MICROBIAL GEOTECHNOLOGY: EVALUATION OF STRENGTH AND STRUCTURAL PROPERTIES OF MICROBIAL STABILIZED MUD BLOCK (MSMB)

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ABSTRACT - Microbial Geo-technology is a new branch of geotechnical engineering that pact with the applications of microbiological methods to geological materials used in engineering. In this research, microbes were isolated and analyzed for various pozzolanic reactions as well as microbial fibrous reinforcement properties. The precipitation of CaCO3 in their micro environment by conversion of urea into ammonium and carbonate were confirmed further. The bacterial spore which is immobilized in soil matrix becomes metabolically active when revived by water entering freshly into the soil. The mycelial network of isolated fungi will provide fibrous reinforcement to the mud block. The micro-organism is identified as Sporosarcina pasturii with 98% query sequence similarity. After the detailed review done by NCBI members allotted unique Accession number KP938 774 for S. pasturii. Hence now onwards this bacterium known as S.pasturii-GRI-Auro. The objective of the present investigation is to study the potential influence bacteria Sporosarcina pasteurii GRI-Auro, and fungus Aspergillus sp. on the strength, durability and mechanical characteristics of compressed earth block. The studies are carried out in red soil. Different concentrations of microbial consortium (105,107,109 cells/ml) were introduced in mud block. Microbial Stabilized Mud Block (MSMB) were prepared by using Tara Balram soil press and cured by spraying an optimized nutrient media for 28 days. Microbial Calcite Precipitation (MCP) was identified and quantified by X-ray diffraction (XRD), Fourier Transform Infrared (FT-IR) analysis and visualized by Scanning Electron Microscope (SEM). Results showed that the rate of water absorption was significantly reduced and compressive strength was enhanced in microbial treated bricks. The results suggested that the microbial activities can significantly improve mechanical properties of microbial stabilized mud block. Hence microbial isolates can be used as additive to stabilize soil.

Key words: Microbial Stabilized Mud Block (MSMB), Microbial Calcite Precipitation (MCP), X-ray diffraction (XRD), Sporosarcina pasteurii, soil stabilization.

INTRODUCTION

The natural properties of soil can be altered through the process of soil stabilization. The idea of stabilizing soil with micro-organisms is taken from termite mounds. Termites construct mounds from the mineral matrix with feces and saliva depending on termite species (Grass'e, 1984). These mounds increase the microbial density as a result of high organic matter. Diverse micro-organisms inhabit the intestinal tract of all termites (Brune, 1998; Brawmen *et al*, 2001). A group of micro- organisms capable of mineral precipitation inhabit termite mounds (Lee and wood, 1971).The idea of isolating the bacteria from termite mounds was that the termites are the effective agents in consolidating soil and making a hard cemented soil similar to bricks. Soil stabilization is the process of modifying the soil properties in relation to its strength, texture, voids and water resisting properties compatible with a particular application.

Application of microbiological methods to improve mechanical properties of soil is known as Microbial geo- technology (Ivanov and chu, 2008). Microbial soil stabilization is one of the alternatives for eco-friendly management of low cost building. Microbial induced calcium carbonate precipitation resulting from metabolic activities of some specific micro-organism in soil (Stockfisher et al, 1999; Achal et al., 2009). This research is looking for the precipitation of calcium carbonate

crystals is by the heterogeneous or exogenous nucleation on the bacterial cell wall which is help to soil stabilization.

EXPERIMENTAL STUDIES

Methodology of this research consists of two phases - Microbial phase of the study and engineering phase of the study. The microbial phase starts with collection of termites which are recognized as the ecosystem engineers in tropical soils. In the study an attempt has been made to collect termite and termitorium soil from selected pockets of sirumalai hills, Dindigul district, Tamil Nadu. Sirumalai is a part of Western Ghats situated between 10^0.07'-10^0.19' north latitude and 77^0.55'- 78^0.12' east longitude.

Isolation of micro-organisms

Termites were collected from termite mounds. The head and gut regions were dissected and separately homogenized by using mortar and pestle. Collected samples including termite gut and mouth parts and termite soil were serially diluted from 10^{-2}- 10^{-7} and subsequently placed over the solidified Nutrient Agar medium and Rose Bengal Agar.

