

PRODUCTION OF BIOBRICKS USING MICROBES

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ABSTRACT

*Brick is an important construction material from ancient time. Since 7000 BC, Brick is considered as one of the oldest known building materials. The first brick was made in areas with warm climates, where the mud bricks were dried in the sun for hardening. The Egyptians were first to invent mortar using the material gypsum as a base. The Romans later developed the concept further, using a mixture of lime, water and sand which is the process still used today. The term **brick** refers to a unit composed of clay, but it is now used to denote any rectangular units laid in mortar. A brick can be composed of clay-bearing soil, sand, and lime, or **concrete** materials. The brick is manufactured with a raw material of clay through three sequential stages such as molding, drying and burning process. All over the world brick manufacture is nearly 1.3 trillion bricks each year out of which 10% of bricks is made through hand in coal-fired ovens. Coal-fired brick emits 1.4 pounds of carbon per brick, which pollutes the atmosphere severely all over the world. The large export countries like India and China face this problem of carbon emission. The manufacturing of conventional bricks requires a high temperature in burning process. The high temperature produced from the woods leads to deforestation and at the same time it releases carbon and pollutes the atmosphere severely. Thus the Manufacturing of conventional bricks leads to high amount of carbon emission and pollutes our environment severely. The alternative to this conventional brick would be a biobrick which does not require any heat and does not cause any pollution. Thus the present study is aimed at producing Biobrick using Microbes.*

KEYWORDS: *Brick, Moulding, Drying, Burning, Microbes and Biobrick.*

INTRODUCTION

Brick is a material which maintains durability of building structure and conservation of cultural heritage. Although hundreds and thousands of successful concrete and buildings are annually constructed worldwide, there are large number of concrete structures (including historical monuments), that deteriorates or become unsafe in loading. Hence in order to overcome the short comings of conventional sealing agent, materials with self healing capability can be used effectively. Use of urease producing microbes addresses these problems effectively as these continue to survive and grow within the concrete structure after the initial use. These unique properties make it particularly suitable for many applications in civil engineering (concrete structures, plasters, mortars, prefabricated elements, refractory elements, cement and stones) (**Karunakaran, 2014**).

Biobrick is a special type of brick and it is an innovative construction material for polluted countries. The manufacturing of conventional brick requires a high temperature, hence recent researches focus on developing a biobrick with a help of bacteria, which has characteristic of calcite precipitation. The present research helps the construction industry as well as public to increase the brick durability and reduce the carbon emission, which results pollution free environment. The brick manufactured by bacteria, reduces the carbon emission nearly 800 million tonnes per year. Biobrick has automatic ability to repair itself. Microorganism has the ability to precipitate the minerals, the bacteria continuously precipitates a calcite over a brick with a high impermeable layer, which increases the compressive strength and prevents the water into the brick, which increases automatic durability of the brick (**Ramakrishnan et.al., 2005**).

MICROBIOLOGICALLY INDUCED CALCIUM CARBONATE PRECIPITATION

This process uses the microbiologically induced calcium carbonate precipitation (MICP) to settle down the soil and creates more stability to the brick and increases the potential invention of the new material (**Chahal**

et.al., 2012). In this process, the microbiological calcium precipitate sticks as microbes in the sand joins together like glue which results the brick to turn to sand stone. Microbiologically induced calcium carbonate precipitation (MICP) is a bio-geochemical process that induces calcium carbonate precipitation within the soil matrix (Laxmana Reddy *et. al.*, 2015). Calcium carbonate can be precipitated in three polymeric forms, which in the order of their usual stabilities are calcite, aragonite and vaterite. The main groups of microorganisms that can induce the carbonate precipitation are photosynthetic microorganisms such as Cyanobacteria and Microalgae, Sulfate-reducing bacteria and some species of microorganisms involved in nitrogen cycle (Savitha Jose *et.al.*, 2018). Several mechanisms have been identified by which bacteria can induce the calcium carbonate precipitation, including urea hydrolysis, denitrification, sulphate production and iron reduction. Two different pathways, Autotrophic and Heterotrophic pathways, through which calcium carbonate is produced, have been identified. However, all pathways results in depletion of carbon dioxide and favors calcium carbonate precipitation. In heterotrophic pathway metabolic cycles involved are the nitrogen cycle and the sulphur cycle. Several applications of this process have been proposed, such as remediation of cracks and corrosion prevention in concrete (Suthar *et. al.*, 2016).

