



SATHYABAMA INSTITUTE OF SCIENCE & TECHNOLOGY
SCHOOL OF BIO & CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY



BIO-CONCRETE

TEAM MEMBERS

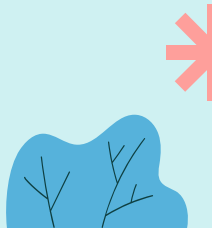
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PROBLEM STATEMENT

- To culture bacteria that can survive hostile condition of concrete
 - Creating a bio concrete that helps the construction industry to minimize cost of repairing and undertaking an eco friendly solution as well.
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ABSTRACT

- Concrete is the mostly used material for the construction having high compressive strength and low tensile strength. Cracking of concrete is a common phenomenon. Without immediate and proper treatment, cracks in concrete structures tend to expand further and eventually require costly repairs. Even though it is possible to reduce the extent of cracking by available modern technology, remediation of cracks in concrete has been the subject of research for many years.
 - The "Bacterial Concrete" is a concrete which can be made by embedding bacteria in the concrete that are able to constantly precipitate calcite. This phenomenon is called microbiologically induced calcite precipitation. It has been shown that under favourable conditions for instance *Bacillus Pasteruii*, a common soil bacterium, can continuously precipitate a new highly impermeable calcite layer over the surface of an already existing concrete layer. The favourable conditions do not directly exist in a concrete but have to be created.
 - Concrete structures usually show some self-healing capacity, i.e. the ability to heal or seal freshly formed micro-cracks. This property is mainly due to the presence of non-hydrated excess cement particles in the materials matrix, which undergo delayed or secondary hydration upon reaction with ingress water.
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- The mechanism of crack healing in bacterial concrete presumably occurs through metabolic conversion of calcium lactate to calcium carbonate what results in crack-sealing.
- A new type of self-healing concrete in which bacteria mediate the production of minerals which rapidly seal freshly formed cracks was developed, a process that concomitantly decreases concrete permeability, and thus better protects embedded steel reinforcement from corrosion.
- Initial results show that the addition of specific organic mineral precursor compounds plus spore-forming alkaliphilic bacteria as self- healing agents produce up to 100. I sized calcite particles which can potentially seal micro- to even larger- sized cracks.
- Further development of this bio-concrete with significantly increased self-healing capacities would represent a new type of durable and sustainable concrete with a wide range of potential applications.

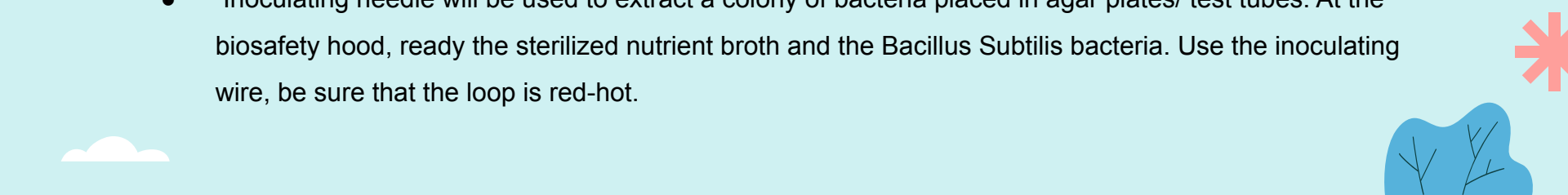




METHODOLOGY

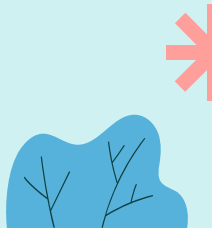


Culturing Bacillus subtilis:

- The bacteria are cultured in mass on the culture medium.
 - The culture is prepared using nutrient broth which consists of Urea Broth, Sodium Bicarbonate, Ammonium Chloride and Calcium Chloride Dehydrate.
 - The culture medium is sterilized in autoclave at 121°C at the pressure of 151 ps.
 - Dilute the 2.10 grams of nutrient broth, 1.50 grams of sodium bicarbonate, 7 grams of ammonium carbonate, 7 grams of urea broth and 5 grams of calcium chloride dehydrate in 1 litre of water. Stir well. Sterilize the apparatus needed for the inducing of Bacillus Subtilis bacteria to reduce contamination.
 - Sterilize the mixed nutrients on the autoclave machine at a pressure of 151 ps (pressurized steam) for 30 minutes.
 - Inoculating needle will be used to extract a colony of bacteria placed in agar plates/ test tubes. At the biosafety hood, ready the sterilized nutrient broth and the Bacillus Subtilis bacteria. Use the inoculating wire, be sure that the loop is red-hot.
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- Test tube openings are run through the heat to make sure that there are no other contaminants in the tube entrance.
 - Close the test tubes tightly with cotton to reduce contamination.
 - Put the test tubes and the Erlenmeyer flasks with Bacillus Subtilis bacteria at the incubator for 18 to 24 hours at room temperature for it to grow

