# Bio Cementation: A Novel Technique and Approach Towards Sustainable Material

## Sweety Parmar, Darshan Marjadi

Abstract— A large number of human activities and natural process creates disturbance in concrete structures that ultimately reduces the service life of a structure. The cement industry produces about 5% of the global anthropogenic carbon dioxide (CO2) emissions. Calcium carbonate is one of the most common minerals widespread on earth. Microbial induced carbonate precipitation is a process by which living organisms produces inorganic solids. Bacteria are incredibly diverse and abundant and many bacterial species contribute to the precipitation of mineral carbonates in various natural environments. Production occurs in an alkaline environment and which leads to calcite precipitation. The hydrolysis of urea by the enzyme urease is unique in that it is one of the few biologically occurring reactions that can generate carbonates. The ubiquity and importance of microbes in inducing calcite precipitation make "Bio cement" a most important metabolic product of Biomineralization which can remediate and restore such structure. Feasibility studies on the use of sludge to produce cement as a means of ultimate sludge disposal have been initiated.

*Index Terms*— Bio cement, Biosilica, Calcium Carbonate, Calcite precipitation, Sustainability.

## I. INTRODUCTION

Due to urbanization, nuclear families and other reasons lead to increase in construction. For construction, soil is treated by chemicals or else cement to increase its strength. The addition of cement makes soil unfertile and irreversibly damaged soil [1]. Existing chemical grouting technique to strengthen soil is toxic [2]. Globally demand of cement increase by 4.7% annually. During the production of Portland cement, it is heated at 1500oCand emits 5% of CO2 emission thus give rise to global warming as Biocement to improve the strength of soil and reduce [3] Portland cement need in the high amount which acts as binding material during construction. Thus Biosilica used need for Portland cement, ultimately decreasing CO<sub>2</sub> emission [3]. Another problem is weathering leads to dissolution of stone minerals and cracks appear [4]. In construction buildings, concrete is widely used as it is cheaply available but it is weak in tension as a resulting crack appears. Micro cracks form the network to form big crack and then it allows ingress of aggressive material and reduces the durability of the building [5]. Generally, micro cracks are not harmful but when aggressive material ingress in it leads to increase in porosity which is dangerous and decrease the life of structure [6]. Cracks are treated by water

**SweetyParmar,** Department of Biotechnology, Shree Ramkrishna Institute of Computer Education and Applied sciences, M.T.B, Campus, Athwalines, Surat-395001, India.

**Darshan Marjadi,** Department of Biotechnology, Shree Ramkrishna Institute of Computer Education and Applied sciences, M.T.B, Campus, Athwalines, Surat-395001, India



repellent and consolidants which have a limitation that effect of these treatments are irreversible and limited long-term performance[4]. There are many research papers show Microbial Calcium Precipitation, it is used as Biocement for remediation cracks. Bacteria-induced precipitation by urease production. Thus Microbial Induced Calcium Precipitation (MICP) used for remediation and restoration of Building. MICP is Biomineralization process helps in the formation of silica, iron oxide, calcium carbonate, etc. Calcium carbonate precipitate is obtained by Urea Hydrolysis, Denitrification, Sulphate reduction, Iron reduction. Out of all these methods, Urea hydrolysis is a better option as this process can be easily controlled [7].Biocement may be liquid or solid thus liquid flows like water and easily penetrate in soil or crack as compare to cement which does not easily penetrate. Portland cement takes28 days for full strength but Biocement need very less time. Thus Biocement is an environment-friendly alternative [1].

## II. BIOCEMENT

It is easy to produce Biocement using microorganism as it requires less time and requirement. Biocement is also cost effective and non-pathogenic and environment-friendly microorganisms are used to produce Biocement [8].

## A. Calcium precipitation

Types of Biocement:

There are two types of calcium precipitates used as Biocement:

## (i) Calcium Carbonate Precipitates:

In nature, many rocks are cemented by the formation of calcium carbonate. A building having good strength, due to humidity there is expansion in structure leads to crack. When this type of building treated with MICP producing microorganism then cracks can heal by reducing porosity [7]. Widely studied calcium carbonate precipitation occurs by urea hydrolysis by urease enzyme from the microbe. Urea is widely used as fertilizer, waste product obtained from mammals and decomposition of uric acid excreted by birds. Increase in urea cause contamination of soil, water and produce harmful algal blooms. Microorganism produce urease exists both within the cell and as an extracellular enzyme on cell death. During microbial urease activity, one mole of urea converts to one mole of ammonia and one mole of carbonate (1), which spontaneously hydrolyse to another mole of ammonia and carbonic acid as follows [7].