Microbiologically induced calcium carbonate precipitation has been tested over more than a decade as a technique to enhance concrete properties. Application of bacteria for surface consolidation has been shown to reduce water absorption and increased durability (Ravindranatha *et. al.*, 2014). Microbial self-healing of cracks in concrete shows promising results at the laboratory scale. Especially the use of self-protected mixed cultures opens perspectives for practical application. However, their self-healing efficiency needs to be further proven in larger concrete elements, and under non-ideal conditions. The use of denitrifying cultures for concurrent self-healing and production of corrosion inhibiting nitrites is a promising new strategy (Meera CM and Subha V. 2016).

MICP in civil engineering has been studied mainly for application in the fields of surface protection of natural stone, crack remediation in concrete and soil improvement. Also strength enhancement by mixing bacteria into concrete has been investigated (Mageswari *et al.*, 2017). The principle of bacteria-based self-healing concrete is that carbonate precipitating bacteria are added into concrete during the mixing process. When cracking occurs, the bacteria will be activated to precipitate CaCO_3 to in situ heal concrete cracks (Navneet Chahal *et. al.*, 2011). This 'self-healing' property results in a recovery of water-tightness, and hence limits the penetration of corrosive substances into concrete structures and improves concrete durability. Thus Self-Healing concrete is a new era of construction industry and it makes concrete structure crack free and our Eco-friendly bricks have this property of self-healing (Kumarappan *et. al.*, 2018).

MATERIALS AND METHODOLOGY

COLLECTION OF SEWAGE SAMPLE

The sewage samples were collected from Retteri, Chennai and was used for the isolation of microorganisms. The microbes present in sewage were innumerable but specific microbes were isolated for the present study.

CHARACTERIZATION OF SEWAGE SAMPLE

The sewage samples were characterized for parameters such as Determination of Total Dissolved Solids in Water, Dissolved Oxygen Concentration, Biological Oxygen Demand and Chemical Oxygen Demand in water. The value of TDS, BOD and COD were calculated using standard protocols. Following this, the Microbes were isolated and were morphologically identified Gram staining technique. Biochemical Tests such as Indole Test, Methyl Red Test, Voges – Proskauer Test, Citrate Utilization Test, Triple Sugar Iron Test, Nitrate Reduction Test, Catalase Test, Oxidase Test, Lactose Utilization Test, Gelatin Hydrolysis Test and Urease Test were conducted following standard protocols and the results were tabulated.

UREA- TOLERANCE LEVELS OF BACTERIAL STRAINS

The best urea hydrolyser inoculums (3%) was added to 1,1.5,2,2.5,3,3.5, and 4 M urea concentration and incubated at 37 ° C .The visual turbidity was determined by cell cultures density at 600nm using UV spectrophotometer.

TEST FOR CALCIUM CARBONATE PRECIPITATION FROM BACTERIA

To 50 ml of Minimal media, 2 grams of urea, 2grams of calcium chloride and 1 ml of culture was added. It was incubated for 3 days and the obtained biomass was filtered, dried and weighed. The biomass was considered as a calcium carbonate precipitation. The tubes which were kept in shaking produced higher amount of Calcium carbonate precipitation than the tubes kept without shaking.

STANDARDIZATION OF CALCIUM CARBONATE PRECIPITATION

To a minimal media, Different concentrations of urea and calcium chloride along with a same concentration of culture was added and incubated 48 hours. The highest concentration of calcium carbonate produced was determined and standardized.

CONFIRMATION OF CALCIUM CARBONATE BY CONDUCTING A CHEMICAL TEST

The presence of Calcium Carbonate was confirmed by performing the Calcium Carbonate Test, Ammonia Test, Calcium Test, Dry Flame Test, Carbonate Test, Solubility Test and Dilute Hydrochloric Acid Test.

