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Review

Electro-Biogrouting and Its Challenges

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Biogrouting is a new method to stabilize sandy soils through precipitation of $CaCO_3$ between grains. This process decreases the permeability about 98% and increases the soil strength up to 12Mpa. It is created by bacterium *Bacillus Pasteurii* and enzyme urease that hydrolyze urea to carbonic dioxide and ammonia. A major problem in biogrouting is the distribution of bacteria injected into the soils. The bacteria grows more in at 30-37°C and pH 9.2, but cannot be distributed homogeneously in soil through biogrouting technique. It depends on the grain size, mineralogy, and properties of the pore fluid. The electrokinetic (EK) technique transports charged particles and fluid in porous media. This technology moves a wide range of particles, including ions, metals and organics. The *Bacillus* bacteria are rod-shaped bacterium with many negative charges in the surface. The electrokinetic can probably transport bacteria towards the cathode in an experimental cell and can distribute them uniformly in porous media of soils for fertile biogrouting. It explains a wide range of diffusion of bacteria influenced electric current. However, basic environment electrokinentic phenomena can probably affect bacterial membrane composition and metabolic activity, but it also justifies an increase of soil pH and can provide a positive effect on microbial activity and bacterial community of *Bacillus Pasteurii.* Furthermore distribution of the urease enzyme could be possible in electrokinetic environment since the urease enzyme has a negative charge at pH of more than 5.5. The urease enzyme is a negatively charged at a pH more than 5.5 which is able to move and diffuse in electrical environment.

Keywords: Biogrouting, Bacillus Pasteurii, Urease enzyme, Electrokinetic

1. INTRODUCTION

Grouting is a method to improve ground in many geotechnical engineering applications, where soils have potential for deformation or fluid flow. Biogrouting is a new method to improve soils based on microbiologically induced precipitation of carbonate calcium. In particular, methods are being

developed using microorganisms which are able to increase strength and stiffness of granular soils by inducing the precipitation of calcium carbonate (Whiffin 2004; De Jong et al., 2006, 2010; Whiffin et al., 2007; Ivanov & Chu 2008). Most studies on biological grouting (biogrouting) use microorganisms containing the enzyme urease and, in particular, the bacterium *Sporosarcina Pasteurii* (DSM 33, renamed from *Bacillus Pasteurii*) (Whiffin 2004; De Jong et al. 2006., Whiffin et al., 2007). This process makes induced carbonate calcium precipitation due to increase soil strength of soils.

$$CO(NH_2)_2+2H_2O \rightarrow 2NH4^{+}+ CO_3^{-2} \quad (1)$$

$$Ca^{+2}+CO_3^{-2} \rightarrow CaCO_{3\downarrow} \quad (2)$$

These bacteria (*Bacillus Pasteurii*) impact on the precipitation $CaCO_3$ by the production of urease enzyme. The enzyme hydrolyzes urea to CO_2 and ammonia, resulting in an increase of the pH and carbonate concentration. In the other hand, microbial activity induced $CaCO_3$ precipitation on concrete has been indicated (Ramachandran et al., 2001). Bacillus is a rod-shaped, gram-positive soil bacterium that secretes numerous enzymes to degrade a variety of substrates, enabling the bacterium to survive in a continuously changing environment. (Westers et al., 2004). A large number of Bacillus species are participated the$CaCO_3$ in the environment the urease enzyme. In addition, bacterial net cell surface charge is negative and absorbs cations (for example Ca^{+2}) from the environment to deposit on the cell surface. (Achal et al., 2010). Because the bacterial cell surface has many negative charges, if $Ca^{+2}$is added first without urea, there will be enough time for Ca^{+2} to be attached to the bacterial surface and the bacteria activity will be greatly influenced and retarded (Chunxiang et al., 2009).

