

An Investigation into the Capability of *Sporsarcina Pasteuri* on Improvement and Refinement of Contaminated Hydrocarbon Soils

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1. Introduction

The extreme dependence of modern economy on fossil fuels has increased the frequency of accidental emissions of petroleum hydrocarbons, a combination of alkanes, aromatic hydrocarbons, and compounds containing nitrogen, oxygen and sulfur. All activities of oil industry, including exploration, extraction, transportation, refining and management of oily waste are potential sources of environmental pollution. There are several methods for refining the contaminated soils with petroleum hydrocarbons, such as physical, chemical and biological methods. The use of bioremediation methods for petroleum hydrocarbons is an effective, economic and environmental friendly technology as a practical solution to improve soils contaminated with petroleum hydrocarbons. On the other hand, the metabolism of microorganisms compatible with these substances breaks them down into simpler substances. That is, it transforms from a passive state into more active molecules for decomposition.

Calcium carbonate microbial deposition improvement method, as a branch of biogeotechnical engineering, is the result of microbial metabolic activity. The urea enzymatic hydrolysis may be employed for calcium deposition, using various methods, of which *Bacillus pasteurii* is the most effective. More recently reclassified as *Sporsarcina Pasteurii* (ATCC 11859), it is an alkaline bacterium highly active with the enzyme urease, which has been used in laboratory researches for calcium deposition. Nowadays there is a relative lack of research on the provision of a suitable solution for the improvement and refinement of contaminated hydrocarbon soils, in such a way to be able to decompose and simplify the hydrocarbon components, recover its lost mechanical properties and somehow reduce the soil permeability to prevent further spread of contaminants.

2. Materials and methods

The materials used in this research are Firouzkouh sand with physical properties summarized in Table 1, two common types of hydrocarbon pollutants (engine oil and gasoline) and *Sporsarcina pasteurii* as the bacterial strain. This type of bacterial strain (ATCC11859), which has a high urea content, was prepared from the Bank of Microorganisms of Iran. For culture and preparation of bacterial suspension, after provision of Nutrient Agar solid culture medium, followed by leaving in autoclave

for 15 minutes, urea 20% and bacterial culture were added. The liquid culture medium (including 20 g yeast extract, 17 molar ammonium chloride, NH_4Cl_2 , 0.1 ml molar nickel chloride, NiCl_2 , and 20 g of urea $\text{CO}(\text{NH}_2)_2$ in one liter of distilled water) was then prepared. The acidity of the culture medium was reached 9.25 by placing the culture medium in an incubator shaker. To prepare bacterial fluke, 100 mM calcium chloride was added to one liter of culture medium containing the bacterium. Calcium chloride causes bacteria to accumulate and coagulate, eventually causes bacteria to settle down in the bottom of the container. After precipitation of bacterial blisters, 85% of the supernatant was removed. The bacterial suspension with CaCl_2 was finally ready to enter the soil in clotted and flocculated form. The mixing of calcium chloride and the bacterial suspension results in the bacterial shell to rupture and releases its urease enzymes.

Table 1. The physical properties of Firouzkouh sand

G_s	e_{\max}	e_{\min}	D_{10} (mm)	D_{60} (mm)	Cc	Cu
2.658	0.94	0.60	0.167	0.40	1.01	2.39

Infection of the samples was achieved by soaking method i.e. mixing sand with 6% engine oil and 6% gasoline by weight. Moreover, to assess the status and changes in the pollutants, the contaminant had to be removed from the soil with which it was mixed and evaluated. A standard sample of gasoline and engine oil (separately) with a concentration of 100 mg/l in dichloromethane solvent was prepared. This sample was used to identify the components in gasoline and engine oil.

3. Test program

The tests performed in this research are based on the evaluation of:

- The degree of soil improvement (based on the strength parameters obtained from direct shear and permeability test results);
- The quantity of purity (using chromatographic method);
- The rate of precipitation and sedimentation of calcium carbonate (using FTIR analysis results).

Table 2 shows the results of classical soil mechanics tests as well as the mass percentage of produced CaO.

Table 2. Summary of classical soil mechanics test results

Sample Code	$(^\circ)\phi$	C (kPa)	Permeability $(\text{cm/s}) \times 10^{-3}$	CaO (%)
WN	38	0	5.03	0.05%
WF	52	630	1.35	9.7%
MN	17	17	4.01	0.05%
MF	19	33	3.99	3.3%
GN	24	10	4.31	0.05%
GF	28	20	1.95	4.5%

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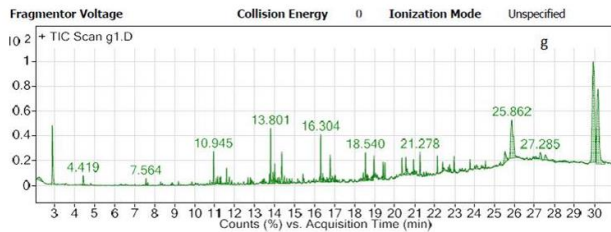
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The WN, MN and GN codes (Table 2) denote sand samples polluted with water, motor oil and gasoline with no improvement. The WF, MF and GF codes represent the same samples after improvement using flocculated method. The increase of the shear strength parameters for different states (non-polluted and polluted) of soil, is an evidence of the MICP process impact on the soil media. The process of Calcium carbonate deposition which is schematically in Figure 1, was observed in all samples. It is worth mentioning that the effectiveness of the relationship between the type of pore fluid and the MICP process, and also how calcium carbonate deposits are involved in different environments (in terms of its pore fluid) with soil grains should be considered.

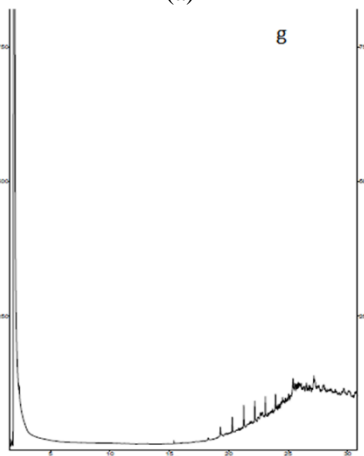


Figure 1. Calcium carbonate deposition among the soil contaminated grains

Mass and gas chromatography (GC) are common types of chromatography used in decomposition chemistry to separate and decompose volatile compounds without degrading them. Figures 2, as an example, is the mass and gas chromatography results for the refined and improved soil samples which have been polluted with gasoline.



(a)



(b)

Figure 2: (a) mass and (b) gas chromatography test results

In reviewing the results, receiving the output of reduced peaks is one of the most important interpretations of chromatograms and comparing them in different soil and pollutant conditions. This represents somehow the bacterial activity. That is, when the area below the chromatographic graph is reduced, the decomposition process is performed. Although the refining and decomposition processes of hydrocarbon materials make it easier to decompose up to 95% in specialized physical, chemical and biological refinements, but the refining rate is about 60% and 50% for gasoline and engine oil, respectively.

4. Conclusion

Based on the chemical and physical test results it can be concluded that:

- The process of improving sandy soils with MICP is an effective method on both polluted and non-polluted soil;
- The slight decrease in the strength parameters of hydrocarbon contaminated soils, compared to non-contaminated soils, can be successfully compensated with MICP methods;
- Soils contamination is one of the major concerns in environmental geotechnics which may be removed by examining the MICP improvement.