Open Science Index, Civil and Environmental Engineering Vol:11, No:5, 2017 publications.waset.org/10007278.pdf

Characterization of the Microbial Induced Carbonate Precipitation Technique as a Biological Cementing Agent for Sand Deposits

Sameh Abu El-Soud, Zahra Zayed, Safwan Khedr, Adel M. Belal

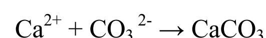
***Abstract*—**The population increase in Egypt is urging for horizontal land development which became a demand to allow the benefit of different natural resources and expand from the narrow Nile valley. However, this development is facing challenges preventing land development and agriculture development. Desertification and moving sand dunes in the west sector of Egypt are considered the major obstacle that is blocking the ideal land use and development. In the proposed research, the sandy soil is treated biologically using *Bacillus pasteurii* bacteria as these bacteria have the ability to bond the sand partials to change its state of loose sand to cemented sand, which reduces the moving ability of the sand dunes. The procedure of implementing the Microbial Induced Carbonate Precipitation Technique (MICP) technique is examined, and the different factors affecting on this process such as the medium of bacteria sample preparation, the optical density (OD600), the reactant concentration, injection rates and intervals are highlighted. Based on the findings of the MICP treatment for sandy soil, conclusions and future recommendations are reached.

***Keywords*—**Soil stabilization, biological treatment, MICP, sand cementation.

I. Introduction

THE MICP technique for soil stabilization is one of the most promising techniques that have been developed in the last decade. Many researches introduced the MICP technique in the United States, Canada, and Europe. Previous researches [1]-[4] were reviewed to determine the type of suitable microbe, the solution input rates and preparation; however, there are some core factors that are further experimented, tested, and modified such as the setup, injection method, soil grades, and outdoor conditions. The MICP technique is a bio-cementation process in which the bacteria are injected in the soil combined with urea and calcium chloride as a source of carbon and calcium respectively. The bacteria hydrolyses urea that leads to the raise in the pH of the soil system and the formation of carbonates. Meanwhile, the presence of a calcium source allows the precipitation of calcium carbonate ($CaCO_3$) that acts as a cementing agent between the sand particles. Consequently, the loose sand is turned into cemented material according to the described below chemical reactions (Al Qabany and Soga [1]):

$$NH_2CONH_2 + 2H_2O \rightarrow 2NH_4^{+} + CO_3^{2-} \quad \text{with raised pH)}$$



$$Ca^{2+} + CO_3^{\,2-} \rightarrow CaCO_3$$

The microbe used in this study is *Bacillus pasteurii*; this bacterium is also named *Sporosarcina pasteurii* (*S. pasteurii*) [American Type Culture Collection (ATCC) 11859]. The *Bacillus pasteurii* was used in many researches as it is the most successful strain in the MICP technique. It is an alkali-philic soil bacterium, which is capable to survive in alkaline (pH roughly 8.5-11) environments [2]. In addition, cells of *Bacillus pasteurii* do not combine; that means a high cell surface to volume ratio, which leads to more efficient cementation between the soil particles [3]. Moreover, there are some factors affecting the MICP technique; the precipitation process is influenced by the availability of calcium and carbon sources in the soil media. The concentration of the chemicals were recommended to be 0.25 mole/litre of reactants every 12 hours, that will occur an input rate the reactants of 0.042 Mole/litre/h [4].

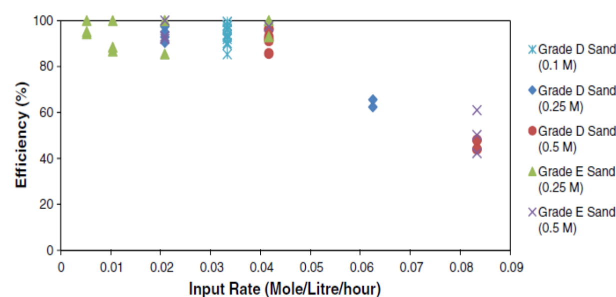


Fig. 1 Reactants efficiency for different input rates quoted from [4]

The bacteria should be in the range (OD600) of 0:8–1:2, which means the number of cells = 107 cells⁄mL [4]. The ability of the microbe to hydrolyze the urea to produce carbonates can be measured by a conductivity test. This test is measuring the ability of the bacteria to hydrolyze urea, and the recommended range after Al Qabany [5] is from 3 to 30 mM urea/hour, and 5–20 mM urea/hour as stated by Al Qabany et al. [4].

