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Review on Biogeotechnics for Soil Treatment

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Abstract- Biogeotechnics is a new and growing branch in geotechnical engineering that deals with the utilization of biomaterials that are easily available in the environment and microbial processes to enhance the properties of the soil. This technique addresses various problems such as poor soil properties, liquefaction and so forth and solves them in a sustainable manner which is cost effective and eco-friendly. Biomaterials such as biochar and natural fibers are reinforced with soil to improve the engineering properties of the soil as it is a part of sustainable geotechnics. This paper mainly focuses on microbial activities carried out to treat the soil. One of the most commonly adopted treatment methods is microbially induced calcite precipitation (MICP). In this method, bacteria injected into the soil undergo chemical reaction and produce calcium carbonate ($CaCO_3$) precipitation at the contact points of soil grain. This process is applied to improve strength and stability of soil; reduction in permeability; and liquefaction mitigation. This review paper clearly discusses about history, process, methodology, applications and limitations of MICP via ureolysis. MICP via denitrification is also discussed.

Keywords: Natural fibers; Biocementation; Microbially Induced Calcite Precipitation (MICP); Ureolysis

1. Introduction

Geotechnical engineers face many problems regarding soil in order to achieve the requirement at that location. These problems include strength improvement, controlling the flow of water and maintaining the hydraulic conductivity, improving the durability, liquefaction mitigation, etc. Some traditional methods which are used to overcome these problems include soil replacement, compaction of soil, grouting for bonding of soil grains, dewatering and pre-compression, structurally reinforcing the foundations and deep foundations. The issues with these traditional methods are, they are costly, more complex, requires more energy or emits greenhouse gases and pollutes the environment. Production of cement alone imparts 5-7% of the total CO_2 emission. As a result, researchers started to work on to find new methods of ground improvement techniques which is sustainable and cause less damage to the environment. This review discusses the usage of bio-materials such as biochar, biopolymers and natural fibers, their composition and its results. Vegetation growth in steep slope and embankment can improve the slope stability as well as environmentally beneficial. Research in "Biogeotechnics" also includes microbial activity in geotechnical engineering, microbially induced carbonate precipitation (MICP) through ureolysis and denitrification. In MICP through ureolysis, urease activity is carried out with the help of ureolytic bacteria. This gained the attention of many researchers over the past decade.

2. Application of Microbial Process

According to present-day research, there are more than 2×109 prokaryotes at the surface of one gram of soil, decreasing to 2×108 prokaryotes at 1–8m depth level. There is still an ample of prokaryotes between 10-300m, with an average estimate of 2.3×107 cells/cm3. This number, however, decreases to 6×106 cells/cm3 between 300 and 500m. Relatively, there are an estimated 6400 to 830000 bacterial species in one gram of soil. Despite the growing diversity and density of microorganisms in soil, their presences have largely been overlooked by geotechnical analysts.

2.1 Natural Microbial Process

The core objective of this review is to analyze the application of the natural microbial process to further efficiency in ground engineering. Hence, it is the responsibility of geotechnical engineers to comprehend the complex mechanisms behind microbial activity. It is, hence, important to understand the function of microorganisms in formation of soil and its structure. To do so, we can analyze various case studies and research papers that investigate the positive and negative effects which have been assigned to microbial action. The role of microbes in soil is very important as they direct the occurrence of stable and easily alterable group of nutrients such as carbon (C), nitrogen (N), etc.. Due to global warming, soil is under major threat; therefore, there is a massive requirement to generate plans for sustainable conservation of soil for hereafter. The information obtained from soil chronosequence can help in recognize the components that operate the formation of soil. Engineered microbial activity is a process in which Microbial Induced Calcite Precipitation (MICP) is carried out. Precipitation process is executed either by MICP via ureolysis or MICP via denitrification.

2.1.1 Function of Microbes in Formation of Soil and its Structure

While many researchers perceive soil as a sterile medium, that is simply not the case. Such a view undermines the importance of microorganisms, giving improper regard to the essential features they perform. Mineralization, for example, is highly dependent on the microorganism within the soil. The process is defined as a phenomenon where the solution's metal cations associate itself with the charged groups on the cell surface. This, as a result, causes nucleation and precipitation, which are also indicators of the final mineral phase. The existence of microbes facilitates the formation of clay, even at low-nutrient and high salinity environments.

2.2 Bioclogging

Chemical grouting is a method in which liquid grouts are used to plug in the soil voids. Solutions of acrylamides, acrylates, polyurethanes and sodium sulphates are some the familiar grouts. Also, biopolymers which are hydrophobic gel like materials obtained from industries such as chitosan, sodium alginate, poly-hyroxybutyrate, xantan and polyglutamic acid are utilized as grouts for liquefaction mitigation, soil remediation and restrain soil erosion.