PRODUCTION OF BIO-BRICKS

Bricks were made in smaller size by using clay sand, biochemical solution (urea and calcium chloride) and microbes. The small size bricks were made by using Ice tray as a mould. The bricks were made by using different microorganisms with different concentration of urea and calcium chloride for the better observation of calcium carbonate deposits over the brick.

OBSERVATION OF CALCIUM CARBONATE DEPOSITS OVER THE BRICKS

The bricks were made by using different bacteria and the highest urease utilizing organism was determined. The calcium carbonate deposits were observed in white colour over the bricks once it dried completely.

PREPARATION OF WOODEN MOULD

The method starts with mold preparation. Mould is prepared by a wooden frame. The inner dimension of the wooden frame is like conventional brick dimension 19 x 19 x 9 cm.

METHODS OF PRODUCING BRICK

Soil is mixed with water and filled in the mold by three-layers. Each layer is compacted well to attain a maximum density and it reduces pores in the brick which results in increased durability and compressive strength of brick without heating process. The solution contains urea, calcium chloride and micro-organism which is mixed and poured into the wooden mold. After few days bacteria consume urea as food and precipitate calcite between the soil grains. In this process chain of Biochemical reactions takes place to harden the brick. The manufactured brick was kept under room temperature for 20 days to attain full growth. After which its strength and durability increased because of the bacterial growth. When the water seeped through the cracks of the brick and reaches the bacteria, again the biochemical reaction is stimulated and calcite precipitate is formed along the brick's crack. The cement was applied all over the bricks and was kept for curing in water for 48 hours. The curing process helped the brick to become more stronger. Property Analysis was confirmed by performing the Hardness Test, Water Absorption Test, Self – Healing Property Test and Tests for Microbial Resistance. The Bricks were further characterized using Scanning Electron Microscope and X – Ray Diffraction Techniques. Biocement was produced from Microbes using Sea water and Water Hardness was tested using EDTA Titration method.

CONSTRUCTION OF WELL USING BIOBRICKS

Finally the well was constructed using three layers of biobrick with cement. It was dried in air and cured in water for increasing its durability and strength.

RESULTS AND DISCUSSION

CHARACTERIZATION OF SEWAGE SAMPLE

The sewage sample was characterized for Physico-chemical parameters such as determination of Total Dissolved Solids in Water, Dissolved Oxygen Concentration, Biological Oxygen Demand and Chemical Oxygen Demand. The values are tabulated in **Table 1**.

PARAMETERS	SEWAGE SAMPLE
Total dissolved solids	0.09 grams
Dissolved oxygen	960 mg/l
Biological oxygen demand	2mg/l
Chemical oxygen demand	5.4mg/l
Ph	6.2

Table 1 : Physico - chemical Parameters of Sewage Sample

ISOLATION AND IDENTIFICATION OF MICROORGANISMS FROM SEWAGE WATER AND FROM AIR

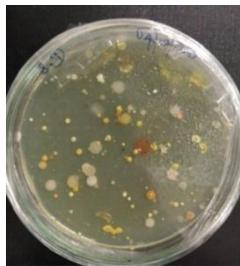


Figure 1



Figure 2

Figure 1: Isolated colonies from air

Figure 2 : Isolated colonies from Sewage Water

IDENTIFICATION OF MICROORGANISMS

The isolated colonies were cultured on Nutrient agar medium, Mannitol salt agar medium, MacConkey agar medium and Christensen's agar medium. The colonies were selected and studied for colony morphology and staining procedures were carried out. The predominantly found bacteria were tabulated (Table 2 & Figures 3 - 7).