Mixing of Conventional Concrete

- The researchers opted to use the concrete mixture 1:2 1/2:5:0.45.
 - Mix the cement, sand and coarse aggregate manually on a platform.
 - Add water and mix until the desired mixture was attained.
 - . Place it in the Mould, fill it in three layers.
 - Add the bacterial sample.
 - Compact each layer with 25 strokes using a tamping rod and smoothen it with a trowel.
 - Level the top surface and smoothen it with a trowel
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PROTOTYPE

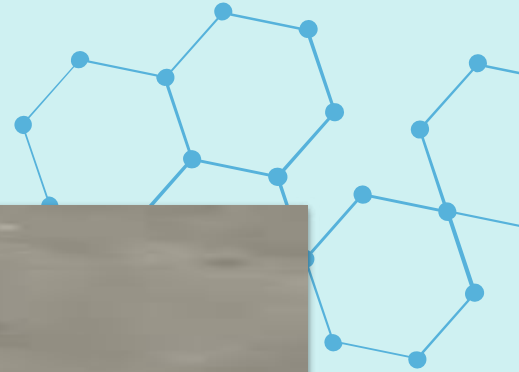
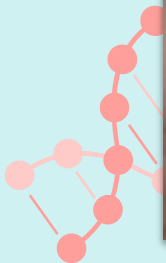
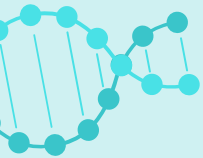




FINAL PRODUCT



VIDEO





THANK YOU!!

Nirmalya Das
39200028

BIO-CONCRETE

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Technology in Biotechnology

By

Amarjeet Yadav (39200005)

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Department of Biotechnology
School of Bio and Chemical Engineering

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

JEPPIAAR NAGAR, RAJIV GANDHISALAI, CHENNAI - 600 119

MAY 2022



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DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the Bonafide work of
AMRIT WILKHU (39230002), NIRMALYA DAS (39200028),
NIKHIL KUMAR SINGH (39200027) & AMARJEET YADAV
(39200005) who carried out the project entitled
"BIO-CONCRETE" under our supervision
from February to April ,2022.

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Dr. V RAMESH KUMAR

Submitted for Viva voice examination held on **29th April,2022**

Internal Examiner

External Examiner

DECLARATION

I, **AMRIT WILKHU (39230002)**, hereby declare that the project
report entitled "**BIO-CONCRETE**" done by me

under the guidance of

Dr. Valli Nachiyar (Internal) and Dr. Vanjinathan (External),

is submitted in partial fulfillment of the requirements for the award
of Bachelor of Technology degree

in Biotechnology Engineering.


SIGNATURE OF THE CANDIDATE:

DATE: 29th April, 2022

PLACE: Chennai

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to Board of Management of SATHYABAMA for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to Dr. V. RAMESH KUMAR, Head of the Department, Dept. of Biotechnology for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide Dr. Valli Nachiyar C, for her valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project.

I wish to express my thanks to all faculty members and technical staff members of the Department of Biotechnology who were helpful in many ways for the completion of the project.

ABSTRACT

Crack formation is very common phenomenon in concrete structure which allows the water and different type of chemical into the concrete through the cracks and decreases their durability, strength and which also affect the reinforcement when it comes in contact with water, CO₂ and other chemicals. For repairing the cracks developed in the concrete, it requires regular maintenance and special type of treatment which will be very expensive. So, to overcome from this problem autonomous self-healing mechanism is introduced in the concrete which helps to repair the cracks by producing calcium carbonate crystals which block the micro cracks and pores in the concrete. The selection of the bacteria was according to their survival in the alkaline environment such as *Bacillus subtilis*. For the growth, the bacteria were kept in a particular medium at a particular temperature for a particular period of time.

