University of Tabriz

Journal of Civil and Environmental Engineering
Volume 52 (2022), Issue 1 (Spring), 41-53

EXTENDED ABSTRACTS

Evaluation of Success in Microbial Bio- Improvement of Motor Oil Contaminated Sandy Soils

Mohammad Taghi Bolouri Bazaz, Jafar Bolouri Bazaz *

Civil Engineering Department, Engineering Faculty, Ferdowsi University of Mashhad, Mashhad Iran

Received: 18 October 2018; **Accepted:** 13 January 2020

Keywords:
Hydrocarbon contamination, Biological method, Bacterial solution, Flocculation.

1. Introduction

Given that advances have been made toward the use of carbon-free or low-carbon energy sources in the modern sciences (Simpson and Tatsuoka, 2008), the development and optimization of more environmentally-friendly methods are required. Biological practices have their own sensitivity due to their dealings with living organisms. The feasibility of the use of the calcium carbonate precipitation method in the sandy soils contaminated with hydrocarbon pollutants and the use of bacterial flocculation have been recently investigated (Cheng and Shahin, 2017). The purpose of the present research is to investigate the variation of geotechnical parameters of sandy soils contaminated with motor oil.

2. Methodology

2.1. Material

The soil used in the current research was Firoozkooh sand with internal friction angle and specific gravity 36° and 2.71, respectively. The maximum and minimum dry unit weights of this sand were found to be 16.38 kN/m^3 and 14.05 kN/m^3, respectively. Also, two common types of hydrocarbon contaminants (motor oil and gasoline) and Sporesarcina pasteuri strain as the main strain of the bacteria with high urease activity were employed.

2.2. Culture and growth of the bacterial strain

The main strain of the bacteria with high urease activity, ATCC11859 Sporsarcina pasteuri, was prepared from the Iran microorganism Bank. Bacterial suspension preparation is conducted based on the process presented in Table 1.

2.3. Improvement method using calcium carbonate precipitation

The microbial improvement process was carried out with two methods:
Method 1: Using the two-stage injection process (Whiffin et al., 2007) in which bacteria enter the soil by injection followed by cementation solution injection.

Method 2: Using flocculation of bacteria (Cheng and Shahin, 2017) in which 100 mM of calcium chloride was added to one liter of culture medium, containing bacteria, to prepare bacterial flocculation. Calcium chloride caused the bacterial cells to accumulate and coagulate, leading to the precipitation of the bacteria at the bottom of the container. To prepare bacterial flocculation, 85% supernatant was removed (Cheng and Shahin, 2017).

* Corresponding Author
E-mail addresses: mbloori@gmail.com (Mohammad Taghi Bolouri Bazaz), bolouri@um.ac.ir (Jafar Bolouri Bazaz).

Table 1. Bacterial preparation process

Step	Process	Description
1	Preparation of Nutrient Agar solid medium and addition of 10 mg/L MnSO4	Adding MnSO4 is to increase the bacterial sporulation
2	Autoclaving 100 ml of Nutrient Agar solid medium	Autoclaving takes 15 minute time
3	Adding 20% urea to the culture medium by filtering	Increasing the culture medium temperature up to 45 ° C when urea is added
4	Pouring the medium into the plate and cultivating the bacteria	This process is carried out beneath the hood.
5	Placing the plate inside the incubator	For 24 hours
6	Preparation and autoclaving of the liquid culture medium (20 g Yeast Extract, 17 M Ammonium Chloride (NH4Cl2), 0.1 mM NiCl2, 20 g Urea (CO (NH2)2) per liter of distilled water	Autoclaving takes 15 minute time
7	Reaching the acidity of the medium to pH = 9.25 by adding (NaOH)	After autoclaving and reaching the temperature up to 30°C
8	Culture of bacteria in a liquid medium and placing it in the incubator shaker	For 48 hours at 150 rpm
9	The bacterial population was measured by spectrophotometer and bacterial suspension	It was measured at a wavelength of 600 nm and when the light absorbance (light density) of the bacterium in this wavelength reached 2.5