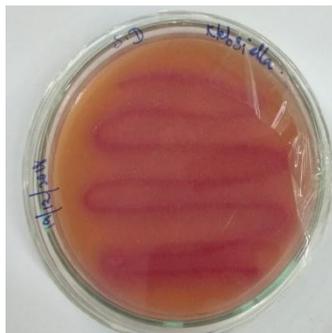


Figure 3



Figure 4



Figure 5



Figure 6

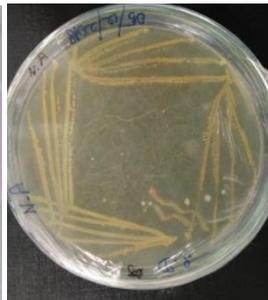


Figure 7

Figure 3 : Isolated colony cultured on Mannitol salt agar medium

Figure 4 : Isolated colony cultured on MacConkey agar medium

Figure 5 : Isolated colony cultured on Nutrient Agar medium

Figure 6 : Isolated colony cultured on Christensen’s agar medium

Figure 7 : Isolated colony cultured on Nutrient agar medium

ORGANISM	COLONY MORPHOLOGY
<i>Proteus</i> spp	Rod shaped,Thin,blue gray,spreading growth
<i>Staphylococcus</i> spp	Cocci,thin,even growth,Acid,rapid reduction
<i>Klebsiella pneumoniae</i>	Rod shaped,slimy,white,translucent,raised growth
<i>Bacillus</i> spp	Rod shaped,large,spreading,irregularly shaped
<i>Serratia</i> spp	Rod shaped,red pigmented,elevated,regular,round colonies

Table 2 : Colony Morphology

MORPHOLOGICAL IDENTIFICATION BY GRAM STAINING TECHNIQUE

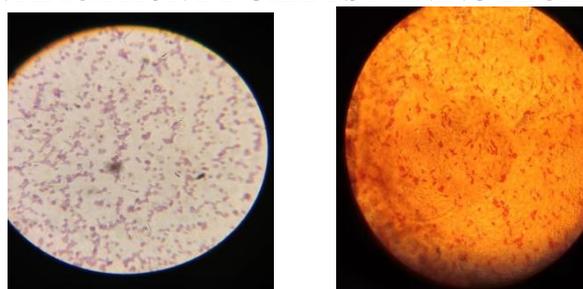


Figure 8 : Gram positive cocci Figure 9 : Gram negative rods

BIOCHEMICAL TEST

The characterization of microorganisms was carried out by various Biochemical tests and the results are tabulated in Table 3.

Organism	Catalase	oxidase	MR	VP	Citrate utilization	Triple sugar iron	Lactose utilization	Nitrate reduction	Gelatin	Indole	Urease test
<i>Bacillus</i> spp	+	+	+	+	+	+	+	+	+	+	+
<i>Serratia</i> spp	+	-	-	+	+	+	-	+	+	-	+
<i>Staphylococcus</i> spp	+	-	+	+	+	+	+	+	+	-	+
<i>Klebsiella</i> spp	+	-	-	+	+	+	+	+	+	-	+

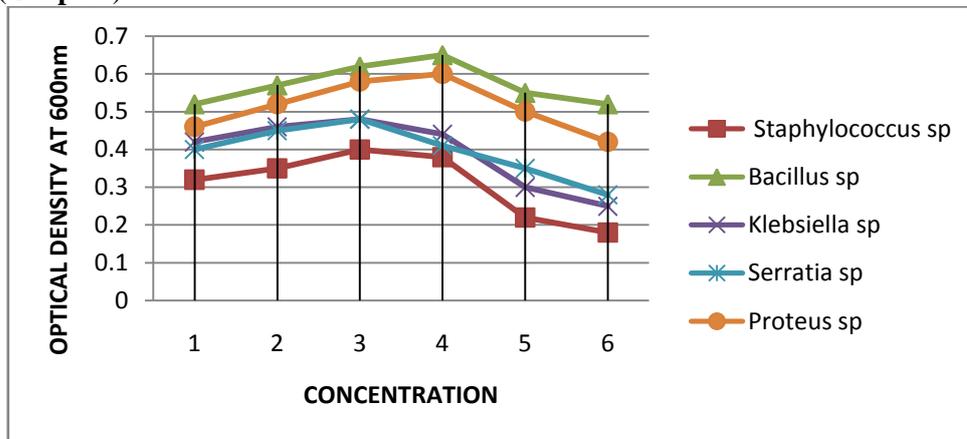
<i>Proteus spp</i>	+	-	+	-	+	+	-	+	+	-	+

Table 3 : Biochemical tests

Where, + indicates Positive result
 - indicates Negative result

UREA-TOLERANCE LEVEL OF BACTERIAL STRAINS

The five bacterial strains (*Proteus spp*, *Staphylococcus spp*, *Klebsiella spp*, *Bacillus spp* and *Serratia spp*) were checked for the best Urea tolerance capability by inoculating the medium containing urea at different concentrations. The optical density was determined at 600nm for Cell Culture Density and the Graph was plotted (Graph 1).



