*Punjab Univ. J. Zool., Vol. 31 (1), pp. 009-012, 2016*
ISSN 1016-1597 (Print)
ISSN 2313-855 (online)

**Original Article**

# Assessment of biocalcification by the water absorption test using microorganism from oil contaminated site

Hareem Mohsin, Azka Asif*, Warda Fatima, Sarooj Nadeem, Anjum Nasim Sabri

*Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore (54590); Pakistan*

(**Article history:** Received: December 31.12.2015; Revised: February 11, 2016)

**Abstract**

The biocalcification is considered as an environment friendly process that includes the precipitation of protective layer of calcium carbonate on the cement based structures or buildings. In the present study, the biocalcification capacity of *Bacillus pasteurii* was studied on the cement samples. The cement samples were treated with urea and calcium salt (calcium carbonate) that act as a source of calcium along with the microorganism under optimum culture conditions. Alkaline conditions produced by the activity of the enzyme urease allowed the reaction between calcium and carbonate ions that leads to the precipitation of the calcium carbonate. The water absorption test was performed to check the presence of a protective layer that provides resistance to water penetration and corrosion to cement. The samples treated with calcium and urea for one hour were found better as shown by less absorption of water compared with the untreated samples. Application of *Bacillus pasteurii* on the cement or the cement based materials has the potential to conserve such materials and increase their durability.

**Key words:** *Bacillus pasteurii, c*ement, biocalcification, urea, calcium carbonate, precipitation, water absorption

**To cite this article:** MOHSIN, H., ASIF, A., FATIMA, W., NADEEM, S. AND SABRI, A.N., 2016. Assessment of biocalcification by the water absorption test using microorganism from oil contaminated site. *Punjab Univ. J. Zool.,* **31**(1): 9-12.

## INTRODUCTION

Biocalcification is a term that refers to the formation and accumulation of calcium carbonate in the presence of microorganisms also known as micrbiologically induced calcite precipitation (MICP) (Sarde *et al,* 2009). This microbial induced calcium carbonate precipitation is linked with the phenomenon of bio-mineralization. This process occurs in the environment created by the microorganism. *B. pasteurii,* soil microorganism and a gram positive endospore forming rod, has the ability of inducing precipitation of calcium. This bacterially induced calcium carbonate precipitation decreases permeability against corrosive substances and water uptake by capillary action (Chunxiang *et al,* 2009). Under optimum conditions, *B. pasteurii* possess enzyme urease which hydrolyses urea and produces ammonia and carbonate ions. The calcium ions are used as a micronutrient therefore are not utilized in large amounts and remain outside the cells. The presence of ammonia provides alkaline conditions and the carbonate ions formed by the hydrolysis combine with the calcium ions. This leads to the precipitation of calcium carbonate outside the cell. It is also called as 'bio cementation' (Chunxiang *et al.,* 2009).

$$\text{Cell} + Ca^{2+} \rightarrow \text{Cell-}Ca^{2+}$$
$$\text{Urea} \rightarrow \text{ammonia} + \text{carbonate ions}$$
$$\text{Cell-}Ca^{2+} + \text{carbonate ions} \rightarrow \text{Cell-}CaCO_3$$
(precipitated outside the cell).

There is a negative charge on the bacterial cell wall because of which the positively charged calcium ions will be attracted to the cell wall and thus precipitated in the form of calcium carbonate (Chunxiang *et al.,* 2009; Talaiekhozan *et al.,* 2014). As cement and cement based materials are mostly used for construction purposes, it provides strength to the buildings and their conservation. But due to a number of corrosive substances that are present in the environment like chlorides, sulphates, nitrates, oxides of sulphur and nitrogen along with atmospheric moisture, this causes damage to the cement buildings and structures (Dhami *et al.,* 2014). These corrosive substances react with cement and create pores and cracks that

32-PUJZ-51021321/16/0009-0012

*Corresponding author: azkaasif@hotmail.com

reduce mechanical strength and durability. This also produces micro-cracks that in turn, cause increased moisture absorption and thus results in the weakening of the whole structure. To counter this problem, the bacterial culture of *B. pasteurii*, along with some essential nutrients is used to restore cracks. This study is done to analyse the capability of *B. pasteurii* to precipitate calcite utilizing different concentrations of cement. The action of this urease producing organism therefore strengthens cement concrete. Not only can it be used for cement but for ornamental stone and granite or the restoration of the micro-cracks.The process is environment friendly and increases the life of cement materials. With this very easy and convenient technique, this method of bio-mediated precipitation of calcium carbonate (Chahal *et al.,* 2011) can bring a revolution in the field of civil engineering as well (Chunxiang *et al.,* 2009; Achal *et al.,* 2011).