The bacteria are growing at 37°C and facultative alkaliphile which grows optimally at pH 9.2 and relatively high amounts of $NH4^{+}$ (Wiley & Stokes, 1962&1963) or urea (Larson & Kallio, 1954, Bornside & Kallio, 1956). The bacteria should have high negative zeta-potential, ureolytic ability, alkalophilic (optimum growth rate occurs at pH around 9, and no growth at all around pH 6.5 (Dick et al., 2006, De Muynck et al., 2007) to rise adhesion, and produce urease enzyme in the presence of high concentrations of ammonium (Kaltwasser et al., 1972, Friedrich and Magasanik, 1977) to promote rate of $CaCO_3$ precipitation and ureolysis (Nemati and Voordouw, 2003).

In bacterium cells, calcium concentrations are high in extracellular compared to intracellular in a normal $CaCO_3$ precipitation environment and low extracellular compared to intracellular proton concentrations (as a result of alkaline pH regimes). The combination of an extracellular alkaline pH and calcium ions present an unavoidable stressful environment for bacteria: because of process $Ca^{+2}/2H^{+}$ electrochemical gradients, the passive calcium rush, and will lead to intracellular calcium build-up and excessive proton expulsion (Hammes & Verstraete, 2002). At the cellular level, this event could be detrimental due to the (1) disruption of intracellular calcium-regulated signal processes, (2) alkalization of intracellular pH, and (3) depletion of the proton pool required for numerous other physiological processes (Norris et al., 1996). The microbiological $CaCO_3$ precipitation began at pH 8 and was completed at 9.0, consolidating 98% of the initial concentration of Ca^{+2}. Calcium carbonate precipitation appeared to be correlated with the growth of B. Pasteurii and was completed within 16 h following inoculation (Stocks-Fischer et al., 1999).

Calcite is produced by *Bacillus Pasteurii* that studied in standard nutrient broth (NB) and Corn steep liquor (CSL). 100 ml of NB and CSL media added with media. 2% urea and 25 mM $CaCl_2$ mixed with 1 mM of overnight grown Bp M-3. The bacteria were grown at 37°C with continuous aeration at 120 rpm. (Achal et al., 2010). The Urease activity was determined for bacterial isolates in NB media containing filter sterilized 2% urea and 25 mM $CaCl_2$ by measuring the amount of ammonia released from urea. One unit of Urease is defined as the amount of enzyme hydrolyzing one micro mole urea per minute (Achal & Pan, 2011).

2. BIOGROUTING AND ITS CHALLENGES

The feasibility of biogrouting is as a ground improvement technique for sandy soils. The crystals of calcium carbonate precipitate in presence of dissolved calcium, which form bridges between the sand grains and hence increase stiffness and strength up to 12 Mpa. (Van Paassen et al., 2010). These methods significantly reduce the permeability of the strengthened soil, which hinders groundwater flow and limits long distance injection, making large scale treatment unfeasible. Biological techniques (biogrouting) can provide the solution (Whiffin, 2004; DeJong et al., 2006; Ivanov and Chu, 2008).

By injecting specific groups of micro-organisms into the soil, in combination with substrates, precipitation of inorganic minerals is induced at the desired location. These minerals connect the existing sand grains, thereby increasing the strength of the material. The product has similar properties as natural sandstone and it remains permeable, thereby enabling large-scale applications (Van Paassen et al., 2010). The microbial grouting decreased the permeability after two injections about 98%. Enzymatic formation of $CaCO_3$ in situ be present at an effective method for reducing the permeability of porous media.(Nemati &Voordouw, 2003). The attachment of bacteria depends on many factors, including grain size distribution, mineralogy, properties of the pore fluid and of the bacteria themselves (Scholl et al., 1990; Torkzaban et al., 2008).

Transport of bacteria is limited in fine grained soils. As bacteria have a typical size of 0.5 to 3μm, they cannot be moved through silt or clay soils, nor induce carbonate precipitation (Mitchell & Santamarina, 2005). Also in fine sands or coarser materials bioclogging could occur when bacteria are adsorbed or strained by the solid grains, which could result in limited treatment distance for ground reinforcement purposes (Van Paassen et al., 2010).