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CHAPTER 1

INTRODUCTION

- Concrete is the mostly used material for the construction having high compressive strength and low tensile strength. Cracking of concrete is a common phenomenon. Without immediate and proper treatment, cracks in concrete structures tend to expand further and eventually require costly repairs. Even though it is possible to reduce the extent of cracking by available modern technology, remediation of cracks in concrete has been the subject of research for many years.
- The "Bacterial Concrete" is a concrete which can be made by embedding bacteria in the concrete that are able to constantly precipitate calcite. This phenomenon is called microbiologically induced calcite precipitation. It has been shown that under favorable conditions for instance *Bacillus subtilis*, a common soil bacterium, can continuously precipitate a new highly impermeable calcite layer over the surface of an already existing concrete layer. The favorable conditions do not directly exist in a concrete but have to be created.
- Concrete structures usually show some self-healing capacity, i.e. the ability to heal or seal freshly formed micro-cracks. This property is mainly due to the presence of non-hydrated excess cement particles in the materials matrix, which undergo delayed or secondary hydration upon reaction with ingress water.
- The mechanism of crack healing in bacterial concrete presumably occurs through metabolic conversion of calcium lactate to calcium carbonate what results in crack-sealing.
- A new type of self-healing concrete in which bacteria mediate the production of minerals which rapidly seal freshly formed cracks was developed, a process that concomitantly decreases concrete permeability, and thus better protects embedded steel reinforcement from corrosion.

- Initial results show that the addition of specific organic mineral precursor compounds plus spore-forming alkaliphilic bacteria as self- healing agents produce up to 100. I sized calcite particles which can potentially seal micro- to even larger- sized cracks.
- Further development of this bio-concrete with significantly increased self-healing capacities would represent a new type of durable and sustainable concrete with a wide range of potential applications.
- The selection of the bacteria is depended on the survive capability of bacteria in the alkaline environment. Most of the microorganisms die in an environment with pH value of 10 or above
- Strains of the bacteria genus Bacillus will be found to succeed in high alkaline environment. The bacteria survive in the high alkaline environment that formed spores comparable to the plant seeds. The spores are of very thick wall and they activated when concrete start cracking and water transude into the structure. The pH of the highly alkaline concrete lowers to the values in the range 10 to 11.5 where the bacterial spores become activated. There many bacteria other than Bacillus which are survive in the alkaline environment shown in Table

Table 1.1- Bacteria other than Bacillus which can survive in alkaline environment

S.No.	Application	Types of Bacteria
1.	As a crack healer	B. pasteurii Deleya Halophila Halomonasrurihalina Myxococcus Xanthus B. megaterium
2.	For surface treatment	B. sphaericus
3.	B. spharicus	Bacillussubtilis B. sphaericus Thiobacillus

CHAPTER 2

LITERATURE SURVEY

1. **S. Reddy et al (2010)** carried out experimental investigation to obtain the performance of the concrete by *Bacillus subtilis*. M20 grade Concrete cubes with and without bacteria were tested. It was observed that compressive strength for cubes with addition of bacteria for a cell concentration of 105 cells/ml was increased up to 13.93% at 28 days, also there was an improvement in split tensile strength by 12.60% at 28 days for the cylinder. From durability studies percentage strength loss with 5% H₂SO₄ revealed that bacterial concrete was more durable. Percentage compressive strength loss in normal concrete after 105days was 14.66 as compared to bacterial concrete where percentage compressive strength loss was 8.64. From “Acid Durability Factor” calculation conventional concrete was 85.34% durable as compare to bacterial concrete which was 91.36% for 105 days studies. *Bacillus subtilis* was used to produce calcite as needed to repair crack and to survive in the concrete.

2. **Srinivasa Reddy et al. (2012)** they studied microbiologically induced calcite by *bacteria subtilis* which converted insoluble limestone which help to seal the crack. Addition of bacteria increase compressive strength of concrete by 23% at 28 days for ordinary, standard and high grades of concrete when compared to controlled concrete with addition of bacteria for a cell concentration of 105 cells/ml. Durability studies carried out in the investigation with 5% H₂SO₄ revealed that bacterial concrete is more durable than conventional concrete. Percentage compressive strength loss in normal concrete after 90days was 12.46, as compared to bacterial concrete where percentage compressive strength loss was 7.61 in immersion of H₂SO₄.