2.4. Test program

The tests performed in this study are classified into three general categories:

1. Classic soil mechanics tests including density , uniaxial compressive strength (UCS) (ASTM-D2166, 1999) and direct shear (DS) tests (ASTM-D3080,1999).
2. Energy-dispersive X-ray spectroscopy (EDS) for the elemental analysis or chemical characterizations of a sample and scanning electron microscope (SEM).
3. Wet chemistry analysis to estimate the amount of CaO compounds (with Ethylenediaminetetraacetic acid (EDTA) salt titration)

3. Results and discussion

Table 2-a, Table 2-b and Table 2-c summarize the UCS test results, strength parameters including cohesion and internal friction angle, and magnitudes of permeability of soil contaminated before and after the MICP improving process respectively.

The test results indicate that with increasing motor oil percentage, the uniaxial compressive strength decreases. On the other hand, using method 2 (bacteria flocculation) in comparison with method 1 leads to better performance. Table 2-b summarizes the direct shear test results of samples with 6% contamination of motor oil, indicating the shear strength parameters have been modified with the MICP improvement process. This leads to a noticeable reduction in permeability after the MICP process, which has been gathered in Table 2-c. Depending on the viscosity of the oil, the oil droplets may be clothed into smaller coagulated units in the vicinity of bacteria solutions, (Vankova et al., 2007). When this happens, the bacteria are not able to grasp the grains directly (Fig. 1). In this case, there is a low chance of crystallization and the formation of a calcium-carbon bridge between the soil particles. Finally, it is worth mentioning that method 2 provides more suitable conditions to connect the bacteria and grains firmly.

Table 2. Classical soil mechanics test results: a) UCS, b) DS, c) permeability results

Sample Code	UCS (kPa)
U2%MS	88.5
U2%MF	113
U6%MS	34
U6%MF	38
U12%MS	10
U12%MF	9

(a)

Sample Code	φ(°)	C (kPa)
D6%MN	17	17
D6%MS	19	33
D6%MF	21	35

(b)

Sample Code	Permeability (cm/s)×10^{-3}
H6%MN	17
H6%MS	33
H6%MF	35

(c)

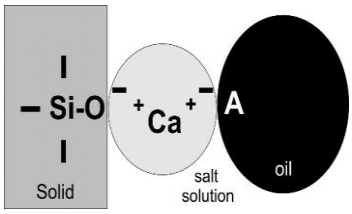

Fig. 1 Interaction mechanism between crude oil and solids

To measure the amount of calcium carbonate produced by the biological process, additional chemical tests were performed on samples, taken from UCS and DS. The amount of precipitated calcium carbonate was higher for samples improved by method 2 (flocculation of bacteria) in comparison with method 1 (bacteria suspension injection). Also, the amount of precipitated calcium carbonate was measured at different parts of a contaminated sample (Fig. 2) with motor oil, which has been summarized in Table 3.

In order to analyze clearly the samples, imaging with SEM was performed. An improved contaminated sample has been shown in Figs. 3-a and 3-b, in which sand grains and CaO bridges can be obviously observed. EDS analysis indicates the precipitation of calcium carbonate between sand grains. Si and Ca peaks in the EDS diagram, shown in Figs. 3-c and 3-d, are related to the sand grain and calcium carbonate respectively.

