Self-healing in concrete using ureolytic species of bacteria and yeast

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Abstract. Structural members made of concrete may develop micro cracks within themselves with time due to shrinkage and temperature effects, which instigates untimely failures. After the second hydration of concrete, some cracks and pores are still left unsealed leading to the auxiliary degradation and lessened service life. The use of ureolytic species of microorganism with concrete aids in the sealing of these cracks without expulsion of any precarious fumes or constituents detrimental to the environment, which is present in various chemical sealants accessible in the market. The ureolytic bacterial species is capable of producing calcium carbonate precipitate in the presence of urea and calcium substrate. The precipitate formed has the ability to cure the cracks and pores that would have occurred during placing or in their service life. In this paper, the comparison between bacteria and yeast infused concrete with the control concrete in terms of durability aspect like compressive strength, behaviour in acidic exposure and water absorption rate has been determined. The amount of calcium carbonate generated with same growth conditions has been determined for both bacteria and yeast infused concrete, which will help in evaluating the nature of different microorganism for self-healing mechanism. The result of this study is that Yeast has the potential to be used as microbe in self-healing of concrete.

Keywords- bacteria infused concrete; yeast infused concrete; acid attack; self-healing; durability

1. Introduction
In 2017, the second rank in cement production of the world is secured by India with the capability of production of 425 million metric tons. In the same financial year, 270 million metric ton was consumed. With the growth rate of construction activities by 6-7% in recent years, the supply will be incapable to meet consumption in coming years [1]. As indicated by the Intergovernmental Panel on Climate Change, 1tonne of cement = 1.25 tonnes of CO2. The modernization trend in construction has given a baggage in the form of pollution. The energy consumed and the limestone needed to produce cement does not substantiate the burning of two particularly stable compounds of Calcium and Carbon to release CO2 gas. So much energy is put to produce concrete at the expense of the environment. Still the cement concrete structures do not have the long life that could complement the energy consumed [2]. They still develop cracks, internal faults and deter with time. Consequently, refurbishment cost increases. Synthetic fillers and treatments are used to resolve this issue which contains hazardous chemicals and epoxy resins. Consolidates of stone and water repellents hastens decay of structure [3,
When chemically bonded construction material is subjected to weathering and harsh atmosphere they tend to deteriorate and become porous with time. Hence, we need something which is not only connected to nature but also instills strength and healing capacity to concrete. Jonkers gave the concept of bio-concrete. His research focuses on interconnection link between nature and human construction. He introduced bacteria of Bacillus family that was capable of generating CaCO3 precipitation around their cells. These organisms are heterotrophs and need organic carbon to grow. This precipitation formed is enough to fill micro-pores in the concrete structure and bind soil particles together. However, this process takes time. To increase its efficiency and rate of precipitation urea and a calcium substrate is added to the concrete or soil mixture [5]. Ureolytic bacteria produce calcium carbonate by hydrolyzing urea in the presence of calcium-rich environment which would fill the pores inside material [6]. Calcite precipitation by micro-organisms which are proficient in producing a good amount of urease enzyme should be used in bio-precipitation techniques [7]. Prerequisites of calcium precipitation by micro-organisms includes good urease production, ability to surviving with urea and other chemical compounds. A basic advantage of this process is that culturing of organisms is not required every time. They can be used 2-3 times [8]. Therefore a lot of currency is saved. When nitrogen is under-supplied the production of urease enzyme takes place. When Bacillus subtilis is subjected to nitrogen deficient source they release 20 to 25 times the urease enzyme otherwise [10]. On the contrary, a calcium ion abundant source would increase urease production 10 times especially when calcium ion provided is 30mM [11].