Graph 1 : Urea-Tolerance Graph

TEST FOR CALCIUM CARBONATE PRECIPITATION BY BACTERIA

Calcium carbonate precipitation with shaking was observed to be 1 gram and calcium carbonate precipitation without shaking was observed to be 0.5 gram (Figures 10 A – D).



Figure 10A

Figure 10B

Figure 10C

Figure 10D

Fig 10A : Calcium Carbonate

Fig 10B : The Filtrate Precipitation

Fig 10C : Filtrate kept for Drying

Fig 10D : Dried Biomass

STANDARDIZATION OF CALCIUM CARBONATE PRECIPITATION

To minimal media, different concentrations of Urea and Calcium Chloride along with the same concentration of culture was added and incubated for 48 hours. The highest concentration of Calcium Carbonate produced was determined and standardized. This standardization method was done by using *Proteus spp*, *Staphylococcus spp*, *Klebsiella spp*, *Bacillus spp* and *Serratia spp* (Table 5).

UREA CONCENTRATION	CALCIUM CHLORIDE CONCENTRATION	OBSERVATION
0.8 gram of urea	0.8 gram of calcium chloride	0.4 gram of CaCO ₃
0.8gram of urea	0.4 gram of calcium chloride	0.2 gram of CaCO ₃
0.8 gram of urea	0.6 gram of calcium chloride	0.3 gram of CaCO ₃
0.8 gram of urea	1 gram of calcium chloride	0.3 gram of CaCO ₃
0.8 gram of urea	1.2 gram of calcium chloride	0.4 gram of CaCO ₃
0.4 gram of urea	0.4 gram of calcium chloride	0.2 gram of CaCO ₃
0.6 gram of urea	0.6 gram of calcium chloride	0.3 gram of CaCO ₃
1 gram of urea	1 gram of calcium chloride	0.6 gram of CaCO ₃
1.2 gram of urea	1.2 gram of calcium chloride	0.7 gram of CaCO ₃
0.4 gram of urea	0.8 gram of calcium chloride	0.3 gram of CaCO ₃
0.6 gram of urea	0.8 gram of calcium chloride	0.4 gram of CaCO ₃
1 gram of urea	0.8 gram of calcium chloride	0.5 gram of CaCO ₃
0.8 gram of urea	1.2 gram of calcium chloride	0.6 gram of CaCO ₃

Table 4 : Standardization of Calcium Carbonate Precipitation

CONFIRMATION OF CALCIUM CARBONATE BY CHEMICAL TESTS

CALCIUM CARBONATE TEST

The presence of calcium carbonate was confirmed by Turbidity test. With an aged precipitant, turbidity occurred owing to the formation of Calcium carbonate (**Figure 11**).



Figure 11 : Turbidity shows the presence of Calcium Carbonate

CALCIUM TEST

The presence of calcium was identified by Flame test. Volatile calcium compound imparts a yellowish-red colour to the Bunsen flame (**Figures 12A and 12B**).



Figure 12A & B : Flame Test

CARBONATE TEST

The presence of carbonate was confirmed by conducting a solubility test which revealed the presence of effervescence. All normal carbonates, with the exception of those of the alkali metals and of ammonium were insoluble in water. Decomposition with effervescence, due to the evolution of carbon dioxide, the gas was identified by its property of rendering lime water (Calcium hydroxide) (Figures 13 and 14).



Figure 13 : Solubility test Figure 14 : Effervescence

PRODUCTION OF BIOBRICKS IN SMALL SIZE

Bricks were made in smaller size by keeping in ice tray as mould. The clay soil was mixed with water and was filled in the mould. The solution containing urea, calcium chloride and micro-organism were poured into the mould. The formation of bricks happened within 7 days (Figures 15 & 16).



Figure 15



Figure 16

Figure 15 : Mixture of Clay with Sand, *Bacillus spp* culture and Biochemical solution (Urea and Calcium Chloride)

Figure 16 : Brick kept for Drying

BIOBRICKS MADE BY USING DIFFERENT BACTERIA

The bricks were made by using *Proteus spp*, *Staphylococcus spp*, *Klebsiella spp*, *Bacillus spp* and *Serratia spp*. The calcium carbonate precipitation over the bricks were observed well in *Proteus spp* and *Bacillus spp*. (Figure 17).