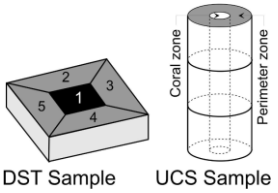

Fig. 2. UCS and direct shear samples for wet chemistry analysis

Table 3. The percentage of calcium carbonate produced from the tested specimens

Sample	Cylinder						Cube				
zone	per.1	per.1	per.1	cor.1	cor.2	cor.3	1	2	3	4	5
6%MS	2.5	2	0.5	0.5	1	0.39	1.5	1.8	1.2	1.5	1.89
6%MF	1.75	1.5	0.5	1.5	1.25	0.94	1.85	1.9	1.39	1.56	1.6

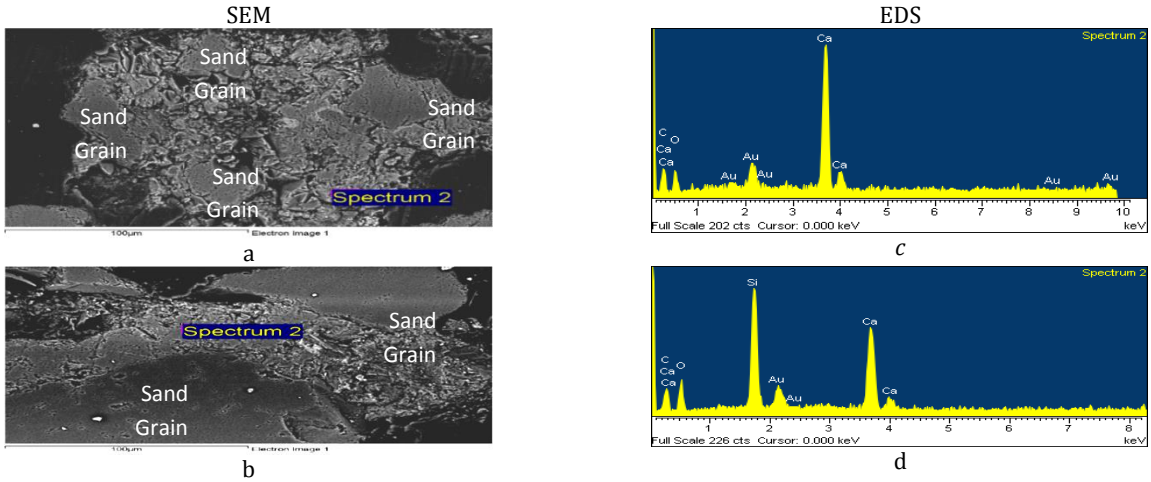

Fig. 3. SEM from the motor- oil contaminated samples and the EDS analyses

4. Conclusions

This paper presents the influence of motor oil contamination on the degree of prosperity with 2 different MICP methods. While the first method, which has been commonly applied in the literature, is two-phase injection method (method 1), the other method (namely method 2) focuses on premixing ureolytic bacterial flocs with motor oil-contaminated soils for the purpose of bacteria fixation. In both methods, precipitation was observed but the quality and quantity of calcium carbonate bridges between grains were differed. In method 2 because of the firm fixation of bacteria flocs on the surface of the grain, MICP process was supplemented on the grain and the bridges were effective. In method 1 despite precipitating of calcium carbonate, no firm connections were performed between oily grains due to non-effective bridges.

5. References

ASTM-D2166, American Society for Testing and Materials D2166 (1999) Standard test method for unconfined compressive strength of cohesive soils, Annual Books of ASTM Standards, 1999.

ASTM-D3080 American Society for Testing and Materials D3080 (1999), Standard test method for direct shear test of soils under consolidated drained conditions, Annual Books of ASTM Standards, 1999.

Cheng L, Shahin MA, "Stabilisation of oil-contaminated soils using microbially induced calcite crystals by bacterial flocs", Géotechnique Letters, 2017, 1-6.

Simpson B, Tatsuoka F, "Geotechnics: the next 60 years", Géotechnique, 2008, 58, 357-368.

Vankova N, Tcholakova S, Denkov ND, Ivanov IB, Vulchev VD, Danner T, "Emulsification in turbulent flow: Mean and maximum drop diameters in inertial and viscous regimes", Journal of Colloid and Interface Science, 2007, 312, 363-380.

Whiffin VS, Van Paassen LA, Harkes MP, "Microbial carbonate precipitation as a soil improvement technique", Geomicrobiology Journal, 2007, 24, 417-423.