There is an important problem with biogrouting, the limited dispersion of bacteria injected into soils. Bacteria often stick fast to solid surfaces. In the absence of a strong hydro geological gradient the organisms remain localized at the origin of injection, resulting in fouling of wells and inadequate dispersion of bacteria. The direct transportation of bacteria from injection wells to other zones would be advantageous to augmentation approaches used for in-situ remediation. (DeFlaun & Condee, 1997). It makes a heterogeneous diffusion of $CaCO_3$ which are less close to injection points.

The lack of CaCO3 close to the injection points could be the result of a higher flow velocity, causing more bacterial flush out and hence lower activity and less $CaCO_3$. Another explanation for the lack of $CaCO_3$ around the injection points, considers the kinetics of $CaCO_3$ precipitation and transport

of crystals. Initially the crystals are still small or not even present if the solution is not yet sufficiently oversaturated that nucleation has taken place, which is likely in quartz sand (Söhnel 1992; Lioliou et al., 2007), that they are still easily transported through the pores. Once flow velocity drops or crystals become bigger, they are more easily trapped in the narrow pores. (Van Paassen et al., 2010).

However, the control and predictability of the in situ distribution of bacterial activity and reagents and the resulting distribution of $CaCO_3$ and related engineering properties in the subsurface are not yet sufficient and form the greatest challenge for further optimization, especially if biogrouting is applied in an open system (Van Paassen et al., 2009). Further research should demonstrate what mechanisms are responsible for the observed heterogeneity in the deposition of carbonate and consequent geotechnical parameters and what are the implications of this heterogeneity for the designed purpose (Van Paassen et al., 2010). Indeed, the great challenge is to establish homogeneous strengthening over larger soil volumes. The relation between heterogeneity and flow direction might also prove beneficiary, as it supposes that the direction of heterogeneity is controllable by changing the injection and extraction well positions. Instead of horizontal layers, vertical walls might be constructed if flow is induced from top to bottom (Van Paassen et al., 2009).

There has been some controversy about improvement of biogroting method by *B. Pasteurii*. These concepts are going to find new ways in using of microbial technique in soil. In this way, the combination of different materials can be applicable For example; Polyurethane (PU) foam was used to immobilize the whole cell of *Bacillus Pasteurii.* The immobilized cells exhibited the rates of calcite, precipitation and ammonia production. Microbiologically induced calcite remains intact in the PU matrix mainly because of the high pH of concrete, at which the solubility of $CaCO_3$ is extremely low. Even though immobilization has reduced the enzymatic activity and, consequently, the rate of calcite precipitation, the overall performance of *Bacillus Pasteurii* in calcite precipitation appears equally effective whether they are immobilized or not (Bang et al, 2001).

3. ELECTROKINETIC TECHNIQUES

The electrokinetic (EK) technique is defined as a physicochemical transport of charge, action of charged particles, and effects of applied electric potentials on formation and fluid transport in porous media with a minimal disruption of soils (Acar and Alshawabkeh, 1993; Barker et al., 2004). The technique has been employed for dewatering, consolidation, stabilization (Casagrande, 1949, Asavadorndeja and Glawe, 2005), and contaminant removal of crystalline mineral soils (Weng and Yuan, 2001, Han & et al., 2004, Kim et al., 2005, Lee et al., 2007; Castillo et al., 2008, Fernandez et al., 2009). The literature reports that the application of EK to a soil results in changes in soil pH due to electrolysis reactions, water electrolysis reactions between the electrodes, and migration of ions towards the electrode of the opposite sign (Acar et al., 1990; Acar and Alshawabkeh, 1993; Mitchell and Soga, 2005; Asavadorndeja and Glawe, 2005; Asadi et al, 2009). Electrokinetic stabilization is a ground improvement method which treats soils without excavation, an advantage over traditional methods. In recent years, several electrokinetic experimental studies have been conducted to find out the feasibility of these techniques on different soils (Asadi et al., 2010). The electrical potential of

particles in EK area is detected by zeta potential (ζ). The value of ζ is less than the surface potential of particle and represents the value at the slip plane, which is located at a small unknown distance from the colloidal surface (Asadi et al., 2011).