$$CO(NH_2)_2 + H2O^{\text{Bacteria}} NH_2COOH + NH_3$$
 (1)

$$NH_2COOH + H_2O \longrightarrow NH_3 + H_2CO_3$$
 (2)

36

This product equilibrates in water to form bicarbonate, produce one mole of ammonia and hydroxide which results in pH increase.

$$H_2CO_3 \longrightarrow 2H^+ + 2CO_3^{2-}$$
 (3)

$$NH_3 + H_2O \longrightarrow OH^{--} + NH_4^+ (4)$$

$$Ca^{+2} + CO_3^{2-}$$
 CaCO<sub>3</sub>(5)

Carbonic acid increase CO<sub>2</sub>conversion and an increase in production of carbonate. Hydrolysis of urea results in accumulation of both bicarbonate and ammonium ion in the cell favors urea and bicarbonate metabolism. Production of carbonate from bicarbonate is strongly pH dependent, an increase in carbonate concentration occurs under alkaline conditions. Therefore, in an alkaline environment, CaCO<sub>3</sub> precipitation occurs outside the cell as calcium and carbonate present in an abundant amount. Calcium carbonates are deposited on the cell due to the production of Biofilm and extra polymeric substances by bacteria [7]. Naturally, the deposition of CaCO3 in the void spaces leads to clogging of porous material [4]. Factors affecting Calcium carbonate precipitation are as follows: (1) calcium concentration, (2) concentration of Dissolved Inorganic Carbon (DIC), (3) pH, (4) bacteria as a nucleation site [9].

#### (ii)Calcium Phosphate Cement:

Calcium Phosphate Cement (CPC) is widely used in biomedical applications such as Bone regeneration. CPC is produced widely by mixing Calcium orthophosphates with aqueous phase generally water which forms paste and can be used [10]. The porosity of CPC can vary from 30% to 50 % depending on the ratio of liquid to powder [11]. There are various commercial CPC available in the market they are generally hydroxyapatite [12]. Very few research being done on an application of CPC in construction as the cost increase a lot when CPC is used. Various applications of Calcium Phosphate Cement are described in the following sections.

#### B.Biosilica

To reduce the use of Portland cement, Biosilica is used to produce Biocement. There are two steps to produced Biocement as follows:

- (i) Organic residues are burnt to obtain ash containing Biosilica
- (ii)Biosilica mix with Portland cement to produce Biosilica.

Thus Biosilica decrease use of Portland cement. Various organic substances can be burnt to obtain Biosilica. Biosilica act as a pozzolanic material.

The pozzolanic reaction between Biosilica and calcium hydroxide as follows:

$$Ca(OH)_2 + H_4SiO_4 \longrightarrow Ca^{2+} + H_2SiO_4^{2-} + 2H_2O$$
  
 $CaH_2SiO_4 \longrightarrow x2H_2O$ 

This reaction is optimized to obtain desired physical and mechanical strength of Biocement.

Si is a second most abundant element on this earth after oxygen. Various plants obtain silica from soil and deposit it in the older leaf. Non-edible parts of a plant contain silica which can be used for a production of Biocement. The following sources are burnt to produce ash containing Biosilica.

- a. Sawdust ash as Biosilica
- b. Paper mill sludge ash as Biosilica
- c. Rice husk and rice straw ash as Biosilica
- d. Sugar cane ash as Biosilica
- e. Oil palm ash as Biosilica
- f. Bamboo leaf ash as Biosilica

Biosilica partially reduce the emission of CO2, as it is mixed with Portland cement to reduce the use of Portland cement thus its demand reduces [3].

#### III. SOURCES OF BIOCEMENT

#### A. Microorganisms:

There are basically three main groups of microorganisms which can produce Microbial Induced Calcium carbonate precipitates [8].

