



Stabilization of dispersive soils by means of biological calcite precipitation

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ABSTRACT

Stabilization of dispersive soils is of great importance and has been looked into in various studies across the globe. In this study, the biological soil stabilization as a novel method has been investigated. In order to improve the erodibility resistance of dispersive soil samples, the bacteria *Bacillus sphaericus* has been employed. In this research, we focus on the improvements induced in dispersive soils as a result of microbial calcite precipitation and furthermore, the controlling factors are looked into. Pinhole experiments are used to investigate dispersivity of laboratory-made dispersive soil samples. Soil samples before and after biological stabilization have been carefully tested to determine their level of dispersivity. Factors such as curing time, as well as bacterial cell density, precipitating agent concentration, and temperature have been examined. The test results demonstrated that microbial calcite precipitation is a promising technique for stabilizing dispersive soils. The pH-decrease during microbial activity, and subsequently the decrease in the double layer thickness, as well as stabilization of exchangeable sodium ions were proposed to be major mechanisms contributing to the observed reduction in erosion potential of the soil samples. Furthermore, microbially-induced calcite precipitation results in further bonding which reduces erodibility potential of the soil samples.

1. Introduction

Dispersive soils are among the problematic soils commonly found in engineering projects in several countries and various geographical regions such as Australia, Greece, Thailand, Vietnam, Mexico, South Africa, Brazil, Iran, New Zealand, and the United States (Sherard et al., 1976; Ludwig, 1979; Goodarzi, 2003; Gutiérrez et al., 2003; Ouhadi and Goodarzi, 2006; Fernando, 2010; Abbasi and Nazifi, 2013; Goodarzi and Salimi, 2015). Alluvial clays and soils originated from mud rocks depositing in a marine environment are known to be dispersive, whereas soils originated from weathering of igneous and metamorphic rocks show less dispersive nature and are usually non-dispersive (Chandra and James, 1984). In dispersive soils, the repulsive inter-particle forces are higher than attractive forces between the clay particles leading to a weak and unstable soil structure. These soils are highly erodible. Even exposure to a slow flow can easily result in the detachment of the particles from the soil structure and soil internal erosion.

The soil dispersion is a rather complex physicochemical phenomenon influenced by various factors such as clay mineralogy, pore size distribution, and the chemical characteristics of the soil pore fluid (Yong and Warkentin, 1966; Sherard et al., 1976; Yildiz et al., 1999; Penner and Lagaly, 2001). The presence of sodium ions leads to an

increase in the double layer thickness of clay particles and consequently, the attractive forces between particles is reduced. The main reason behind the dispersivity of such soils is the electrical repulsive forces between the grains, which conquer the attractive van der Waals forces. In general, the concentration, valence, and size of ions are factors of importance in soil dispersion (Yong and Warkentin, 1966). Therefore, a large amount of sodium ions in the pore fluid as well as active minerals such as montmorillonite contribute to the soil dispersive behaviour (Holmgren and Flanagan, 1977).

Erosion of dispersive soils threatens many earth dams, hydraulic structures and roadway embankments. Dispersion-induced erosion can result in piping processes in earth dams, which are responsible for 37% of the failure of earth dams worldwide (Goodarzi and Salimi, 2015). The cost of repairing major earth structures is substantial and therefore considerable attention has been paid to various methods of soil treatment for dispersive soils. Replacement of the dispersive soils with another soil is the simplest engineering technique to prevent problems associated with the presence of dispersive soils. However, this method has limitations with regard to the economic aspects of the projects, and thus in many cases, soil treatment has been considered as a favourable option. To date, several methods have been employed to stabilize dispersive soils such as stabilization using aluminium sulphate (Bourdeau and Imaizumi, 1977; Ouhadi and Goodarzi, 2006), pozzolan (Vakili

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et al., 2012), lime (McDaniel and Decker, 1979), fly ash (Indraratna et al., 1991), electroosmotic current (Sadrekarimi, 1998), cement and asphalt emulsion (Rahimi and Fakour, 1996), and organic polymeric material (Polyvinyl Alcohol, PVA) (Bolouri-Bazaz and Saghafy, 2003).

Environmentally friendly soil stabilization methods have recently received much attention. Feasibility of replacing chemical soil improvement techniques by methods inspired and/or induced by natural processes in soils has been explored by various researchers (DeJong et al., 2013). One of such bio-mediated soil treatment techniques is microbially induced calcite precipitation. *Bacillus sphaericus* is one of the most used strains for microbially induced calcite precipitation (MICP) (Van Tittelboom et al., 2010; Van Paassen, 2011). In this process, the bacteria decompose urea into ammonia and carbon dioxide and prepare the environment for the formation of calcite. Calcium is usually provided by calcium chloride, which is used usually as a precipitating agent (secondary ingredient) along with the bacteria. While the use of microbially induced calcite precipitation has been explored for various geotechnical engineering problems such as dust aggregation (Bang et al., 2011; Stabnikov et al., 2013), mitigating liquefaction (e.g., Montoya et al., 2013), treating expansive soils (Sajadi et al., 2014), and stabilizing collapsible soils (Sisakht et al., 2015), to the authors' knowledge, its use for the stabilization of the dispersive soils has not yet been comprehensively studied. The only study in the literature in this area is that of the authors (Moravej et al., 2015) where feasibility of stabilizing a dispersive soil with a biological technique was studied, however, several factors which influence the stabilization of a dispersive soil through such a technique had not been looked into.