Apart from bacteria other organisms also possess an ability to produce urease enzyme. The capability of yeast to produce urease enzyme by means of urease test on CHROM agar medium was identified by Bharathi and Meyyappan [12]. When nitrogen is under-supplied the production of urease enzyme takes place. Very less data on yeast was available for bio-concreting. Though, urease activity of yeast cell has been published [13]. Yeast strain of Candida tropicalis was added to concrete with an equimolar mixture of urea and calcium chloride. This was tested for water absorption for 24hours and then 30 minutes of oven air drying. The Yeast’s concentration on the surface of concrete for the process of calcium carbonate has also been studied [12]. ‘Aerated cellular concrete’ is a light-weight configuration where H2 gas bubbles are emitted after reaction between aluminum fine particles with elevated pH of concrete creating a cellular’ structure. However, fine aluminum is a combustible substance. In addition, its surface corrodes easily [14].

The concrete durability is the resistance when exposed to decline conditions during its service life [16]. The chemical attack, as one of the concrete durability aspects, is also a significant investigation part which results in cracking, strength loss and deterioration [17]. However, many investigations have been made for the acid resistance behaviour of ordinary Portland cement (OPC) concrete but very limited information can be found on the bio-concrete durability studies. The relative performance of bacterial structural concrete to that of ordinary Portland cement (OPC) concrete served as a control contained in the sulphuric and hydrochloric acid solutions (H2SO4 and HCl) using Bacillus clostridium, species cannot grow to defend Hydrogen Peroxide (H2O2) due to the enzyme shortage [18]. Sulphuric acid and hydrochloric acid are also considered to be as the most aggressive threat from industrial waters. The acidic attack is influenced by the disintegration processes of the cement paste components [19]. The acid attack risk can also be reduced by blocking the pathways present within the concrete porosity. CaCO3, from the micro-organism as filler material can decrease the porosity and improve the concrete durability. In this paper, two microbes – bacteria (Bacillus subtilis) and yeast (Saccharomyces cerevisiae) are added to concrete in suspension form with water. Durability and exposure to acidic environment tests are performed on bacterial, yeast and control specimen. The precipitation of calcium carbonate on the surface of concrete specimen EDTA test is performed.
2. Material and Methods
Control concrete, Bacteria infused concrete and yeast concrete has been used in research.

2.1 Flowchart
Figure 1 shows the course of the experiment.

![Flowchart of Experimental study](image)

Figure 1. Flowchart of Experimental study

2.2 Micro-organism
The microbes - Bacillus subtilis and Saccharomyces Cerevisiae were selected due to following reasons:

- The production of calcium carbonate precipitation in presence of a calcium substrate and urea.[10-12]
- Availability in research area vicinity.
- Human-safe, Biologically-safe microbes.[22]
- Availability of their chemical precursors.
- Economy.
- Availability of apparatus for producing the microbial solution.
2.2.1 Medium of growth. Saccharomyces cerevisiae strain was obtained from petri plate of Saccharomyces cerevisiae refrigerated in Bio-Engineering Department Lab. A new culture was grown on agar plates by streaking technique as shown in figure no. 2(a). The agar plate consisted of 10 gm/l yeast extract, 20gm/l Peptone, 20 gm/l Dextrose and 20 gm/l Agar. It was suspended in 150ml of distilled water.

Bacillus subtilis strain was obtained from the solution of Bacillus subtilis which was refrigerated in Bio-Engineering Department Lab. It was grown on agar plates by spreading technique as shown in figure no. 2(b). The Agar plate comprised of 2.8gms of Nutrient Agar made of 5.00gm/l of peptic digest of animal tissue, 5.00gm/l NaCl, 1.50 gm/l Beef Extract, 1.50gm/l Yeast Extract and 15 gm/l Agar which was suspended in 150ml of distilled water.

2.2.2 Growing conditions. The suspension containing bacteria and yeast was sterilized by autoclaving at 15 lbs pressure maintained at 121ºC for 15 minutes then a thoroughly mixed sterilized solution was poured into sterilized petri plates. The microbial solution was then applied to solidified petri plates in UV Chamber by spreading technique or streaking technique. Bacteria and yeast were fully grown in 28 hours. Serial dilution was done to reduce the number of bacteria per unit sample volume, then again, streak or spread plate technique was performed using nutrient agar medium. The standard viable plate count was used to determine the colony-forming units (CFUs).