- (a) Photosynthetic microorganisms
- (b) Sulfate reducing bacteria
- (c) Microorganisms involved in Nitrogen cycle

#### i. Bacteria:

Bacteria are prokaryotic unicellular organisms present at almost every place. In nature, many rocks are cemented by the production of Calcium carbonate. Various bacteria found near natural calcium source may be able to produced calcium carbonate precipitate which is called Biomineralization. Many species of Bacillus able to produce Bio calcite are Bacillus pasteurii, В. subtilis, **Bacillus** sphaericus and B.lentus [4]. Bacteria which carry out the metabolic process such as Urea hydrolysis, Denitrification, Sulphate reduction, Iron reduction and produce Carbonic anhydrase enzymes have the ability to produce Calcium carbonate precipitates [7]. The majority of research is focused on Urease-producing microorganisms, to know molecular mechanism very few work has been done on UreC gene which is responsible for the production of one of three subunits of Urease enzyme [13]. Pseudomonas, Variovorax, Leuconostocmesenteroides, etc are microorganisms produces Bio calcite in a various amount [14]. Nonureolytic bacteria helpful in MICP production in mixed culture as they provide the nucleation site for calcium carbonate precipitation e.g. B. subtilis [15]. Halotolerant alkaliphilic bacteria also produce calcium carbonate precipitates [16]. Idiomarinainsulisalsae gives the better result than B. pasteurii for the production of MICP [17].

### ii. Microalgae:

Photosynthetic organisms which produce urease and consume urea can be used for the production of Bio calcite thus microalgae can be used as a nucleation site for Microbial Induced Calcium Carbonate Precipitation. Photosynthetic organisms utilize  $CO_2$  which is in equilibrium with carbonate bicarbonate system as shown in equation (2). Microalgae utilize  $CO_2$  results in a shift in equilibrium and thus increment in pH occurs (3), when this occurs in presence of Calcium there is a production of calcium carbonate (4) as



carbonates are present in an abundant amount due to pH shift. The reaction occurs as follows [8]:

$$CO_2 + H_2O$$
  $\longrightarrow$   $(CH_2O) + O_2(1)$   
 $2HCO_3$   $\longrightarrow$   $CO_2 + CO_3^{2-}(2)$   
 $CO_3^{2-} + H_2O$   $\longrightarrow$   $HCO^{3-} + OH^{-}(3)$   
 $Ca^{2+} + HCO^{3-} + OH$   $\longrightarrow$   $CaCO_3 + 2H_2O$  (4)

The amount of increase in pH is directly proportional to the calcium precipitation, hence microorganisms should be selected which can survive in high calcium and ammonia concentration as urea hydrolysis produces ammonia. Also, microorganisms should be nonpathogenic [3]. Photosynthetic organisms such as green algae from marine as well as fresh water are *Cyanobacteria syncococcus*, *Scyntonema*, *Chlorella*, *Nannochlorisatomus*, *Synechocystis*, *Synechococcus* produce Bio calcite [4].

#### B. Plant:

Several plants contain silicon in abundant amount as some plants have the capacity to absorb silica from the soil. This type of plants is burnt then the biomass having silica mixed with Portland cement to produce Biocement [3]. Plant-derived urease can also be used for Biocement production same way as microbial calcium precipitation occurs the only source differs. Plant-derived urease has benefits that its size is small can penetrate in very small crack also. Families of the plant which produce urease are varieties of beans in leguminous family, watermelons, squash, pumpkin and cucurbits family. The crude extract of jack bean is a good source of urease. Through plant derived urease both calcium carbonate as well as calcium phosphate cement can be produced [18].

## C. Sewage sludge:

Municipalities collect a large amount of waste which is disposed of by land spreading, burial in landfills and incineration. It consumes energy as well as money to treat sewage waste but it can be used for Biocement production and reduces the cost for treatment of sewage sludge. Sewage sludge contains silica hence can be used for the production of Biosilica and can blend with Portland cement. Sewage sludge can be used by two ways to produce Biocement as follows [3]:

- (a) By blending its incinerating ash with Portland cement
- (b) By co-combustion of limestone with sewage before adding in Portland cement

Trace metals are another problem during sludge treatment but when sludge is used for Biocement production then trace metals are immobilized in cement and reduce the risk of metal contamination in the environment. Thus sewage sludge can be used as raw material to produce Biocement and partially eliminate the use of Portland cement which will reduce CO<sub>2</sub> emission and act as environment-friendly [3]. Microorganisms from waste water used for Biocement production are *Caulobacter*, *Blastomonas*, *Roseobacter*, *Gemmatimonas*, *Saccharopolyspora* and *Sphingomonas* [19].

#### IV. APPLICATIONS

#### A. Remediation of Cracks in Building:

As the construction got older there is wear and tear in the concrete leads to micro cracks and thus water enters in the micro cracks leads to the development of macro-cracks. This may eventually lead to a bad appearance of building and building may also collapse if not treated at an early stage, hence microbial culture injects in the cracks of building thus treated at an early stage [4]. This can also be used to conserve cultural heritage of India by remediating cracks in ancient monuments of India [20].