Identification- The micro-organism used in the study was identified as *Sporosarcina pasturii* on MALDI-TOF Mass spectrometry and 16 S rRNA sequence analysis. Bacterial analysis by Matrix Assisted Laser Desorption/ Ionization Time Of Flight were recorded and interpreted with automated Jackknife test and the K- nearest neighbor method. Different similarity measures were evaluated to perform numerical analysis on the Maldi 2 spectra and the final character data set. It showed 99% similarity with *Bacillus cereus*.

On 16SrRNA typing the isolated pure colony of bacterial strain was transferred to nutrient slant and these slants were further sent to sequence of DNA. The DNA were extracted by using sigma Aldrich DNA purification kit 16SrRNA gene amplification was carried out by YAAZH Zenomics private Ltd. Chennai. Later the amplified DNA was sequenced by Sanger's Dideoxy method. The sequence were providing 1484 base pair product, further the sequence were processed for trimming both 5' and 3' ends. The software Applied Bio system sequence scanner VI.O and Mega 5.1 were used to processed sequence data.

Bio informatics Tools: Blastin- The national center for bio informatics USA (NCBI) Online tool Blastin were used to compare DNA sequence with archived NCBI nucleotide database. The Blast of sequence with database showed 98% query similarity with *Sporosarcina pasturii*.

Morphological Characteristics

Gram staining was done with isolates to determine morphological features like form, size and gram reaction. The colony characteristics were also studied by spotting the isolates on nutrient agar plates. The structures of fungal strains were identified as *Aspergillus* sp. through the Lactophenol cotton blue.

Cheap media optimization

A natural medium comprised by jaggery (10g), cane molasses (20g), green gram (1.5g), soya been (1.5g) were prepared. After sterilization inoculate the microbial consortium in the medium.

Table: 1 Geotechnical properties of soil used

Properties	Red soil
Specific gravity	2.58
Grain size distribution	
Coarse sand (%)	10.5
Medium sand (%)	26
Fine sand (%)	57.3
Atterberg's limits	
Liquid limit (%)	25.45
Plastic limit (%)	16.67
Plasticity index (%)	8.78
Consistency index	1.0
Shrinkage limit (%)	16.27
Compaction characteristics	
Maximum dry density (g/cm^3)	2.01
Optimum moisture content (%)	12

Making of mud blocks- The stabilized mud block is made with TARA Balram soil press. The blocks are made with microbial consortium as the stabilizer with different proportions. The size of the block is 230x110x75 mm. The total quantity of mix for a two block is 8 kg.

Field test- The block is tested for penetration in which a penetrometer is used on the top surface of blocks and it should not be penetrated more than 9 mm.

Curing of mud blocks- The blocks are covered with tarpaulin or polythene sheets. The blocks are cured by spraying nutrient media on bricks and water over the polythene sheets the blocks made with microbial consortium as a stabilizer are tested for 7^{th}, 14^{th} and 21^{st} day of curing.

RESULTS AND DISCUSSION

. They are cream colored, moist, spread and gummy colonies on nutrient agar. Optimum pH is 7.5, temperature is 30^0C-40^0C and agitation is 100 rpm. This bacterium has the ability to produce calcium carbonate.

16s rRNA sequence was submitted to NCBI for getting Gene Bank Accession number by using BankIt tool. After the detailed review done by NCBI members, allotted unique accession number KP938774 for *Sporosarcina pasteurii* GRI-AURO. Evolutionary relationships of taxa of *Sporosarcina pasteurii* associated with the other members of the genus *Bacillus.* Distance matrix was calculated on the basis of Jakes Cantor Algorithm and topology was inferred using the neighbour- Joining (nj) based on the Bootstrap analysis of 1000trees.