The size of the soil particles also has a significant effect on this technique as the recommended size of the particles is

Adel M. Belal (Prof.) is with the Structural Engineering, College of Engineering and Technology, Arab Academy for Science, Technology and Maritime Transport, Heliopolis, Cairo, Egypt (e-mail: adel_belal@hotmail.com).
Sameh Abu El-Soud (Assoc. Prof) is with the Geotechnical Engineering, College of Engineering and Technology, Arab Academy for Science, Technology and Maritime Transport, Heliopolis, Cairo, Egypt (e-mail: saelsoud@gmail.com).
Zahra Zayed (Teaching assistant) is with the AUC, Cairo, Egypt (e-mail: z.z@aucegypt.edu).
Safwan Khedr (Prof.) is with the Geotechnical Engineering, AUC, Cairo, Egypt.

Open Science Index, Civil and Environmental Engineering Vol:11, No:5, 2017 publications.waset.org/10007278.pdf

between 50 and 400 µm for the bio-cementation [6]. In this research, the MICP technique is to be experimented to enhance the soil properties and to be used in the Egyptian environment, without temperature or humidity control, and in a non-sterile condition.

II. Research Scope and Objectives

The research will be carried on *Bacillus Pasteurii* bacteria and soils obtained from an Egyptian site using laboratory experimental approach in the AUC laboratories. The main research objectives are:

- Prepare the bacteria outside the biology lab (un-sterilized condition).
- Applying the MICP technique on different types of soil.
- Measuring and comparing the mechanical and physical properties of the treated samples with the original soil properties.

Many variables are involved in MICP technique such as the type of soil, the concentration on the biotic solution, the methods of injection, etc. Therefore, two main criteria were taken into consideration during this research:

- First, testing different concentrations of the biotic solution to choose the optimum concentration of the chemicals.
- Second, the efficiency of MICP treatment in different soil types through measuring and evaluating enhancements of mechanical and physical properties of the soil.

III. Methodology

In the proposed research methodology, it is planned to cover four major tasks:

- Biological aspects of *Bacillus pasteurii* bacteria: Studying of the MICP process and changing the lab conditions and investigate the effect of these changes on the growth of the bacteria.
- Bactria-Soil interaction: Optimization of the concentration and the injection duration for Sand and Sabakha soil.
- Characteristics of (MICP- Soils) system: Testing the effect of MICP on soil permeability and strength and the possibility of using MICP in soil improvement.
- Finally, to evaluate the feasibility of using the MICP process in the proposed engineering applications.

IV. Laboratory Program

The research is based on two main phases which are microbiology and soil injection and testing.

- Microbiology: This phase is about preparing solid and liquid media, and inoculates the bacteria in the media, in order to inject it in the soil samples. The bacteria were brought from the Microbiological resource center (Cairo MIRCEN), Faculty of Agriculture, Ain shams university. Bacteria used research is Sporosarcina pasteurii (American Type Culture Collection (ATCC) 11859).
- Soil injection and testing: Injection of bacteria in the soil samples with different concentrations. Testing and evaluation of cementation efficiency of the samples after the treatment using MICP technique.

V. Preparation of the Samples

Bacteria used in this research are *Sporosarcina Pasteurii*. The bacteria were brought from the Microbiological Resource Center (Cairo MIRCEN), Faculty of Agriculture, Ain shams university. Bacteria cells from the frozen stock were grown on (NH4 –YE) medium plates (20 g yeast extract, 10 g of $(NH_4)_2SO_4$) and 20 g of agar in 0.13 M Tris buffer of pH value which is equal to 9 as shown in Fig. 2.