## B. Development of self-healing concrete:

Inspired by the nature, the scientist tries to create self-healing concrete, thus when there is a crack it will heal itself without any treatment but to have these properties, concretes are pretreated with MICP microorganisms. Microorganism remains in the dormant state in the normal condition but when there will be the formation of the crack, ingress of water results in the activation of the cell and thus Calcium carbonate is produced and crack can heal itself [21]. Self-healing concrete is able to increase the durability of building [22]. This technology will not allow cracks to appear hence this is preventive measure for cracks and leads to use of environment-friendly Cement and sustainable building [6]



Figure: 1 Cracks healed with calcium carbonate Source: Society for Applied Microbiology

### C. Soil improvement using MICP:

For construction soil needs to be treated with chemicals or cement to change it properties otherwise construction at soft soil does not sustain. Therefore, MICP microorganisms in the liquid medium added to soil using the various instrument to harden it. It also reduces the use of chemicals and cement thus saves cost as well as environmental friendly [2].

## D. To make sustainable deserts:

In deserts when there is storm then it's very difficult to survive there. Even people may die traveling in the desert. Many other problems occur to the people living in arid and semiarid areas, coexisting with the shifting sands, struggle to sustain in the harsh conditions. Thus a novel concept of architecture in desserts can be helpful. Dunes move rapidly with air, in this concept MICP is used for the localized cementation of granular material. Thus cementation done through microorganisms must be a controlled process so it produces the pattern we want and serves as architecture [23].



Table 1 Possible microbial processes that can lead potentially to biocementation[31]

Physiological group	Mechanism of	Essential conditions	Potential Geotechnical
of Microorganisms	Biocementation	for Biocementation	Applications
Sulphate reducing	Production of	Anaerobic conditions;	Enhance stability for
Bacteria	undissolved sulphides	presence of sulphate	slopes and dams
	of metals	and carbon source in	
		soil	
Ammonifying	Formation of	Presence of urea and	Mitigate liquefaction
Bacteria	undissolved carbonates	dissolved metal salt	potential of sand.
	of metals in soil due to		Enhance stability for
	increase of pH and		retaining walls,
	release of CO <sub>2</sub>		embankments, and
			dams; Increase bearing
			capacity of foundations
Iron-reducing	Production of ferrous	Anaerobic conditions	- Density soil on
Bacteria	solution and	changed for aerobic	reclaimed sites and
	precipitation of	conditions;	prevent soil avalanching
	undissolved ferrous and	presence of ferric	-Reduce liquefaction
	ferric salts and	minerals	potential of soil
	hydroxides in soil		
	hydroxides in soil		



## World Journal of Research and Review (WJRR) ISSN:2455-3956, Volume-4, Issue-3, March 2017 Pages 36-41

applied here to reuse RCA. But before using RCA is soaked in water for a long time then it is dried. In this method, more amount of MICP organisms is used as the porosity of RCA is high [26].

## G. Application of CPC as Biocement:

Calcium Phosphate Cement is used majorly in Biomedical as compare to the construction industry. Therefore, this section focuses on medical applications of CPC.

#### a. Bone regeneration:

Collagen and non-collagen protein present in the extracellular matrix of bone cause regeneration and self-healing of bone[27] But the application of CPC give rapid regeneration and provide strength till cells regenerate themselves as a result people will no longer need to have plasters for two months. CPC is a temporary method to fill gaps in bone till bone cells regenerate [28].

#### b. Skull regeneration:

It experimented, holes were created in rabbit skull and it was treated with the CPC which is available in the market and  $\beta$ -dicalcium silicate [29]. Results were found positive in both the treatment but  $\beta$ -dicalcium silicate gives better results. This shows future potential for utilizing CPC in skull defects and treatment of various major problems occur due to skull defects [29].





(A) Skull defect of Rabbit, (B) treatment of skull with (a) CPC, (b)  $\beta$ -C2 silicate, (c) in the defect site, (d) as a negative control [29].

H.CPC as Drug delivery system [30]:

Carriers for antibiotic

Carriers for analgesic, anticancer, anti-inflammatory drugs.

