Utilization of Ultramicrobacteria for Soil Stabilization

Introduction

The stability and permeability of soil is a function of years of geological and hydrological processes. The intrusion of seawater into coastal aquifers is a common occurrence throughout the world. This is due to the overdrafting of the aquifer by local populations. Other soils are subject to compression or liquefaction as a result of seismic activity. In both cases, the source and potential solution of the problems mentioned above is the permeability of the soil matrix.

Bacteria account for approximately half the biomass on the planet. When bacteria are starved there are three possible outcomes: they die, they form a resting spore, or they shrink. There are certain species of bacteria that when starved shrink to approximately one-hundredth their normal size as a survival mechanism. These kinds of bacteria are called "ultramicrobacteria"(1). There are some ultramicrobacteria that occur naturally in this size range ($<0.1\ \mu m^3$) and others that can be starved to achieve this size (2).

There is a wealth of information on bacterial biofilms and the process of soil and aquifer clogging by naturally occurring bacteria (3,4,5). The extent of the clogging is limited to the ability of the bacteria to penetrate into the soil or rock matrix. In their normal growth state, bacteria can penetrate between a few centimeters to tens of meters depending on the volume of the bacteria and the pore size of the matrix. Using native bacteria, it is possible to create a group of ultramicrobacteria that can be introduced into a matrix such that there is complete and uniform penetration of the matrix by the bacteria. Due to their smaller size, the consortium of ultramicrobacteria can be evenly distributed throughout the matrix and with the addition of low concentrations of nutrients, be revived to their normal state. Once the bacteria have returned to their normal state they can develop extensive biofilms that will fill in the void spaces with the solid matrix. Extensive clogging like this could prevent or at least inhibit salt water intrusion and could stabilize soils subject to liquefaction. A recent study showed that bacterially clogged sediment could further be stabilized by the formation of minerals such as calcium carbonate and calcium sulfate within sediment/bacterial matrix (6). Researchers at Montana State University have used ultramicrobacteria for produce "biobarriers" to prevent contaminated groundwater from mixing with surface water (7,8,9). To date, there is no mention in the literature for using ultramicrobacteria to prevent salt-water intrusion or to stabilize soils by the formation of biofilms and microbially facilitated mineral deposition.

Purpose

The purpose of this study is to determine if ultramicrobacteria can be used to uniformly clog soil columns and if the development of the biofilm over time and subsequent mineral deposition leads to further soil stability.

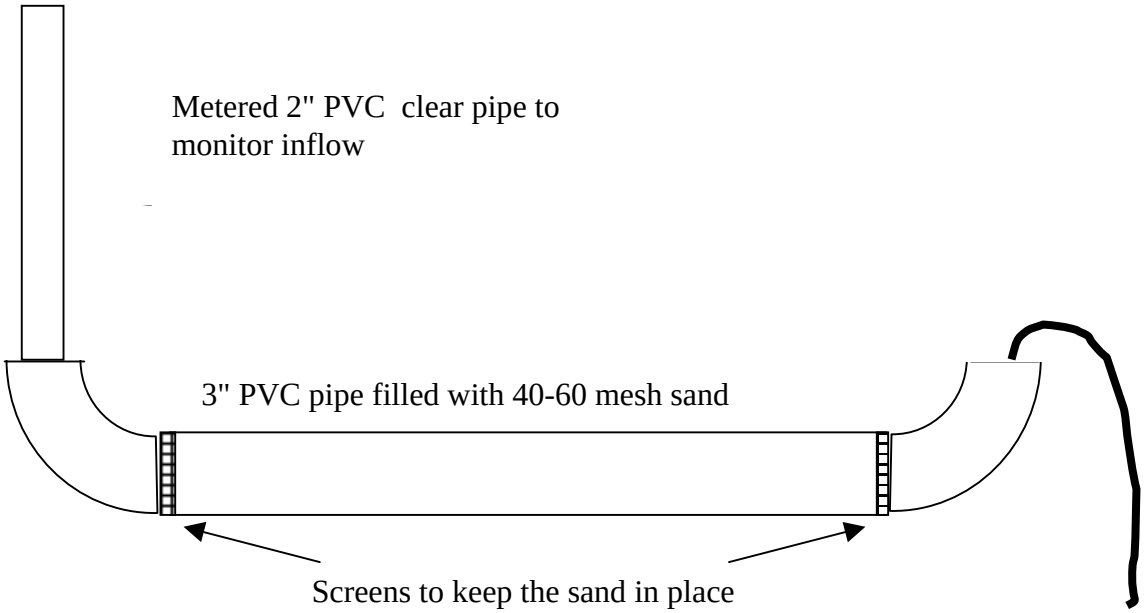
Objective

The objectives of this project are to study the processes of biofilm formation through a review of the current literature and through a series of experiments that involve the construction of soil columns, the monitoring of hydraulic conductivity of the columns, physical and chemical analysis of the soils using Scanning Electron Microscopy (SEM) and SEM Electron Dispersive X-Ray Spectroscopy (SEM-EDS) and soil stability tests.