Fig. 2 Adjusting the pH of the Tris buffer

Then, it is transferred to (NH_4 –YE) liquid medium to be pre-cultured and incubated in 37 °C for 24 hours, with a control tube that contains a clear media without bacteria to ensure that there is no contamination occurred as shown in Fig. 3. A suction pump of 0.5 HP was connected to a feeding reservoir which contains the abiotic solution (reactants without bacteria). The outlet of the pump was connected to a pressure regulator to regulate the pressure of the reactants flowing in four samples connected in parallel. The pre-culture was then added to 1 liter of fresh (NH_4 –YE) liquid medium and divided into five flasks of a capacity 1 liter to fill only one fifth of the volume, to ensure enough aeration for the bacteria. The flasks were put in incubator shaker at temperatures 37 °C and 220 rpm. After 30 hours, the optical density (OD600) reached 0.86. The bacterial activity was tested at this stage by adding 1 ml of bacteria to 9 ml of 1.1 mole urea which was 18 mM urea/hour.

Fig. 3 Pre-culture and the control tube

The bacteria then were added once at the beginning of the experiment. The bacteria in the liquid media were packed with the non-sterilized sand in the experiment beakers, then the reaction solution was injected to the samples every 12 hours for four days with the old excess solution which was drained, to allow the new reactants to saturate the soil sample.

The abiotic solution used was 0.25 M calcium chloride and

0.25 M urea in addition to 3-g nutrient broth and 2.17 g of sodium bicarbonate per liter of distilled deionized water. The input dosage of the abiotic solution was approximately 1.5 the pore volume of the sample to grantee the saturation of sample.

The proposed setup as shown in the schematic drawing (Fig. 4) and laboratory setup photo (Fig. 5) contains one pump connected to the inlet reservoir that contains the abiotic solution. The outlet of the pump was connected to a pressure gauge and an overflow outlet valve to regulate the pressure of the injected solution in order to avoid any heave or boiling that may happen due to the pump head. A suction pump of 0.5 HP was connected to a feeding reservoir which contains the abiotic solution (reactants without bacteria). The outlet of the pump was connected to a pressure regulator to regulate the pressure of the reactants flowing in four samples connected in parallel.

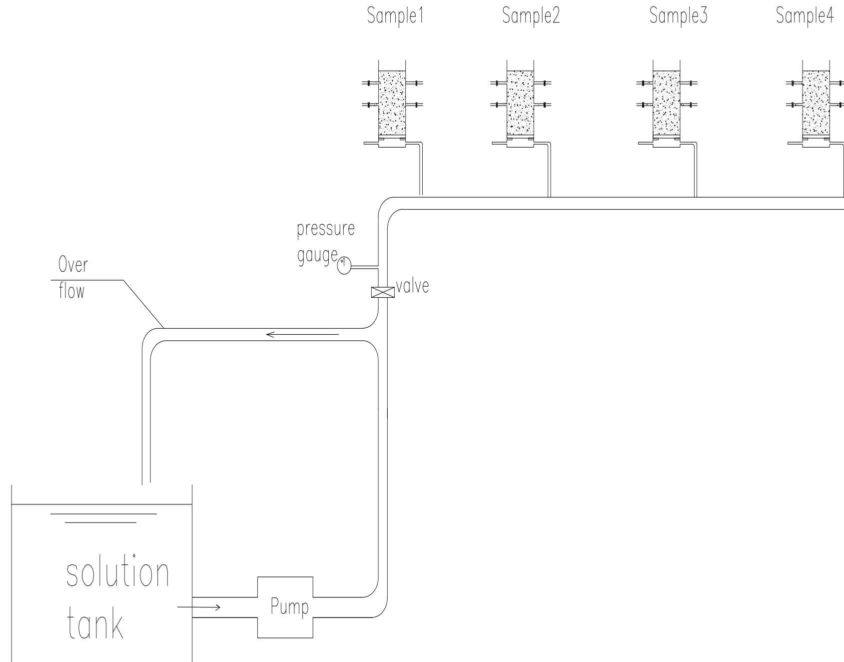


Fig. 4 Schematic diagram of the laboratory setup for bacterial treatment for sandy soils