4. PROBABLE TRANSPORTATION OF BACTERIA USING ELECTROKINETICS

Electrochemical remediation can remove potentially a wide range of pollutant materials as an in situ treatment technology from the subsurface, including both organics and metals (Lageman et.al, 1989, Trombly, 1994, Bruell et al., 1992). This same technology can use to move microorganisms through soil. This application would be different for electrochemical remediation from others in that it is an in situ destruction technology and the organism being transported are strains of bacteria capable of degrading organic contaminants in the groundwater and adsorbed to aquifer solids (Ensley & DeFlaun, 1996). The attachment of bacteria on a solid matrix is affected by the characteristics of bacterial and mineral surfaces and the characteristics of fluid phase in porous media, e.g., flow rate and solution chemistry such as pH and ionic strength. The attachment appeared to be a major control of the extent of bacterial movement (Harvey et al, 1991). The main advantage of EK usage is that this process can be performed in situ and for soil with low permeability. Recently, the application of EK has extended to site infected with hydrophobic organic compound (HOC) that has low mobility, low solubility, low volatility, and low degradability (Park et al., 2007, Saichek and Reddy, 2003). There have been some studies to combine EK and bioremediation (EK-bioremediation) to improve the movement of bacteria for active biodegradation (DeFlaun and Condee, 1997, Schmidt et al., 2007, Shi & et al., 2008a; Wick et al., 2004). These studies has shown, the transport of bacteria was detected but there were no any data on the influence of electric current on soil microorganism (Kim et al., 2010). The Electrokinetics applies to transport any organic materials that adsorb to solids particles (DeFlaun & Condee, 1997).

The effect of electric current on microbial activity and viability was studied (Luo et al., 2005, Cang et al., 2009, Tiehm et al., 2009, Shi et al., 2007).There are a wide range of changes in, bioavailability, physico-chemical properties and toxic electrode-effect of bacteria because of applying electric current which can be depended on the amperage, treatment period, cell type, and medium (Wick et al., 2007). Many of researches suggest that bacteria able to endure environmental stress and can be impossible in EK-bioremediation. In addition, although electric current can influence on membrane composition of bacteria and metabolic activity, many studies indicated that weak DC does not have negative effect on microbial viability (Lohner and Tiehm, 2009, Shi et al., 2008c, Tiehm et al., 2009).

The direct electric current and soil pH are the main factors that make changes in microbial activity. EK remediation decreases soil microbial number by changing soil pH, but the direct electric current increases biodegradation of hydrocarbons and soil enzyme activity. This indicates that the combination of electrokinetic remediation and bioremediation can be promising by increasing microbial activity. A successful combination of electrokinetics and bioremediation can be achieved by detecting of soil parameters, electrode, electric current, and electrolyte (Kim et al., 2010).

Often, the most bacteria have a net negatively charged surface at high pH values and at low pH a net positive charge due to a number of polymers which carry ionizable groups in the membrane. The experiments indicated at pH7.0 with a net negative surface charge, the unidirectional transport to the anode for all of the bacterial strains was determined (DeFlaun & Condee, 1997).

Previous studies have shown that EK process raised the number of Bacillus (Lear et al., 2004). Bacellius Pasteurii is a negative charges bacterium which demonstrates in high rate of pH (7-9). It will be able to move in an electrochemical environment. The applied current produces hydrogen ions (H^+) at the anode and hydroxyl ions (OH^-) at the cathode, with a resulting pH gradient (Acar and Alshawabkeh, 1993). It can cause to increase of PH in cathode (more than 9) and a great reduction in anode (less than 3) during process. Because of being negative charges on the surface of bacteria and strongly growing in alkaline situation, the Bacillus bacteria can move in electrokinetic process. In the other hand, the movement of bacteria towards the cathode in electrokinetic cell are made by direct impact of electrokinetics on the microbial community, and may have caused further distribute of bacteria in prose media of soils.