![Figure 2 (a). Growth of Saccharomyces cerevisiae on the agar plate using Streaking and (b) Growth of Bacillus subtilis on the agar plate using Spreading Technique.](image)

2.3 Preparation of Microbial Solution

2.3.1 Nutrient Medium. In a 250 ml flask, Nutrient broth of bacteria and yeast (without agar) with 100 ml of distilled water was added and shaken until fully mixed (separately of each micro-organism). It was then sterilized by autoclaving at 15 Lbs. pressure maintained at 121ºC for 15 minutes. When it cooled down at normal room temperature, a loopful of bacteria & yeast from agar plates was inoculated to this solution in UV chamber as shown in figure 3. This prepared solution was then carefully placed in Rotating Incubator at 100rpm & 35.7ºC for at least 24 hours.

2.3.2 Microbial Culture. 10gms Urea, 25 gms Calcium chloride and 8 gms Nutrient Broth were added to 2 litre flask to make media for microbial culture. This powder was then shaken with distilled water until it reached 1 litre mark. The obtained solution was then sterilized by autoclaving at 15 Lbs pressure maintained at 121ºC for 15 minutes. When the solution cooled down to normal room temperature, it was distributed equally into 4- 500 ml flask (250 ml each) as shown in figure 4. In UV Chamber, 200μml of microbial solution was inoculated in each flask. This prepared solution was then carefully placed in rotating incubator as shown in figure no.5 at 100rpm, 35.7ºC and for atleast 24 hours.
2.4 Concrete Specimens
Cement conforming to IS 1489 Part-1 [20] of PPC 33 Grade was used. Preliminary information of concrete specimens is tabulated in table 1. The casting and curing were performed at 19.4° C and 63% relative humidity.

Table 1. Properties of concrete specimen

| Preliminary tests                                      | Results                                |
|-------------------------------------------------------|----------------------------------------|
| **Cement**                                            |                                        |
| Consistency of Cement                                 | 34%                                    |
| Initial Setting Time                                   | 31 minutes                             |
| Final Setting Time                                     | 610 minutes                            |
| Fineness Modulus                                      | 2.5                                    |
| Specific Gravity                                      | 2.53                                   |
| Apparent Specific Gravity                             | 2.62                                   |
| Water Absorption                                      | 1.04%                                  |
| Silt Content                                          | 2.04%                                  |
| Zone of Sand Specified by Sieve Analysis               | Zone II of IS 383-1970 [21]            |
| **Fine Aggregate**                                    |                                        |
| Size and Ratio                                        | 20mm and 10mm, 50:50                   |
| Specific gravity                                      | 2.07                                   |
| apparent specific                                     | 2.74                                   |
| Water absorption                                      | 0.45%                                  |
| **Coarse aggregate**                                  |                                        |
| Water pH                                               | 7.0 at zero turbidity                  |
| CaCO₃ content                                         | 152.67 mg/l                            |
| **Concrete mixture composition (per m³):**            |                                        |
| Cement                                                | 442.6 kg                               |
| Fine Aggregate                                        | 629.83 kg                              |
| Coarse Aggregate                                      | 1194.94 kg                             |
| Water                                                 | 160.94 L                               |
| water-cement ratio                                     | 0.4                                    |
| **Microbial Solution to Cement Ratio**                | 0.1                                    |

2.5 Slump value test
Slump value decides the degree of workability of concrete mix. It is obtained by using slump cone of dimension top and bottom diameter 100mm and 200mm respectively with 300mm height. Concrete
mix is then poured in the cone in three subsequent layers, each tamped 25 times. Cone mould is then removed. The height of fall from the top surface of the concrete is measured as slump value.