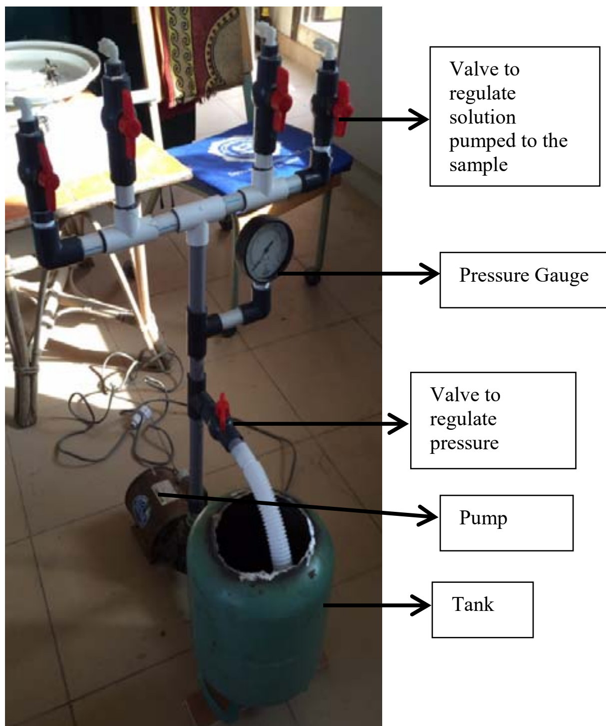


Fig. 5 Laboratory photo of sample treatment setup

Fig. 6 The beakers used in reactant injection

Regarding the reactant injection intervals, the bacteria were mixed with sand, and every six hours, excess solution was drained and new solution was pumped.

Glass beakers which are shown in Fig. 6 have a diameter of 7.5 cm and height of 20 cm with inlet and outlet nozzles for injection of the reactants.

VI. Results and Discussion

A. Bacteria

In the first trial, bacteria cells from the frozen stock were

Open Science Index, Civil and Environmental Engineering Vol:11, No:5, 2017 publications.waset.org/10007278.pdf

grown on (NH4 –YE) medium plates (20 g yeast extract, 10 g of $(NH4)_2SO_4$) and 20 g of agar in 0.13 M Tris buffer in pH 9.0). Bacteria are then transferred to (NH4 –YE) liquid medium at 37 °C for 40 hours, and the optical density (OD600) reached 3.8. This value is exceeding the OD600 that is recommended in the previous studies, which was between 0.8 and 1.2. The bacteria were added once at the beginning of the experiment, and the bacteria were packed with the sand in the experiment beakers, then the reaction solution was injected to the samples every 12 hours for four days.

Bacteria are added once at the beginning, every six hours excess solution was drained, and new solution was added. The concept of this setup was to have an over tank supply (Fig. 7) connected through a plastic tube that would provide the sample with the abiotic solution by the force of gravity. The point of injection was at the bottom of the sample, as it was left to rise and fully saturate the sample until it reaches the top of sample. The primary setup was very simple and had many problems that affected our results:

- The pressure was not enough to push the solution up through the saturated soil that affected all the injections after the first injection.
- The size of sample (60 ml syringe) was very small to observe any precipitation occurred between sand particles. The size also affected the injection of the solution.
- Lack of air circulation to provide the bacteria in the sample with oxygen to keep the reaction occurring

Fig. 7 Addition and drainage of the reactant into the MICP-Treated sand sample

The dose was approximately 1.5 the pore volume of the sample to grantee the saturation of sample. The abiotic solution used was 0.25 M calcium chloride and 0.25 M urea in addition to 3 grams of nutrient broth and 2.17 grams of sodium bicarbonate. A rotten smell was recognized after the experiment termination and apparently, no reaction happened, and by further examination of the samples, no cementation occurred as expected.

