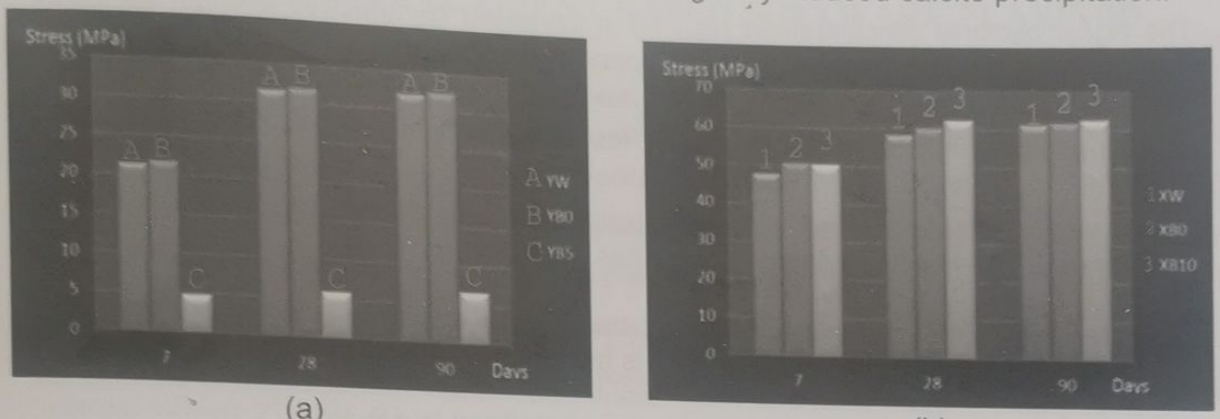


Fig 6 – Summary of Compressive Strength of mortar cubes

From the test results, it was observed that cement mortar cubes containing all the nutrients, the yeast, peptone, beef extract and nutrient broth, showed a significant reduction in compressive strength up to 70% at 90th day compared with specimens with medium ($CaCl_2$ and Urea) only and control group. On the other hand, mortar cubes with only medium ($CaCl_2$ and Urea) showed a significant increase in compressive strength up to 20%, relative to control group. However, with the inclusion of bacteria (Sporosarcina pasteurii) in addition to the nutrient 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 as compared to specimens with nutrient only can be due to microbiologically induced calcite precipitation.



(a) (b)

Fig 7 – Summary of Compressive Strength of Concrete (a) w/c=0.575 and (b) w/c=0.35

And when we see results of compressive strength test of concrete cubes, the effect of medium ($CaCl_2$ and Urea) 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. The medium, $CaCl_2$ and Urea, has a positive effect on compressive strength of concrete specimens. However, a significant reduction in compressive strength was observed on concrete cubes with water-cement ratio 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; as a result the bacterial concentration and hence the bio-mineralization was not enough to compensate for the reduction in strength. The yeast affects the efficiency of calcite precipitation. In this research yeast, beef extract, $CaCl_2$, Urea were used as a medium and nutrient for the bacteria. On other researches the beef extract, $CaCl_2$, Urea, peptone was used and results showed that there was an improved compressive strength due to calcite precipitation on mortar specimens [2]. However, results of this research showed that for lower bacterial concentration, the compressive strength was found significantly reduced compared to the control group. But as the bacterial concentration increased (1.5 OD), in the case of water-cement ratio of 0.35, the compressive strength was improved compared with the control group. Researches also showed that type of medium is one of the factors which affect the efficiency of calcite precipitation [7]. In this experimental investigation the yeast is the main factor which significantly affects the compressive strength as well as efficiency of the calcite precipitation.

With higher concentration of bacteria on mortar and concrete mixes (1.5 OD for mortar and 1.0 OD for the concrete with water-cement ratio of 0.35), there is an increase in compressive strength on both mortar and concrete cubes, although the yeast increases the void and negatively affects the compressive strength. These can indirectly prove the occurrence of calcite precipitation.

Conclusion

Based on the results, the following points are hypothesized and concluded:

1. Lower bacterial concentration Portland cement mixes resulted in decreased compressive strength due to yeast extract of the nutrient medium, which was responsible for the observed void spaces, volumetric change and consequently reduction of strength.

2. The higher bacterial concentrations resulted in increased compressive strength compared with control specimen; this increase in compressive strength might be due to two possible reasons:
   - The bacteria have consumed the yeast, lessening the effect of volumetric change and reduction in strength,
   - As many literatures suggest, bio-mineralization has occurred and that more precipitation following higher bacterial concentrations has happened.

3. Bio-mineralization has potentially occurred; but this statement is only backed by results obtained from compressive strength tests. It is believed that this can be strongly proven with the employment of direct tests and visual examination of the microstructure of the crushed samples. Hence primarily, we recommend an in-depth direct investigation

4. It is also identified that the yeast extract in the nutrient was responsible for the reduction of strength and also affected the efficiency of calcite precipitation. But inclusion of bacteria into the mix resulted in precipitation of calcite and improvement of strength. Therefore, the focus in the future should find means of reducing effects of yeast extract by optimization or through replacement. Having gotten rid of the effect of the yeast, the results indicate that the concrete precipitation will be the only major process left and that can provide extra performance for the concrete and mortar mixes.

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