Approach

Research Conducted till Present

Horizontal sand columns were constructed using 3" ABS pipe as shown in the diagram below. Four columns were constructed in the Structures Test Hall at UCI. They were filled with washed 40-60-mesh sand. Flow rates were taken from the column outflow while a constant head of pressure was maintained in the inflow. The columns were periodically flushed with a dilute bleach solution to prevent biofilm formation.



Sediment and pond water taken from a lake in Anaheim was supplemented with dilute R2A broth to grow the bacteria in the sediment. After one week at room temperature, a 100 ml sample of the solution was placed in 2 L of autoclaved pond water and left for one month. A sample of that water was transferred to another flask containing 2 L of autoclaved pond. This flask was left for three months to starve the bacteria.

Spiking the water with sodium chloride and measuring the change in conductivity over time in the column effluent determined the residence time for each column. Once a base line of data was collected on the columns, three were inoculated with the ultramicrobacteria. Samples of the water were taken from the effluent and put on to 1% trypticase soy agar plates to verify that the bacteria passed through the columns. Two of the columns receive 2ml of trypticase soy broth per week. The third column that received the inoculum of bacteria served as a control. The fourth column without the bacteria served as a second control. Flow rates are monitored three times per week using a sterile solution containing deionized water

and 450 mg/l of a commercial aquarium salt solution. This provided a total of dissolved solids level similar to the native pond water minus any organic compounds that could be used by the bacteria.

Research to be Accomplished Under this Proposal —Project Overview

Hydraulic conductivity will be followed for the next four months in the columns. Smaller columns will be constructed to develop biofilms for the stabilization and mineral formation tests. Half of these columns will have the same 450 mg/l of total dissolved solids and the other half will have increasing levels of salts to accelerate the deposition of minerals within the sand/biofilm matrix. After three to five months some of these columns will be used for stability experiments where they will be subject to the mechanical equivalent of seismic activity. Other columns will be used for the analysis using Scanning Electron Microscopy to observe the fouling layer and the possible deposition of minerals. Using SEM-Electron Dispersive X-Ray Spectroscopy, it will be possible to determine the chemical makeup of the clogging layer.

Student's Responsibilities:

Hydraulic Conductivity

Saturated hydraulic conductivity is usually assumed to be constant through time at any given location in porous media studies. However, it is known that this parameter varies extensively with time. Hydraulic conductivity is an indicative of a medium's ability to transmit water. A substance with high hydraulic conductivity will allow fluid to flow through much faster than a substance with lower conductivity. The time dependence of the saturated hydraulic conductivity varies according to the medium's physical resistance to water flow resulting from reduction in size of the pore space or from changed friction coefficients or fluid viscosity (1). The cause of these structural and rheological changes is due to physical, chemical, and microbial.

Some physical factors that may lead to a reduction in hydraulic conductivity are compaction by superimposed loads, smearing of the surface, migration of fines and suspended solids. The percolating aqueous solution and the chemical properties of the solid particles of soils or aquifer materials is linked to the geometry of the pore space. Precipitation/dissolution that takes place in chemical reactions all affect the shape and stability of the pores, thus the chemical properties determine the value of the saturated hydraulic conductivity of the medium (3).

Clogging can be induced by the activity of bacteria, and the addition of growth substrates is expected to accelerate the process such as additions of carbon and energy sources enhances soil clogging (3). Since saturated hydraulic conductivity depends on porous space or changed friction coefficients or fluid viscosity; bacterial cells can affect it mainly by altering the pore geometry, by reducing the cross-sectional area. Also some bacteria can excrete slimy or gummy materials on their surface, which refers to as exopolymers. As a result, exopolymers could affect the hydraulic conductivity by either increasing the viscosity of the fluid or by decreasing the volume and size of the pores. It can also causes high frictional resistance (3).

Laboratory measurement of the saturated hydraulic conductivity, K_s (L/T), can easily be done if the flow rate and the hydraulic head gradient are measured simultaneously. K_s can be calculated through Darcy's law:

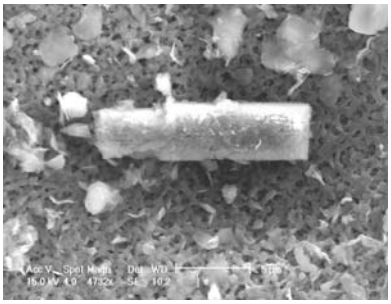
$$Q = AK_s \frac{\Delta H}{z}$$

where Q (L^3/T) is the flow rate, A (L^2) is the cross sectional area of the column, H is the hydraulic head (water potential per unit weight, L), $\Delta H \equiv H_{in} - H_{out}$, and z (L) denotes the distance between the two points where H is measured (3).