Figure 17 : Calcium Carbonate precipitation over the bricks

PRODUCTION OF BIOBRICKS IN REGULAR SIZE

This method starts with mould preparation. Mould was prepared by using a wooden frame. The inner dimension of the wooden frame is similar to the Conventional brick dimension 19 x 19 x 9 cm (**Figures 18 & 19**).



Figure 18 : Wooden Mould



Figure 19 : Biobrick

CURING THE BRICKS

The cement was applied over the smaller brick and water was sprinkled over the brick frequently for 2 - 3 days and was kept for curing. This curing process made the bricks to be more stronger and without any deformation (**Figure 20**).



Figure 20 : Curing of bricks

ANALYSIS OF BRICK

HARDNESS TEST

Bio bricks were tested for its hardness with the help of fingernail. The bricks did not show impression on the surface of the brick, which implies that the bricks were harder in nature.

WATER ABSORPTION TEST

The rate of water absorption is the important parameter of the brick because it affects mortar and grout bonding during the wall construction. The cured brick was immersed in water for 48 hours and was noted down for its water absorption rate by calculating the difference between its dry weight and wet weight (**Figure 21**).



Figures 21 : Water Absorption Test

The present study reveals the fact that the biobrick does not absorb water more than 20 percentage of its own weight. The present study shows the water absorption rate as 8% and that the water absorption rate is low.

DRY WEIGHT = 49.533 grams

WET WEIGHT = 53.432 grams

PERCENTAGE = 8%

STRENGTH

The strength of the bricks was observed by trying to break the bricks by hands and its physical strength was determined by throwing the bricks from the top to the floor. The bricks did not brake when it was thrown and thus the brick seems to be stronger.

SELF-HEALING PROPERTY

The produced brick was analysed for its self-healing property by the observation of cracks (Figure 22).



Figure 22 : Self - Healing Property of the Biobrick

MICROBIAL RESISTANCE

The cured brick was tested for other microbial attack through the observation of the brick. The brick did not show any microbial attack such as fungi and it remains same without changing its colour and position.

CHARACTERIZATION OF BIOBRICK

SCANNING ELECTRON MICROSCOPY

The present study deals with the product produced by *Bacillus spp* and was confirmed through SEM analysis in which calcite was present in the form of calcium carbonate. The SEM images were in the range of 0- 50µm, 0-20µm and 0- 5µm. The calcite was observed clearly in the form of calcium carbonate (Figures 23 - 25).

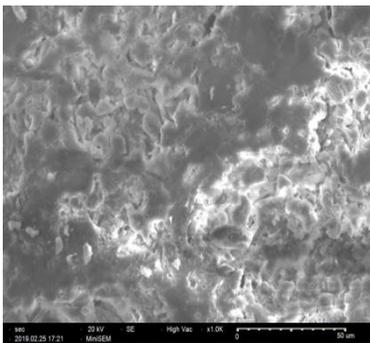


Figure 23 : SEM IMAGE 1
(Range 0 - 50µm)

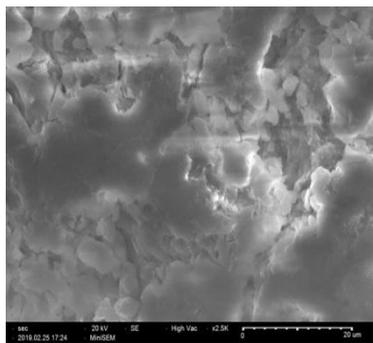


Figure 24 : SEM IMAGE 2
(Range 0 - 20µm)