2.6 Compressive strength test
For compressive strength test, cube moulds of dimension 150X150X150mm have been used for 7th & 28th days for control, bacterial and yeast concrete.

2.7 Water absorption test
To verify the resistance towards water penetration, water absorption test was performed. After 28 days, concrete cubes were surface dried and kept in the oven at 100ºC for not less than 24 hours and weighed. Later, cubes were immersed in water for next 24 hours and weighed. This test was performed on 3 concrete cubes specimens to obtain average value of absorption. The percentage of water absorption was calculated using equation (1).

\[
\text{% WaterAbsorption} = \frac{W(\text{saturated}) - W(\text{ovendried})}{W(\text{ovendried})} \times 100
\]

2.8 Acidic attack test
To study the durability of structural concrete against aggressive agents such as acidic conditions, the specimens were immersed in acidic solution (HCl). When cubes gained their desired strength after 28 days, they were immersed in an acidic solution maintained at pH=2.0 for 15 days. All the results of acid attacks have the concrete age of 43 days.

2.9 EDTA test
To check the precipitation of calcium carbonate on the surface of concrete specimen, EDTA test was performed. After achieving 28th day strength & performing compressive strength test, the surface material obtained was scratched & powdered. This powder was later prepared into solution (3 gms powder diluted to 50ml distilled water). This solution was then filtered to obtain a clear solution through filter paper and finally then titrated against (N/50) EDTA solution using EBT as an indicator.

3. Results & Discussion

3.1. Morphological Characteristics
There morphology and characteristics are illustrated in Table 1.

Table 2: Morphology and characteristics of micro-organisms

| Description                  | Bacteria          | Yeast          |
|------------------------------|-------------------|----------------|
| Micro-organism               | Bacillus subtilis | Saccharomyces cerevisiae |
| Growth temperature           | 30ºC              | 35ºC           |
| Colony-Forming Unit (CFU)    | 20X10⁷ Cells/ml   | 30X10⁷ Cells/ml |
| Colony Morphology : (i) Shape| Circular          | Circular       |
| (ii) Elevation               | Flat              | Convex         |
| (iii) Edge                   | Entire            | Entire         |
| (iv) Colour                  | Dull-White        | Pearly         |
| (v) Surface                  | Smooth            | Smooth         |
| (vi) Opacity                 | Opaque            | Shiny          |
| Cell Morphology              | Rod-Shaped        | Ovoid          |
| Gram reaction (+/-)          | +                 | +              |
| O₂ Use                       | Aerobe            | Aerobe         |
| Endospore (Y/N)              | Yes               | Yes            |
| Hydrolysis of Urea           | Yes               | Yes            |
3.2 Slump and Workability

The slump value of control, bacterial and yeast concrete was obtained as 65mm, 75mm and 80mm respectively. Therefore, the workability of bio-concrete specimens (bacterial & yeast) is better than control specimen. Yeast concrete has the highest workability. Figure 6 summarizes the result of slump test.

![Slump Value Graph](image)

**Figure 6.** Slump value describing the degree of workability by slump cone. Error bars show standard deviation (n=1).

![Water Absorption Graph](image)

**Figure 7.** Water absorption value after 24 hours of oven air-drying and immersion in water for next 24 hours. Error bars show standard deviation (n=1).

3.3 Water absorption capability and Bio-precipitation

The least amount of water was absorbed by yeast concrete (1.22%) after 24 hours of oven air-drying at 100°C. Figure 7 shows that Bacterial concrete absorbed 2.98% water and control specimen absorbed 4.61% water. The process of bio-precipitation was noticeable even during the curing process. The bio-precipitation of CaCO$_3$ in the micro-pores and internal fissures has led to decrease in water absorption content in bio-concrete specimens.