My responsibility is to monitor hydraulic conductivity of the columns by collecting flow rate each week. If the flow rate begins to decreases, then it means that the conductivity is getting lowered. Addition of energy source will be fed to the ultramicrobacteria in the first two columns as growth substrate to see if it'll dramatically clog up the two columns as compared to the third column where there is no growth substrate to the bacteria. Thus, my responsibility is to look at the process of change of changes in hydraulic conductivity as a function of biofilm development.

Mineral Formation

My responsibility is to look at the mineral formation in a column subject to bacterial clogging and frequent hydraulic flow. After 3 to 5 months of hydraulic conductivity, I will first examine samples from the columns using light microscopes to observe the existence of any mineral deposits. I will then take samples from the columns and examine the chemical composition of the column matrix using SEM-Electron Dispersive X-Ray Spectroscopy.



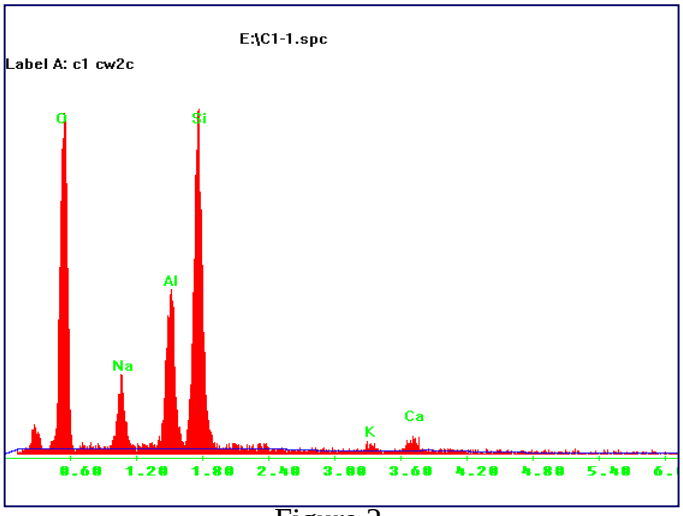


Figure 2.

Figure 1 is a sample image of a feldspar crystal from a SEM-EDX, and from the reading in Figure 2 we see the chemical profile of the crystal at the left which is a sodium aluminum silicate. This method allows us to determine the nature of the original matrix (e.g. silica) and the formation of mineral deposits such as calcium carbonate or calcium sulfate. Samples will also be drawn from separate columns that were inoculated with ultramicrobacteria, fed with a dilute nutrient solution and saturated with a carrier solution ranging in total dissolved solids concentrations ranging from 450 mg/l to 1200 mg/l to enhance the likelihood of mineral formation. Under ambient conditions of 450 mg/l, mineral formation was observed in local lake sediment after one year. (6)

Soil Stabilization

Among many aspects of this project, my study will focus on how the growth of micro-bacteria in and around the voids of soil interaction can help to stabilize soil structure that can eventually lead to prevention of liquefaction during earthquakes. The smaller columns used in the mineral formation experiments will be placed on the seismic shake tables at the UCI Structures laboratory. They will be subjected to the equivalent earthquake conditions ranging from 4.0-7.0 on a Richter Scale. Then the control and experimental columns will be observed to determine slumping, compression, or fractures within the column matrix. It is anticipated that the polymer formation would provide some stabilization while the columns with mineral formations should be the most stable of all the columns.

Project Timeline

- Spring-- experimental design and construction of columns and waterproof chamber
- Summer-- baseline data of flow rates and creation of a mixed culture of ultramicrobacteria
- Fall-- baseline residence time determined, inoculated three columns with bacteria, more flow rates, begin adding nutrient to two of the columns, build more columns for stability and mineral formation studies.
- Winter--more flow rates of the four columns, flow rates of the new columns
- Spring--stability tests of the columns and SEM & SEM-EDS analysis of the columns, summarize data and prepare for the final report.

Itemized Budget

1.	SEM & SEM-EDS fees	600
2.	Office Supplies	
	a. photo copies	50
	b. printing/poster supplies	50
	c. printer	200
3.	Building Supplies & Chemicals	
	a. tubing, glue, pipes, tools	200
	b. machine shop fees and materials	300
	c. chemicals	200
		$ 1,600.00

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