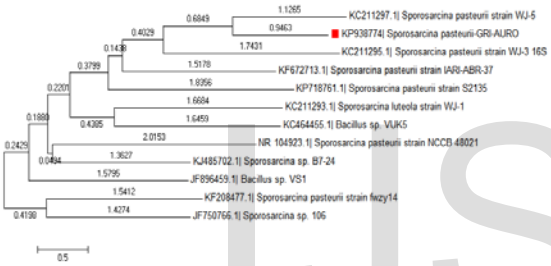

Fig. 1. Phylogenetic Analysis of Submitted strain *S. pasteurii* GRI-Auro (KP938774)

Table: 2 Representative 16S rRNA cloned fragment and their phylogenetic affiliation.

Representative Clone Sequenced (GenBank acc.no)	Bestmatch with database (the closest sequences)	Similarity (%)	Microbial group affiliation
KP938774	*Sporosarcina pasteurii* (KC211297.1)	99%	*Sporosarcina pasteurii*

Phylogenetic Analysis of Submitted strain *S. pasteurii* GRI-Auro (KP938774)

Sporosarcina pasturii is a gram positive, aerobic bacterium

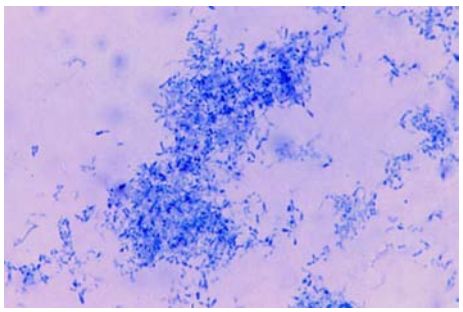

Fig. 2. Micrograph of *Sporosarcinapasteurii-GRI-Auro*

Fig. 3. Micrograph of isolated *Aspergillus sp.*

Fig. 4. 72 hour batch culture of *Aspergillus sp.* (TMF-1) with optimized medium

72 hour incubation of fungal culture *Aspergillus sp* showed cotton ball like mycelia chitin network may act as a potent natural reinforcement to the mud block.

Compressive strength test- The compressive strength of mud block was obtained by testing them in compression testing machine after 7, 14 and 28 days of curing. The compressive strength of microbially treated mud block at 10^9 cells/ ml concentration was found out to be 4.97 MPa while that of control bricks was 3.12MPa which showed 35% improvement. The improvement in strength is due to bio-mineralized calcium carbonate on bacterial cell, cell surface and within the soil. The

plugging of pores in between the soil structure of block leads to enhanced strength.

Water absorption test – The presence of bacteria resulted in a significant decrease of the water uptake compared to untreated specimens (control). Reduction of water is about 40% compare to the control specimen.

Immersion test for briquettes- The two treated and untreated briquettes were analyzed after the incubation time of immersion test. While the treatment, briquettes showed different water absorption rate. The untreated control briquettes were crumpled within 10 minutes. The 24 hour treated ones remained for 18 minutes but the 72 hour culture treated briquette showed less absorption of water during incubation (Incubation time 30 minutes).

Morphological studies

The formation of calcite by means of bio-mineralization was analyzed using various characterization techniques- SEM, EDS, XRD, FTIR and TGA. Techniques are specialized or involved all modes of microbial analysis like imaging, diffraction and spectroscopy including X-ray, neutrons and electrons as primary radiation.

To confirm microbial calcite formation in microbial stabilized blocks, the powdered samples from tested specimen was studied using SEM (fig. 5) and EDX.

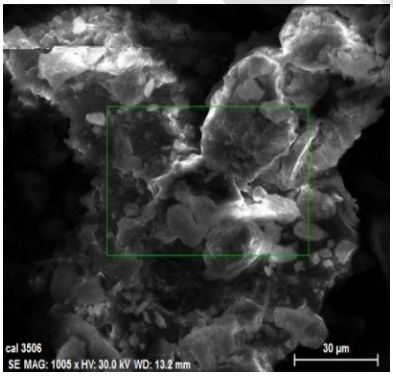

Fig. 5. Scanning Electron Microscope image of a treated soil

Mapping EDX: EDX Mapping were carried out to measure mineral profiling (fig. 6) the map showed this test were confirmed localized and specific mineral profile of an area measured 30um x 30um .it is confirmed the presence of CaCO3 and ammonium sulphate precipitation.