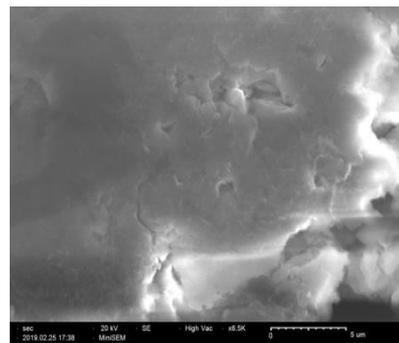
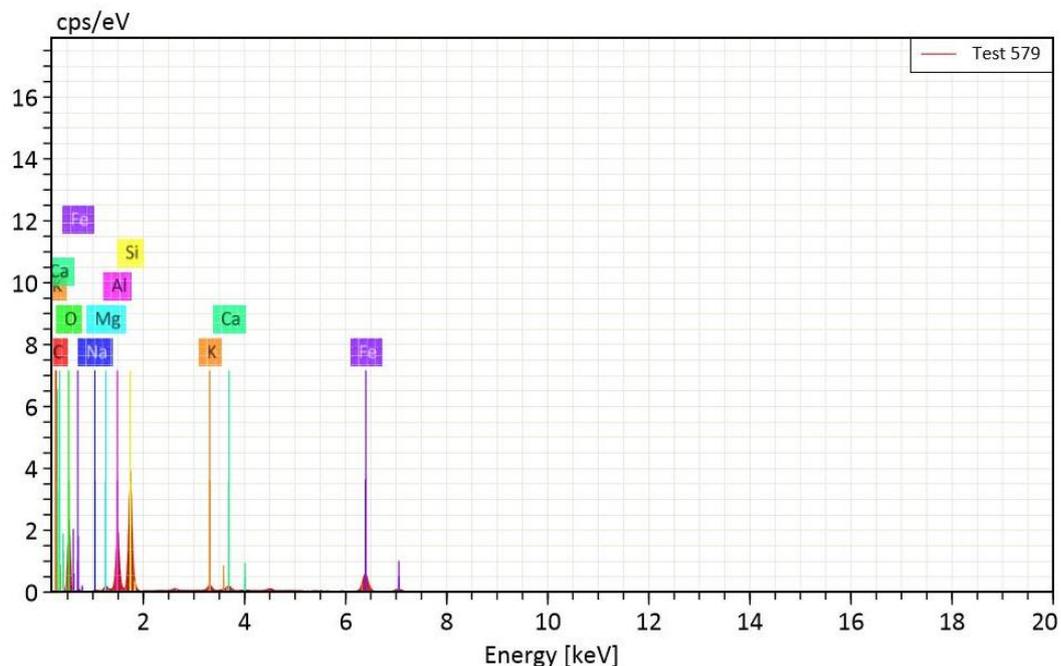


Figure 25 : SEM IMAGE 3
(Range 0 - 5µm)

SEM WITH EDX

The composition of the giving biobrick sample was analysed and studied using EDX (Figure 26).



Test 579

Element	At. No.	Netto	Mass [%]	Mass Norm. [%]	Atom [%]	abs. error [%] (1 sigma)	rel. error [%] (1 sigma)
Carbon	6	1966	11.97	11.15	17.11	2.30	19.23
Oxygen	8	27657	57.03	53.13	61.18	7.10	12.45
Sodium	11	663	0.68	0.64	0.51	0.08	12.46
Magnesium	12	2522	1.38	1.29	0.98	0.11	8.09
Aluminium	13	31510	11.14	10.38	7.09	0.56	5.07
Silicon	14	63830	17.27	16.09	10.55	0.77	4.44
Potassium	19	3118	0.68	0.63	0.30	0.05	7.40
Calcium	20	2803	0.67	0.62	0.29	0.05	7.41
Iron	26	15763	6.52	6.08	2.01	0.21	3.18
Sum			107.33	100.00	100.00		

Figure 26 : SEM WITH EDX IMAGE

X RAY DIFFRACTION TECHNIQUE

The carbonate deposits as calcite crystals was observed by XRD (Figures 27 & 28). The present study shows the peak in the concentration between 60-80 and it was compared with a standard graph of Navneet chahal *et.al.*, (2011).