This may be a result of the high (OD600) 3.8 value that was exceeding the OD600 that is recommended in the previous studies, which was between 0.8 and 1.2. According the relevant studies, when the OD600 exceeds 1.2, the bacteria in the medium then reached the stationary state, which means that most of the cells present in the media are dead.

In the second trial, bacteria were grown in (NH4 –YE) liquid medium at 37 °C for 30 hours, the optical density (OD600) reached 0.84. The bacteria were packed with the sand in the experiment beakers, and the abiotic solution was added as previously mentioned in trial 1. The experiment was terminated after four days, the samples were drained, and oven dried. Partial reaction happened, a white layer was precipitated at the top surface of the sample with high collapsibility potential as shown in Fig. 8.

Fig. 8 Sample with high collapsibility potential after treatment from second trail

In the third trial, bacteria were grown in (NH4 –YE) liquid medium at 37 °C for 30 hours, the optical density (OD600) reached 0.92 (Fig. 9). The bacteria were packed with the sand in the experiment beakers, and the abiotic solution was prepared and added as in the previous trails. Moreover, in this trail, before injecting each new dose of the abiotic solution, it was subjected to airflow to insure a sufficient the presence of dissolved oxygen needed for the activity of the bacteria.

Fig. 9 Sample with a good cementation after treatment from third trail

Open Science Index, Civil and Environmental Engineering Vol:11, No:5, 2017 publications.waset.org/10007278.pdf

Open Science Index, Civil and Environmental Engineering Vol:11, No:5, 2017 publications.waset.org/10007278.pdf

B. Reactant Input Rate and Concentration

The input rate that we used was 0.042 mol/L/h. We chose this rate since it was proven, by previous experiments, to be the highest urea-$CaCl_2$ input rate that can still maintain a chemical efficiency of over 90%. Using this input rate, it was required to determine the best combination of concentration and injection interval. The higher the concentration is, the longer the interval is, and vice versa.

The lower concentrations at more frequent injection times are said to be more efficient than higher concentrations with smaller number of injections as it results in a more uniform distribution of the precipitation without areas of concentrated precipitation. According to previous research, the results were optimum at an injection concentration of 0.25 M every six hours. This is the concentration and injection interval that we used in our research, which thus leads to an input rate of 0.042 mol/L/h.

VII. Conclusion and Recommendations

Based on the laboratory test results for the biologically treated sandy sample using the MICP technique, the following conclusions may be drawn;

1. The treatment using the MICP technique showed significant enhancement of the sand cementation, yet it has many challenges due to the working with a microorganism that is highly effected by the surrounding conditions in lab and out-door.
2. The recommended ranges of the optical density (OD600) 0.8-1 showed efficient activity of the bacteria compared to lower optical density, with a maximum cementation effect at a value of 0.92.
3. The lower concentrations at more frequent injection times are said to be more efficient than higher concentrations with smaller number of injections.
4. Further testing, development, and research in this technique is recommended, it can rise to be a very resourceful and efficient soil stabilization technique and can add substantial value to the construction industry.

References

[1] Al Qabany, A. & Soga, K. (2013). Effect of chemical treatment used in MICP on engineering properties of cemented soils , Ge´otechnique 63,No .4, 331–339
[2] Ferris, F.G., & Stehmeier, L. G. (1996). Bacteriogenic mineral plugging. Journal of Canadian Petroleum Technology, 35(8), 56-61.
[3] DeJong, J. T., Fritzges, M. B., and Nusslein, K. (2006). “Microbially induced cementation to control sand response to undrained shear.”J. Geotech. Geoenviron. Eng., 132(11), 1381–1392.
[4] Al Qabany, A., Soga, K. & Santamarina, J. C. (2012). Factors affecting efficiency of microbially induced calcite precipitation. J. Geotech. Geoenviron. Engng 138, No. 8, 992–1001.
[5] Al Qabany, A. (2011). “Microbial carbonate precipitation in soils.”Doctoral dissertation, Univ. of Cambridge, Cambridge, U.K.
[6] Rebata-Landa, V. (2007). Microbial activity in sediments: effects on soil behavior. PhD thesis, Georgia Institute of Technology, Atlanta, GA, USA.