#### CONCLUSION

Bacterial concrete, an inherent and self-repairing biomaterial that can remediate the cracks and fissures in concrete. Though concrete is quite strong mechanically, it suffers from several drawbacks, such as low tensile strength, permeability to liquid and consequent corrosion of reinforcement, susceptibility to chemical attack and low durability. Modifications have been made from time to time to overcome such difficulties of concrete but all those processes are not easy and good. MICP is a complex biochemical process that utilizes the urea hydrolysis that takes place between the sand particles for improvement of soil engineering properties. There is an increasing need for a ground development method, and one of the methods is to improve the strength of soil particles by utilizing the cementation technique. Even though there are various chemical methods available that are currently in practice, many of them have adverse environmental effects.



(a) Pneumatic balloons inserted in desert



(b) MICP on pneumatic balloons



(c) Growth of plants and increase In MICP



(d) People can live under such construction

Figure 2: Application of MICP and future life of deserts Pneumatic balloons are inserted to inject microbial culture in sand and thus dunes get solidify where people can stay safe, get shelter [23].

#### E. Removal of calcium ion:

Waste water or sludge is used as raw material for Biocementation. It serves two purposes it produces Biocement thus reduce need for Portland cement ultimately lead to decrease in CO2 emission. Also, it removes Calcium from waste thus decrease the cost of waste treatment [24]. In paper mill industry MICP microorganisms can be used to remove calcium [25].

## F. Recycle Concrete Aggregate (RCA):

RCA cannot be reuse due to its high water absorptive capacity and low binding efficiency. Thus it considered as waste but to dispose of waste is also costly hence MICP is



#### REFERENCES

- [1] Chu, J. (2016). Solutions to Sustainability in Construction: Some Examples. Procedia Engineering, 145, 1127-1134.
- [2] Soon, N. W., Lee, L. M., Khun, T. C., & Ling, H. S. (2014).
- Factors affecting improvement in engineering properties of
- residual soil through microbial-induced calciteprecipitation. Journal of Geotechnical and Geoenvironmental Engineering, 140(5), 04014006.
- [3]. Hosseini, M. M., Shao, Y., & Whalen, J. K. (2011). Biocement production from silicon-rich plant residues: Perspectives and future potential in Canada. Biosystems engineering, 110(4), 351-362.
- [4]. Bharathi, N. Calcium Carbonate Precipitation with Growth Profile of Isolated Ureolytic Strains.
- [5]. Vekariya, M. S., &Pitroda, J. (2013). Bacterial concrete: new era for construction industry. International Journal of Engineering Trends and Technology, 4, 4128-4137
- [6]. Wiktor, V., &Jonkers, H. M. (2011). Quantification of crack-healing in novel bacteria-based self-healing concrete. Cement and Concrete Composites, 33(7), 763-770.
- [7]. Qiu, J., Tng, D. Q. S., & Yang, E. H. (2014). Surface treatment of recycled concrete aggregates through microbial carbonate precipitation. Construction and Building Materials, 57, 144-150
- [8]. Ariyanti, D., & Handayani, N. A. (2012). Hadiyanto (2012) Feasibility of Using Microalgae for Biocement Production through Biocementation. J Bioprocess Biotechniq, 2(111), 2.
- [9]. Fisher, K. A., Yarwood, S. A., & James, B. R. (2017). Soil urease activity and bacterial ureC gene copy numbers: Effect of pH.Geoderma, 285, 1-8
- [10]. Jia, J., Zhou, H., Wei, J., Jiang, X., Hua, H., Chen, F., ... & Liu, C. (2010). Development of magnesium calcium phosphate biocement for bone regeneration. Journal of The Royal Society Interface, rsif20090559.
- [11]. Vater, C., Lode, A., Bernhardt, A., Reinstorf, A., Heinemann, C., &Gelinsky, M. (2010). Influence of different modifications of a calcium phosphate bone cement on adhesion, proliferation, and osteogenic differentiation of human bone marrow stromal cells. Journal of Biomedical Materials Research Part A, 92(4), 1452-1460.
- [12]. Horiuchi, S., Hiasa, M., Yasue, A., Sekine, K., Hamada, K., Asaoka, K., & Tanaka, E. (2014). Fabrications of zinc-releasing biocement combining zinc calcium phosphate to calcium phosphate cement. Journal of the mechanical behavior of biomedical materials, 29, 151-160
- [13]. Fisher, K. A., Yarwood, S. A., & James, B. R. (2017). Soil urease activity and bacterial ureC gene copy numbers: Effect of pH.Geoderma, 285, 1-8.
- [14]. Hammes, F., Boon, N., de Villiers, J., Verstraete, W., &Siciliano, S. D. (2003). Strain-specific ureolytic microbial calcium carbonate precipitation. Applied and environmental microbiology, 69(8), 4901-4909.
- [15]. Gat, D., Tsesarsky, M., Shamir, D., & Ronen, Z. (2014). Accelerated microbial-induced CaCO3 precipitation in a defined coculture of ureolytic and non-ureolytic bacteria. Biogeosciences, 11(10), 2561.
- [16]. Stabnikov, V., Jian, C., Ivanov, V., & Li, Y. (2013). Halotolerant, alkaliphilic urease-producing bacteria from different climate zones and their application for biocementation of sand. World Journal of Microbiology and Biotechnology, 29(8), 1453-1460.
- [17]. Carmona, J. P., Oliveira, P. J. V., &Lemos, L. J. (2016). Biostabilization of a Sandy Soil Using Enzymatic Calcium Carbonate Precipitation. Procedia Engineering, 143, 1301-1308
- [18]. Dilrukshi, R. A. N., & Kawasaki, S. (2016). Effective Use of Plant-Derived Urease in the Field of Geoenvironmental. Geotechnical Engineering. J Civil Environ Eng, 6(207), 2.
- [19]. Torres, A. R., Martinez-Toledo, M. V., Gonzalez-Martinez, A., Gonzalez-Lopez, J., Martin-Ramos, D., &Rivadeneyra, M. A. (2013). Precipitation of carbonates by bacteria isolated from wastewater samples collected in a conventional wastewater treatment plant. International Journal of Environmental Science and Technology, 10(1), 141-150.
- [20]. Marjadi, D. S. Conservation and restoration of cultural heritage: A biotechnological approach. Advances in Applied Science Research, 2016, 7(4): 159-167.
- [21]. Achal, V., Mukherjee, A., & Zhang, Q. (2016). Unearthing ecological wisdom from natural habitats and its ramifications on development of biocement and sustainable cities. Landscape and Urban Planning, 155, 61-68.
- [22]. Talaiekhozan, A., Keyvanfar, A., Shafaghat, A., Andalib, R., Majid, M. A., Fulazzaky, M. A., ... &Marwar, N. F. (2014). A review of self-healing concrete research development. Journal of Environmental Treatment Techniques, 2(1), 1-11.
- [23]. Larsson, M. (2010). Dune: Arenaceous Anti-Desertification Architecture. In Macro-Engineering Seawater in Unique Environments (pp. 431-463). Springer Berlin Heidelberg
- [24]. Reddy, M. S. (2013). Biomineralization of calcium carbonates and their engineered applications: a review. Frontiers in microbiology, 4, 314