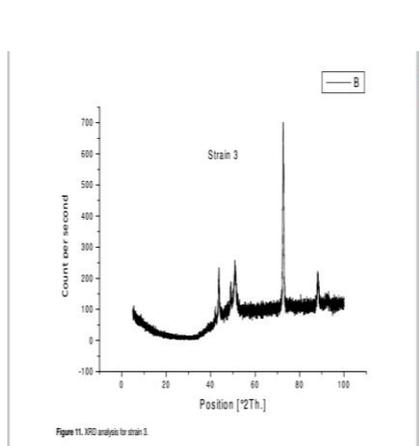


Figure 27: Standard XRD image

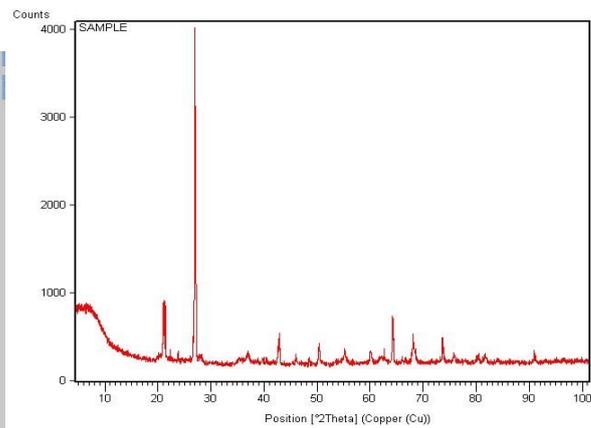


Figure 28: XRD Result of Biobrick

PRODUCTION OF BIOCEMENT FROM MICROBES USING SEA WATER

DETERMINATION OF WATER HARDNESS BY EDTA TITRATION

The EDTA draws the calcium and magnesium ions into a complex which is present in the water sample, so neither ions are free in the solution.

Hardness In Sea Water and In Soft Water

The change of colour from wine red to steel blue by reducing EDTA was observed.

Total hardness of sea water = 2144mg/l

Total hardness of soft water=80.593mg/l

The sea water has more calcium, magnesium and mineral ions when compared to the soft water and so it was confirmed that the hardness of sea water is greater than the soft water. This Confirms that the calcium deposits in the seawater is more than the soft water (**Figure 29**).



Figure 29 : Determination of Hardness of sea water and soft water by EDTA Titration

PRODUCTION OF BIOCEMENT

The Calcium carbonate precipitation in sea water was more than the soft water, it has been proved that the hardness of sea water is greater than the soft water and *Bacillus spp* organism precipitated Calcium carbonate at the higher amount. 250 ml of sea water produced 2 grams of Biomass. Biocement was produced at the incubation of one week (**Figure 30**).



Figure 30 : Production of Biocement

CONSTRUCTION OF SMALL WELL

A small well was constructed using Biobricks and cement. The well was cured in water for 48 hours (**Figure 31**).



Fig 31 : Small well constructed using Cement and Biobricks

CONCLUSION

The goal of the present study was to produce biobrick in an eco-friendly way. The process involves the production of Biobricks using microbes. Since bacteria has multifaceted applications, it is also used in civil construction and in the process of brick manufacturing. The urease utilizing organisms (*Proteus spp*, *Staphylococcus spp*, *Klebsiella spp*, *Bacillus spp* and *Serratia spp*) were isolated from sewage sample and from air. The isolated bacteria were morphologically identified and confirmed by various Biochemical tests. These bacteria are non-pathogenic bacteria which produce calcium carbonate with the help of urea and calcium chloride as a substrate. The amount of calcium carbonate precipitation was higher in *Proteus spp* and *Bacillus spp*, when compared to *Staphylococcus spp*, *Klebsiella spp* and *Serratia spp*. The urea tolerance level was said to be higher in *Proteus spp* and *Bacillus spp* and had a highest urea tolerance level upto a concentration of **4 gram/ml**. Further study has been conducted using microbes. The calcium carbonate was confirmed by Chemical Tests such as Solubility Test, Turbidity Test, Flame Test and by Effervescence. Brick has been produced using Clay Sand, Microbes and Biochemical solution (urea and calcium chloride) in smaller and regular size. The property of biobricks such as Water Absorption, Physical Strength, Hardness and Self-Healing property were analysed. The product was characterized and its composition was studied using Scanning Electron Microscopy with EDX and X-Ray Diffraction Technique. The calcium carbonate was observed in SEM and calcite crystals using XRD. The other application was the production of Biocement from microbes using Seawater and construction of a Small Well using Biobrick and Cement.

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