![Healing Graph](image)

**Figure 8.** Healing of Bacterial Concrete

![Healing Graph](image)

**Figure 9.** Healing of Yeast Concrete
Figure 8 and 9 represent the healing of concrete surface for several days. The bacterial concrete showed impressive healing at 28th day at the surface but healing in yeast concrete surface was not that much healed. On the contrary; it absorbed less water and bacterial concrete.

3.4 Compressive strengths and effect of the acid attack

The control concrete, bacterial concrete and yeast concrete took 24 hours, 27 hours and 36 hours respectively to set (at 15°C) completely. The compressive strength tests were taken at 7th, 28th and 43rd day. There was 20% and 8% increase in compressive strengths of bacterial & yeast concrete at 28th Day respectively as shown in figure 10. After acidic exposure of 15 days in HCl acid (maintained at pH=2.0), the bacterial and yeast concrete retained its 85 to 87% strength in comparison in control concrete which lost 43.03% of this compressive strength after immersion in acid as shown in figure 11.

![Figure 10. Compressive Strength of Concrete at 7th Day, 28th Day (before immersion in acid) & at 43rd Day (after immersion in acid). Error bars shows standard deviation (n=1).](image)

3.5 Weight Loss and change in appearance after acid attack

After concrete cubes were subjected to acidic exposure at pH=2.0, the loss in weight was observed in addition to the deterred surface. The normal concrete lost maximum weight and its surface was scarred such that the sand particles could be easily felt by placing the hand on it. Its edges were rough and with no intactness. But the bacterial and yeast concrete lost 1 to 2% of their weight as shown in figure 12. Their outer surfaces were less deterred in comparison to the control concrete. This can be easily seen in figure 13.

![Figure 11. Comparative compressive strength loss after acid attack. Error bars show standard deviation (n=1).](image)

![Figure 12. Weight lost after the acid attack. Error bars standard deviation (n=1).](image)
3.6 **Calcium carbonate generation**

The potable water used has water hardness of 152mg/l, lowest of all specimens. The control concrete contains calcium carbonate naturally however by adding ureolytic micro-organisms such as bacteria and yeast the bio-precipitation takes place and seals the micro-pores and fissure which are not sealed in conventional concrete specimens. The increased amount of hardness as calcium carbonate on the bio-concrete specimens is shown in data provided in figure 14.

![Control, Bacterial, Yeast Concrete Specimens](image)

**Figure 13.** Change in appearance after acidic exposure for 15 days in low pH medium (pH = 2.0).

![Calcium Carbonate Content](image)

**Figure 14.** Hardness as calcium carbonate accumulated from the surface of concrete specimens. Error bars show standard deviation (n=1).

4. **Conclusion**

The experimental study conducted shows that the Yeast, a common and locally available microorganism can also be effectively used for self-healing mechanism for Bio-concrete. It can be
used as an alternative for other micro-organisms like Bacillus subtilis. The conclusions drawn from are illustrated below:

- Bio-concrete (especially yeast infused concrete) is suitable for areas having high temperatures. At 15º C, the yeast concrete mix took approximately 36 hours to set completely. This slow setting time can be used for construction that requires longer period of execution.
- Bio-concrete can be used on the top-most or outer surface of any structure such as roof & exterior walls because of low water absorption rates. Self-healing mechanism will be activated each time a crack appears. The minimal amount water entry is enough for sporulation of microorganisms for calcium carbonate precipitation.
- Water absorption rate of bio-concrete is less than that of control concrete. The micro-pores and cracks subjected to any damp atmosphere will result in generation of calcium carbonate precipitate. It can be concluded that bio-concrete reduces chances of wall dampness.
- Due to the reason that the bioconcrete can certainly survive acidic exposure and tolerance up to pH=2.0, the structures made from bio-concrete will not deteriorate in acid rains.
- It can be used for sewer pipes and tanks because of good compressive strength which is capable of withstanding ground forces from above and soil pressure from beneath. Water absorption is less hence, the seepage of harmful water is minimized and reduces the chances of contamination with groundwater. The pH tolerance factor is advantageous in this case.
- Corrosion of steel reinforcements is heavily reduced as water absorption rate is very less. It should be used in constructions involving heavy reinforcements which are susceptible to corrosion. Calcium Carbonate filled in micro-pores and micro-cracks would not allow seepage or ingress of any foreign material, be it liquid or gas.
- Yeast infused concrete has scope in construction industry. Yeast (like Saccharomyces Cerevisiae) is locally available, less price, non-pathogenic; workability is good.