- [25]. Erşan, Y. Ç., De Belie, N., & Boon, N. (2015). Microbially induced CaCO 3 precipitation through denitrification: an optimization study in minimal nutrient environment. Biochemical Engineering Journal, 101, 108-118
- [26]. Qiu, J., Tng, D. Q. S., & Yang, E. H. (2014). Surface treatment of recycled concrete aggregates through microbial carbonate precipitation. Construction and Building Materials, 57, 144-150.
- [27]. Lode, A., Reinstorf, A., Bernhardt, A., Wolf-Brandstetter, C., König, U., &Gelinsky, M. (2008). Heparin modification of calcium phosphate bone cements for VEGF functionalization. Journal of Biomedical Materials Research Part A, 86(3), 749-759.
- [28]. Hempel, U., Reinstorf, A., Poppe, M., Fischer, U., Gelinsky, M., Pompe, W., & Wenzel, K. W. (2004). Proliferation and differentiation of osteoblasts on Biocement D modified with collagen type I and citric acid. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 71(1), 130-143.
- [29]. Sun, M., Liu, A., Ma, C., Shao, H., Yu, M., Liu, Y., ... & Gou, Z. (2016). Systematic investigation of  $\beta$ -dicalcium silicate-based bone cements in vitro and in vivo in comparison with clinically applied calcium phosphate cement and Bio-Oss®. RSC Advances, 6(1), 586-596.
- [30]. Ginebra, M. P., Traykova, T., &Planell, J. A. (2006). Calcium phosphate cement as bone drug delivery systems: a review. Journal of Controlled Release, 113(2), 102-110.
- [31]. Ivanov, V., & Chu, J. (2008). Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ. *Reviews in Environmental Science and Bio/Technology*, 7(2), 139-153.



www.wjrr.org

41