Due to the complex bio-chemical processes involved in biological stabilization, a comprehensive study inclusive of macro-scale and micro-scale observations, exploring various influential factors such as precipitating agent concentration, bacterial concentration and curing time is essential prior to the field scale application of the method. This study aims to investigate the effect of MICP on mechanical, chemical and physical properties of dispersive soils. For this purpose, various techniques have been employed such as Atterberg limits, pH measurement, X-ray powder diffraction (XRD), scanning electron microscope (SEM), and pinhole tests to look into the effects of this treatment on soil basic properties, pore fluid chemistry, soil microstructure and dispersibility potential, respectively. These experiments have been performed on the laboratory-made dispersive soil sample with and without treatment with *Bacillus sphaericus*. In order to investigate the effect of treatment on dispersivity under various treatment conditions, several samples have been prepared and tested and the effect of curing time, bacterial concentration and precipitating agent (calcium chloride) has been carefully examined. The analysis of pore fluid chemistry (pH test) and the use of mineralogical and microstructural studies (XRD and SEM) elucidate the governing mechanisms, which can contribute to the efficacy of the method at different soil environmental and practical conditions.

2. Material and methods

The soil basic properties are presented in Table 1. The soil samples were prepared by soil passing sieve no. 100. Following method A of ASTM 4647 (the pinhole test standard), the soil sample was identified as ND2 level in dispersivity classification which means it was non-dispersive (Standard ASTM, 2006). To make it dispersive, 3% sodium hexametaphosphate was added to the soil sample resulting in high dispersivity of the soil sample (type D1 according to ASTM 4647). The reason to make a laboratory-prepared synthetic sample is the fact that pore fluid chemistry, and soil mineralogical characteristics are important factors affecting the soil dispersivity. Further to that, we are going to study a very complex phenomenon which is the interaction of a calcite precipitating microorganism and a dispersive soil where the effects of mineralogy, soil-water-microorganism interaction and all biogeochemical processes are of major influence. Thus, in order to be able

Table 1
Soil properties.

Unified classification	CL
Maximum dry density(kN/m ³)	16.5
Optimum water content (%)	20.7
LL	42
PL	23
PI	19
G _s	2.72
Finer than sieve number 200 (%)	95
Finer than 2 μm (%)	43

to prepare controlled samples with known composition and mineralogy and to examine the effect of biological treatment in a rather precise manner, laboratory-prepared dispersive samples were employed. Moreover, the use of laboratory made dispersive samples has also been preferred in the previous studies on dispersive soils such as Ouhadi and Goodarzi (2006) and Goodarzi and Salimi (2015).

2.1. Sample and bacterial solution preparation

Bacillus sphaericus PTCC¹ 1487 (CIP S25 001) was employed to stabilize the soil sample. Lyophilized vials of bacteria, required for the experiments, were obtained from Iranian Research Organization for Science and Technology (IROST). The ingredients of the culture medium, utilized for cultivation of bacteria, are presented in Table 2. The culture medium was prepared by autoclaving (120 °C) the sodium bicarbonate, nutrient broth, and ammonium chloride in 1 l volumes distilled water, cooled, and supplemented with filter-sterilized (0.22 μm, Jet Biofil) urea. The initial pH was 7.8 but increased to 8.0 after urea addition.

Bacillus sphaericus was cultured through the activation of lyophilized bacteria. After inoculation, the medium was incubated at 28.5 °C with shaking (190 rpm). The organism was grown to late exponential/early stationary phase before harvest, and stored at 4 °C no longer than 48 h prior to use. It should be noted that whole culturing process was performed under sterile conditions. *Bacillus sphaericus* is a Gram-positive strain (Miteva et al., 1990). Thus, to make bacteria more visible and ensure that cultures had not been infected by other types of Gram-negative microorganisms, the Gram stain method was used.

To determine the bacterial concentration in the solution, **optical density (OD) measurement technique was used**. In this technique, OD is employed as an indicator and a measure of bacterial concentration. To determine OD, visible light passes through the suspension of microorganisms and is scattered where greater scatter indicates higher bacterial concentration.

The optical densities of the bacterial suspensions were determined immediately before their use in the experiments. For this purpose, an optical spectrometer (UV/VIS Spectrophotometer SP-3000 Plus) was used for measuring the optical density (OD₆₀₀) of the solution, i.e., the wavelength of spectrometer is 600 nm. The bacterial suspension with desired OD₆₀₀ was mixed with the soil along with the precipitating agent (Calcium chloride) dissolved in distilled water. In all experiments, the same volume of precipitating agent (Calcium chloride) as that of culture medium was used.

In order to explore the effect of bacterial solution and precipitating agent concentration, different scenarios were considered, details of which are explained in the next sections. Furthermore, to prepare and compact the soil sample, the Harvard miniature compaction apparatus was employed.

¹ Persian Type Culture Collection

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