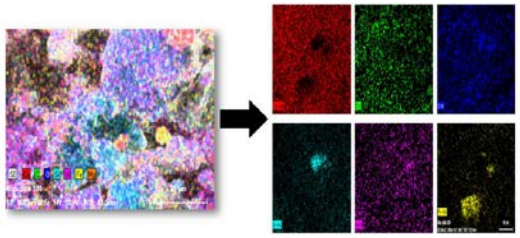

Fig. 6. Compiled EDAX mapping data of treated soil BRUKER softer generated data showed various mineral profile

EDAX mapping was explain the localized Si and Fe-KA accumulation by microbial action and support the formation of mineral deposits such as calcium carbonate or calcium sulfate. The involvement of bacteria in calcite bio mineralization was very evident as bacterial cells in close contact with calcite crystals were visible. Rod shaped impressions of bacterial cells within calcite crystals proved that they had been occupied by bacteria at some stage of crystallization or the cell had completely colonized by the crystals. The presence of calcite associated with bacteria proves that bacteria served as nucleation sites during the mineralization process.

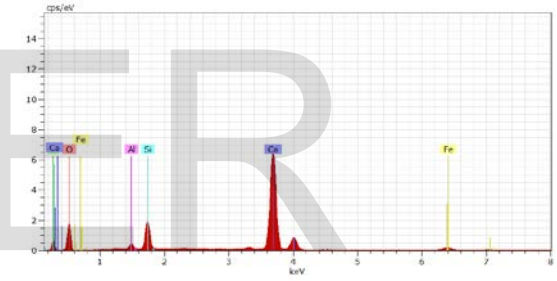

Fig. 6. Energy dispersive X ray analysis of microbial stabilized mud block

More number of calcite peaks suggests that minimum calcite precipitation which would thereby reduce the pores in blocks. In addition to calcite peaks the XRD spectrum database also confirmed the presence of minor peaks for calcium derivative-dolomite.

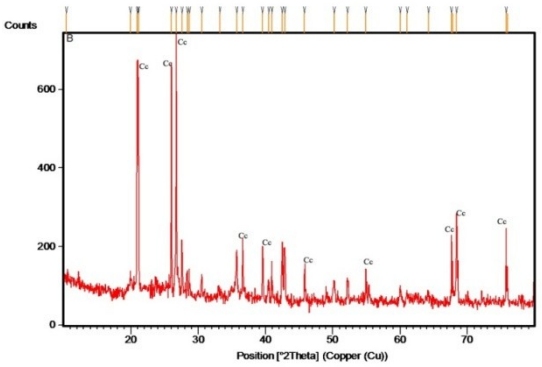

Fig. 7. XRD Profile of MSMB sample (10^9 cells/ml)

FTIR spectroscopy is a simple and accurate technique to identify and quantify $CaCO_3$ polymorphous. The C=O bonds (of carbonate group) would exhibit in plane blending and out of plane bending about 713 and 875 cm^{-1}.

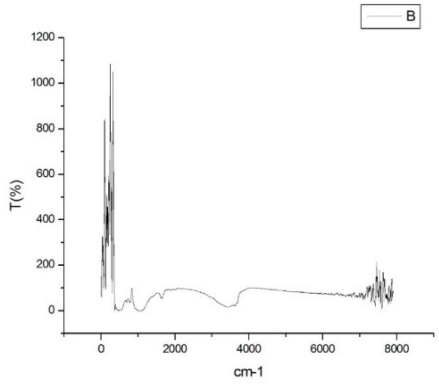

Fig. 8. FT-IR Profile of Microbial stabilized Mud Blocks

CONCLUSION

The technique of microbial treatment of soil significantly improved the mechanical properties of mud blocks. From the laboratory results, it was confirmed that the maximum compressive strength obtained by the application of microbial consortium having 10^9 cells/ ml concentration. Microbial calcite precipitation was identified and quantified by X-ray diffraction (XRD), Fourier Transform Infrared (FT-IR) analysis and visualized by Scanning electron Microscope (SEM) with EDX. The development of the "Microbial stabilized Mud Blocks (MSMB)" will provide the basis for an alternative and pores sealant that is cost effective and environmentally safe and ultimately leads to enhancement in the durability of building materials.

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