## MATERIALS AND METHODS

***Samples***

Specimen used was a hard cement paste formed by adding 45g cement in 100ml water and blocks were made. Blocks were placed at room temperature for 21 days prior to bacterial treatment. This treatment was given so that the cement blocks acquire a compact structure and cracks are minimized so that false positive results can be eliminated.

***Bacterial Strains and Culture Media***

The bacterial strain used was biochemically identified using the Bergey's Manual identification scheme. A wet mount of the strain showed large and uniform rods which indicated towards a *Bacillus* species. The culture was gram positive rods after gram staining. Spore staining was done using malachite green of a month old culture which showed endospore bearing rods. Further biochemical testing is summarized in table I (Garrity *et al.,* 2011).

**Table I: Sequential biochemical testing for the identification of *B. pasteurii.***

| Sr. No. | Tests | Results |
|---|---|---|
| 1 | Starch hydrolysis (amylase) | Negative |
| 2 | Catalase test | Positive |
| 3 | Spore staining | Positive |
| 4 | Nitrate reduction | Positive |

***Experiment***

Nutrient broth was prepared in autoclaved flasks and the inoculum of the organism was given aseptically. The flask was put on a rotary shaker for 24hours incubation at 37ºC. The pre-prepared cement blocks were weighed. The blocks were made from 45g cement. 10 blocks were made. 5 were used for the application of bacteria while other 5 were used as negative controls. After incubation of the culture for one day, the cement samples were added in autoclaved beakers having 100ml culture, 3g calcium source and 3g urea. After that beakers were sealed and incubated for 7 days at 37ºC (Chunxiang *et al.,* 2009).

***Water Absorption Test***

Cement blocks after the bacterial treatment were dried and weighed. These were then immersed in water for 24 hours to check the absorption. After 24 hours blocks were blot dried and weighed again to evaluate change in weight (Chunxiang *et al.,* 2009; Banga *et al.,* 2010; Achal *et al.,* 2011; Dhami *et al.,* 2012).

## RESULTS AND DISCUSSION

Samples after the water absorption test are shown in Fig. 1. The figure shows the cement block samples when treated with calcium carbonate and urea together in A and the samples after the treatment in which urea was added one hour later in B. The sample in C acts as a negative control *i.e.,* no addition of bacteria, calcium source and urea. A total of 10 blocks were used in the experiment to check the biocalcification ability of bacteria. Table II shows results of water absorption test in all those cement blocks. The samples treated with either calcium carbonate or urea alone absorbed water more as the bacteria couldn't perform the activity of biocalcification. While in case when urea and calcium carbonate were added positive results were obtained which showed almost no absorption of water because the calcium carbonate was precipitated in the micro cracks of samples (Raut *et al.,* 2014).The blocks B4 and B5 showed a little decrease in mass which may have occurred due to the removal of excess calcium carbonate precipitated on the samples. The sample that was given calcium carbonate and urea one hour late showed better results and better resistance to the water as compared to the sample that was provided with the calcium carbonate and urea together. It may be due to the fact that when calcium source and urea are added together, there is not sufficient time forcalcium to get in the cracks;urea is hydrolysed readily at the same time due to which a thin layer of calcium carbonate is deposited. But when urea is added one hour late, the calcium gets enough time to stick to the cracks.