3. **M. V. Rao et al. (2013)** they studied Microbiological action done by *bacillus subtilis* to produce CaCO₃precipitates. Improvement of compressive strength for cement mortar at cell concentration of 105 cells/ml for all ages. The study showed that 25% increase in 28 days compressive strength of cement mortar was achieved. The study also showed that for M20 grade of bacterial concrete 13.92% increase in 28 days

compressive strength, and 12.60% improvement in 28 days split tensile test was achieved as compared to conventional concrete. The strength improvement is due to growth of filler material within the pores of the cement-sand matrix as shown by scanning electron microscopy (SEM). SEM also confirmed the role of microbiologically induced precipitation within the mortar matrix. More CaCO_3 precipitate higher is the healing effect

4. **Didier Snoeck et al. (2014)** they studied Microfibre reinforced concrete is durable and provides reliable tensile ductility and crack controlling capabilities to prevent cracking failures. Super Absorbant Polymer (SAP) particles promote the self-healing ability by renewed internal curing upon crack formation and this lead to regain of mechanical properties. Optimum dosage of SAP was 1 % of cement weight.

5. **Mignon et al. (2014)** carried out an investigation to discuss addition of higher amount of Super Absorbant Polymer (SAP) result in a superior self-sealing effect. The addition of SAP of 0.5% of cement weight to cement mortar leads to significantly stronger sealing effect of cracks over period of 28 days compared to reference mortar without SAP. Water permeability test shown high sealing effect in SAP mortar when compare to mortar without SAP. Stalactites have been observed on samples in depth characterization showed the product forming around was CaCO_3 .

CHAPTER 3

AIM AND SCOPE

1. To isolate culture *Bacillus subtilis*
2. To form bio-concrete using *Bacillus subtilis*

CHAPTER 4

MATERIALS AND EXPERIMENTAL METHODS

4.1 MATERIALS

- **Cement**

Pozzolan Portland cement of 53 grade is used in concrete. Cement used has been tested as per IS 4031-1988.

- **Crushed sand**

Crushed sand having specific gravity of 2.70 and confirming to IS-383 II is used.

- **Coarse aggregate**

The maximum size of coarse aggregate should be 20mm and minimum size should be 10 mm. It is angular in shape and rough surface texture is used.

- **Water**

Locally available portable water confirming to standard specified in IS 456-2000 is used

- Urea
- Bacillus subtilis
- Sodium bicarbonate
- Ammonium carbonate
- Calcium chloride dehydrate
- Nutrient broth
- Autoclave
- Test tubes
- Erlenmeyer flasks
- Inoculating wires
- Laminar air hood

4.2 EXPERIMENTAL METHODS:

4.2.1 Culturing *Bacillus subtilis*:

- The bacteria are cultured in mass on the culture medium.
- The culture is prepared using nutrient broth which consists of Urea Broth, Sodium Bicarbonate, Ammonium Chloride and Calcium Chloride Dehydrate.
- The culture medium is sterilized in autoclave at 121°C at the pressure of 151 ps.
- Dilute the 2.10 grams of nutrient broth, 1.50 grams of sodium bicarbonate, 7 grams of ammonium carbonate, 7 grams of urea broth and 5 grams of calcium chloride dehydrate in 1 litre of water. Stir well.
- Sterilize the apparatus needed for the inducing of *Bacillus Subtilis* bacteria to reduce contamination.
- Sterilize the mixed nutrients on the autoclave machine at a pressure of 151 ps (pressurized steam) for 30 minutes.
- Inoculating needle will be used to extract a colony of bacteria placed in agar plates/ test tubes. At the biosafety hood, ready the sterilized nutrient broth and the *Bacillus Subtilis* bacteria. Use the inoculating wire, be sure that the loop is red-hot.
- Test tube openings are run through the heat to make sure that there are no other contaminants in the tube entrance.
- Close the test tubes tightly with cotton to reduce contamination.
- Put the test tubes and the Erlenmeyer flasks with *Bacillus Subtilis* bacteria at the incubator for 18 to 24 hours at room temperature for it to grow

4.2.2 Mixing of Conventional Concrete

- The researchers opted to use the concrete mixture 1:2 1/2:5:0.45.
- Mix the cement, sand and coarse aggregate manually on a platform. 2.
- Add water and mix until the desired mixture was attained. 3
- . Place it in the Mould, fill it in three layers.
- Add the bacterial sample.
- Compact each layer with 25 strokes using a tamping rod and smoothen it with a trowel. 5.
- Level the top surface and smoothen it with a trowel.



Fig 4.1 Coarse aggregate



Fig 4.2 Mixture of sand, coarse aggregate, bacteria, cement and water



Fig 4.3

CHAPTER 5

RESULTS AND DISCUSSIONS



Fig 5.1 - Bioconcrete was demoulded

CHAPTER 6

SUMMARY AND CONCLUSION:

1. Bacillus subtilis was sub cultured
2. Bio concrete was de moulded and left for 28 days for bacteria to fully adjust and grow

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