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References
[1]. Nomura Research 2015: India Cement
[2]. The UNFCCC COP21 2015 New Climate Regime to Take Place in Paris: Ministry of Environment: Paris, France
[3]. Rodriquez D 2001 Consolidation of decayed stone a delicate problem with few practical solutions. In: P.B. Lourenco, P.Roca (Eds), Historical constructions, Guimares
[4]. Soundharya S, Nirmalkumar K 2014 Study on the effect of calcite precipitating bacteria on self-healing mechanism of concrete Intern. J. Eng. Res. Mang. Tech. I 202
[5]. Jonkers H.M. 2011 Bacteria-based self-healing concrete Heron 56 1
[6]. Tiano P, Biagiotti L, Mastromei G 1999 Bacterial bio-mediated calcite precipitation for monumental stones conservation: Methods of evaluation Journal of Microbiological Methods 36 139
[7]. Stocks-Fischer S, Galinat J K, Bang S S 1999 Microbiological precipitation of CaCO3 Soil Biology and Biochemistry 31(11) 1563
[8]. Whiffin V.S. 2004 Microbial CaCO3 precipitation for the production of bio cement Ph.D dissertation Murdoch University Western Australia
[9]. Thawadi Al, S M 2008 High strength in-situ biocementation of soil by calcite precipitating locally isolated ureolytic bacteria Ph.D dissertation Murdoch University Western Australia
[10]. Mobley H L T, Island M D, Hausinger R P 1995 Molecular biology of microbial urases Microbiological Review 59 451
[11]. Hammes F et al 2003 Strain-specific ureolytic microbial calcium carbonate precipitation Applied and Environmental Microbiology 69(8) 4901
[12]. Bharathi N, Meyyappan R M 2015 Production of urease enzyme from ureolytic yeast cell International Journal of Engineering Research and General Science 3 Issue 2 Part 2 March-April

[13]. Bharathi N, Meyyappan R M 2014 Potentiality of Yeast Strain On Cement Concrete specimen International Journal of Science Engineering and Technology Research 3 Issue 12 December

[14]. Tietz S B 1968 Some Problems with Aerated Concrete 1st International Congress on Lightweight Concrete 2 Cement and Concrete Association London pp. 188-189.

[15]. https://www.researchitaly.it/en/news/enea-brewer-s-yeast-and-water-for-new-bio-concrete/

[16]. Mullick A 2007 Performance Of Concrete With Binary And Ternary Cement Blends Indian Concr. J. 81(1)15

[17]. Prasad J et al 2006 Factors Influencing the Sulfate Resistance of Cement Concrete And Mortar Asian J. Civil Eng. Housing 3(6) 259

[18]. Andalib R et al 2014 Durability Improvement Assessment In Different High Strength bacterial Structural Concrete Grades Against Different Types Of Acids Sadhana 39 1509

[19]. Turkel S et al 2007 Influence Of Various Acids On The Physico–Mechanical Properties Of Pozzolanic Cement Mortars Sadhana 32 683

[20]. IS 1489-1 1991 Specification for Portland pozzolana cement, Part 1: Flyash based

[21]. IS 383 1970 Specification for Coarse and Fine Aggregates from Natural Sources For Concrete

[22]. Lefevre M et al 2017 Safety assessment of Bacillus subtilis CU1 for use as a probiotic in humans Regulatory Toxicology and Pharmacology 83 54