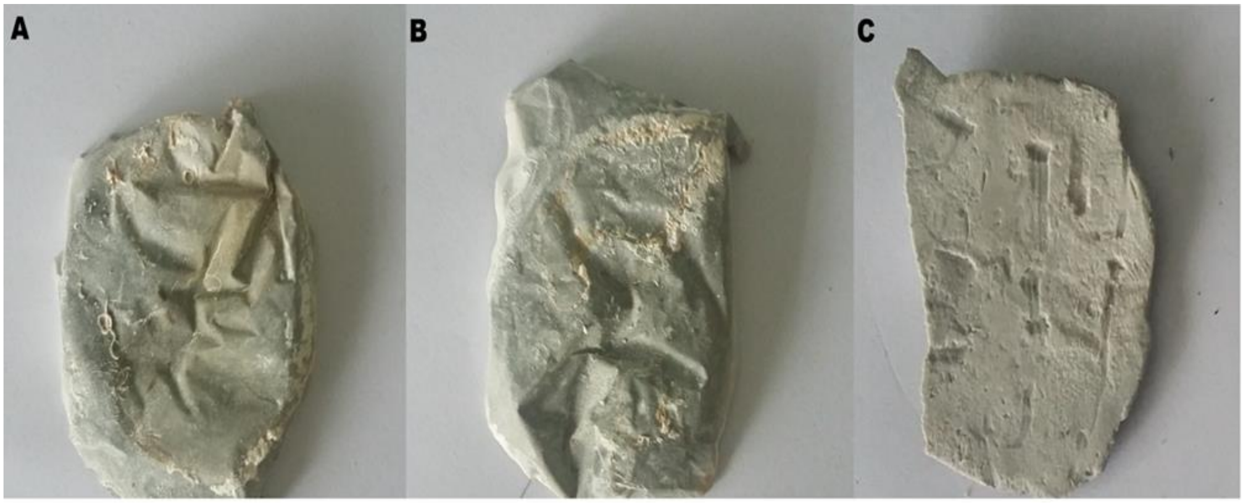


**Figure 1: 45% Cement blocks before and after treatment with calcium carbonate and urea.**

**Table II: Representation of blocks before and after water treatment**

| **Components of the beaker** | **Positive controls for the bacterial treatment** | | | **Negative controls Plain broth is added without the culture** | | |
|---|---|---|---|---|---|---|
| | **Block** | **Weight before water treatment (g)** | **Weight after water treatment (g)** | **Block** | **Weight before water treatment (g)** | **Weight after water treatment (g)** |
| Block + culture | B1 | 6.4 | 9.6 | B6 | 5.3 | 9.4 |
| Block + culture + calcium carbonate | B2 | 4.9 | 7.5 | B7 | 6.2 | 11.1 |
| Block + culture + urea | B3 | 7.8 | 10.9 | B8 | 7.8 | 11.6 |
| Block + culture + calcium carbonate + urea | B4 | 8.6 | 8.4 | B9 | 5.9 | 11.6 |
| Block + culture + calcium carbonate + urea( 1h late) | B5 | 9.7 | 9.6 | B10 | 6.5 | 11.7 |

After 1 hour when urea is added, it is hydrolysed and then more carbonates can bind with the calcium in the cracks. This deposits a thick layer of calcium carbonate and thus the cracks are healed. The precipitation of calcite done by the microorganism can be by various metabolic processes but most commonly used pathway is the conversion of organic carbon into inorganic carbon (Kraus *et al*., 2013). On the hydrolysis of urea by the action of urease, ammonia and carbamate are produced. The carbamate will be hydrolysed into ammonia and hydrogen carbonate. This hydrogen carbonate is dissociated in bicarbonate and hydrogen ions. The ammonia after reacting with water will lead to the production of hydroxide ions that will create the alkaline conditions. This causes the increase in pH (Mahanty *et al*., 2014)which will cause the bicarbonate equilibrium production. Thus the amount of carbonate ions in the solution increases and this leads to the saturation that is increased in the carbonate concentration (Chahal *et al.,* 2011; Talaiekhozan *et al.,* 2014). This is because the number of the bacterial cells increased and thus the activity of microbial induced calcium carbonate precipitation is enhanced (Chou *et al.,* 2011).