The soil pH change by electrokinetics reduced microbial cell number and microbial diversity. Especially the number of culturable bacteria decreased significantly and only Bacillus and strains in Bacillales were found as culturable bacteria.

The use of EDTA (a complex agent that use to enhance the transport of contaminants in EK remediation) as an electrolyte seemed to have detrimental effects on the soil microbial activity, particularly in the soil near the cathode. On the other hand, the soil dehydrogenises activity was enhanced close to the anode and the analysis of microbial community structure showed the increase of several microbial populations after Electrokinetics (Kim et al., 2010).

5. TRANSPORTATION OF UREASE ENZYME

The urease enzyme is negatively charged at a pH value over its isoelectric point (pH = 5.5) (Kuralay et al., 2005). The structure of *Bacillus Pasteurii* urease with the two Ni ions is bridged by the carboxylate group of the carbamylated. The two Ni ions in the active site are separated by a distance of 3.53 Å (Benini et al., 2000).

The dependence of urease adsorption on NaCl concentration suggested that the enzyme is bound to the carrier gel through electrostatic interactions. The immobilized enzyme showed increased stability, with respect to the free enzyme, with increasing time or temperature, and in the presence of photolytic enzymes. The pH activity profile revealed that the adsorbed enzyme showed no change in the optimum pH: (8.0), but it was more active than the free form in the pH range 5-8 (Ciurli et al., 1996). These enzymes are absorbed by soil colloids (Karaca et al., 2000). It seems that because of electrolytic features of urease enzyme, it can be diffused in electrokinitic cell. The urease is an extracellular enzyme; it depends on conditions of soil parameters, electric current. Furthermore the type of electrolytes should be considered.

6. CONCLUSIONS

Biogrouting is a new method to precipitation of $CaCO_3$ in sandy soils by microbial activity for improving strength. The *Bacillus Pasteurii* is a kind of bacteria with urease enzyme which hydrolyzes ammonia and produce Ca^{+2}. In a solution of $CaCl_2$, the crystals of $CaCO_3$ are created between particles of soil. Biological grouting has many challenges to operate. First, it could not be utilized homogeneously because of a higher flow velocity and distribution of the bacteria. Second, limited dispersion of the bacteria injected into the fine soils because of small prose and big size of the bacteria.

Electrokinetic is an applicable technique to transport of charged particles and fluid in an electric potential. EK demonstrate changes in soil pH due to electrolysis reactions, water flow between the electrodes, and migrate ions towards the electrode. Application of electrokinetics can have positive effect to transport and distribute microorganisms for biogrouting in soils. When the elecrokinetic hydrolyses hydrogen and hydroxyl ions from water, a transportation of charged particles to anode and cathode are produced. It led to make acidity in anode and alkalinity in cathode. However, changing acidity and alkalinity in two side of experimental cell may be intensive, but it can be effective for growing bacteria in alkaline situation. In addition, the bacellius bacterium has negative charge with a tendency toward to move the cathode. Electrokinetic technique is able to distribute charged particles, metals and most of microorganisms in soil. Considerably, EK will probably use for distribution of bacteria homogeneously in soil for biogrouting.

It is confirmed that the direct electric current and soil pH in EK method can increase the ability and microbial activity. Therefore, the Bacillus action should be same as well as other microorganisms. At the high pH value, the microbial activity and rate of bacterial population of these bacteria are increased. It seems that Bacillus can transport in EK area in high rate of growing.

Transport of bacteria is limited in clay and silt soils because of grain size. This limitation led to disuse of Bacillus for biogrouting in fine soils. Even so, it appears able to be done with transportation of Urease enzyme in fine soils by EK method. The Urease have negative charged which can diffuse with electric potential. Indeed, produced urease should mix with ammonia and, transport in fine soils by electric method. Finally, the calcium chloride solution adds as injection process. This method can make induced carbonate precipitation (CaCO3) for improving in soil. It can operate in fine soils like clay, silt and peat which do not have ability transit in many of microorganisms and bacteria.

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