**CONCLUSION**

Our study revealed that when bacteria is provided with the required optimum conditions it is able to heal cracks and enhance durability of cement (Achal *et al.,* 2009) as a there is a formation of a layer calcium carbonate on the surface (Chuab *et al.,* 2012) that strengthens the structure. Therefore this study is highlighting the calcite precipitating ability by the hydrolysis of urea. As cement is widely used in buildings and architectural structures therefore its durability and improvement in structural integrity by the aid of these bio-binders is a wide area of interest.

## REFERENCES

ACHAL, V., BASU, P.C., MUKHERJEE, A. AND REDDY, S.M., 2009. Strain improvement of *Sporosarcina pasteurii. J. Ind. Microbiol. Biotechnol*., **36:** 981-988.

ACHAL, V., MUKHERJEE, A. AND REDDY, M.S., 2011. Effect of calcifying bacteria on the permeation properties of concrete structure. *J Ind. Microbiol. Biotechnol*., **38:** 1229-1234.

BANGA, S.S., LIPPERTA, J.J., MULUKUTLAB, S., RAMAKRISHNANB, V. ANDYERRAB, U., 2010. Microbial calcite a bio-based smart nano material in concrete remediation. *Int. J. Smart. Nano. Mat*., **1:** 28-39.

CHAHAL, N., RAJOR, A. AND SIDDIQUE, R., 2011. Calcium Carbonate precipitation by different bacterial strains. *Afr. J. Biotechnol*.,**10:** 8359-8372.

CHOU, C.W., AYDILEK, A.H., LAI, M. ANDSEAGREN, E.A., 2011. Biocalcificaion of sand through ureolysis. *J. Geotech. Geoenviron. Eng*., **137:** 1179-1189.

CHUAB, J., IVANOVA, V. AND STABNIKOVA, V., 2012. Microbially Induced calcium carbonate precipitation on surface in the bulk of soil. *Geomicrobiol. J*., **26:** 544-549.

CHUNXIANG, Q., LIANG, C., JIANYUN, W. AND RUIXING, W., 2009. Corrosion protection of cement-based building materials by surface deposition of $CaCO_3$ by *Bacillus pasteurii., Mater. Sci. Eng. C*., **29:** 1273-1280.

DHAMI, N.K., MUKHERJEE, A. AND REDDY, M.S., 2014. Application of calcifying bacteria for remediation of stones and cultural heritages. *Front. Microbiol*., **5:** 1-12.

DHAMI, N.K., MUKHERJEE, A. AND REDDY, M.S., 2012. Improvement in strength properties of ash bricks by bacterial calcite. *Ecol. Eng*., **39:** 31-35.

GARRITY, G., BOONE, D.R. AND CASTENHOLZ, R.W., 2011. The Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, & Planctomycetes. *Bergey's Manual of Systematic Bacteriology*., Springer-Verlag, New York, NY.

KRAUS, C., HIRMAS, D., *ET AL.,* 2013. Microbially indurated rammed earth: a long awaited next phase of earthen architecture. ARCC Conference Repository.

MAHANTY, B., KIM, S. AND KIM, C.G., 2014. Dissolved inorganic carbon inventory and $CO_2$ sequestration in ureolytic biocalcification process with *Bacillus pasteurii. Geomicrobiol J*., **31**:145-151.

RAUT, S.H., LELE, S.S. AND SARODE, D.D., 2014. Biocalcification using *B. paeteurii* for strengthening brick masonry civil engineering structures. *World J. Microb. Biot*., **30:**191-200.

SARDE, D., CHOONIA, H.S., SARODE, D.D AND LELE, S.S., 2009. Biocalcification by *Bacillus pasteurii* urease: a novel application. *J. Ind. Microbiol. Biot.,* **36**: 1111-1115.

TALAIEKHOZAN, A., ABDMAJID, M.Z., ANDALIB, R., FULAZZAKY, M.A., HAIDAR, H.I., HAMZAH, N., HUSSIN, M.W., KEYVANFAR, A., LEE, C.T., MARWAR, N.F., SHAFAGHAT, A. AND ZIN, R.M., 2014. A review of self-healing concrete research development. *Enviro. Treat. Tech*., **2:** 1-11.