Collection of organic substances and increasing amount of microorganisms in the ground is termed as "bioclogging", which can reduce the soil permeability. Bioclogging could be difficult specifically in voids, filter and in geosynthetics. Recently, engineers have appraised that bioclogging could be advantageous in certain practices where hydraulic conductivity has to be reduced by improving the growth of microorganisms in the laboratory.

Microbial fabrication of hydrophobic polysaccharides in site is one of the methods of bioclogging. Even though, many hydrophobic gel like microbial polysaccharides are obtained from industries, these are very costly, and hence, they are not used for grouting. Therefore, hydrophobic microbial slimes which are obtained from cheap raw materials are used. Microbes are applied along with element that commences bioclogging. Properties of soil in site are altered by obtaining bacterial exopolymers. This has been used for oil recovery (bioremediation of soil).

Table 1: Bioclogging using various microbes

Microbial class	Method of clogging	Circumstances for clogging	Application in geotechnical engineering
Iron reducing bacteria	Production of ferrous solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil.	Presence of ferric minerals.	Averts piping of earthen dams and dykes.
Oligotrophic bacteria (bacteria which have the ability to grow under less nutrient environment)	Production of slime in soil.	Low concentration of oxygen and carbon.	Controls seepage and lowers soil erosion in drainage channels.
Algae and cyanobacteria (bacteria which gets energy via photosynthesis)	Thick impermeable layer of of biomass is produced for clogging.	Availability of light and nutrients.	Controls seepage and lessens the intrusion of water in slopes.
Algae and facultative anaerobic heterotrophic slime producing bacteria.	Production of slime in soil.	Presence of oxygen and medium with ratio of C:N > 20	Forms cover to improve soil erosion control and protects slope.

2.2.1 Reduction of soil permeability

Bioclogging method is used to reduce the permeability of soil in dams and dykes, it is also used to decrease infiltration and leakage in landfills, also to avert soil erosion and retrieval of oil from reservoir. In soil which has poor drainage, organic waste substance such as manure is added which leads to decomposition during the absence of oxygen and forms oxygen free condition. This comes along with reduction of Fe and reduction in hydraulic conductivity of soil. When ground water is recharged artificially by using surface water or effluents obtained from waste water treatment plant, then exopolysaccharides (EPS) is produced by oligotrophic bacteria which produces a layer of clogging in organic soil [1].

2.2.2 Limitations of Bioclogging

- The stability of soil is deprived due to usage of polysaccharides in site.
- Microbial EPS are debased by many microbes present in soil. Hence, notable bioclogging is feasible under soil which is convenient for microbes that produce EPS, and not convenient for microbes that debase EPS.
- Slow growth rate of microbes such as oligotrophic and nitrifying bacteria is a limitation while applying, therefore, they produce EPS at low rate and it is a long term process for the microbes to clog the soil. The growth rate of bacteria is around 1% to 10% per day, so it might take 69 to 690 days for the microorganisms to multiply 1000 times of its initial content.
- Infiltration of microbial cells in soil is restricted due to bioclogging as the pore size is between 0.5 & 2 µm, hence, this process is constrained for soil with preferable permeability.
- As bioclogging takes place in soil, by-products such as nitrate and organic acid are produced due to nitrification and fermentation, which must be discarded from the soil or should be transformed to neutral products like CO_2, N_2 gases and water.

2.3 Bio-cementation

In chemical cementation, chemical grouts are used to bind soil particles to transform them into sandstone like formation to improve their load carrying capacity. This technique is very common in civil engineering applications. Some chemicals that are used in this process shall comprise Na2SiO3, CaCl2, Ca(OH)2, acrylamytes, acrylates, cement and polyurates.

Similarly, microbial cementation or biocementation is a process to form bond with soil fragments using the intrusion of microbes and other additives into the soil. The microorganisms crystallizes calcium, aluminium, magnesium, manganese and iron into hydroxides, sulphates, phosphates, carbonates and silicates. Biocementation is less harmful and relatively cheap when compared to chemical cementation. Table 1 shows the cost analysis for chemical and microbial grouting. It can be seen that the cost of raw materials for chemical grouting ($2 to $72 per m^3) which is much higher than the cost of raw materials for biogrouting ($0.5 to $9 per m^3). The cost shown in table 1 is the cost of raw material at market alone, it can increase for processing and application of those raw materials.

Table 2: Cost of Raw materials

Process	Material	Price ($/kg)	Quantity required (kg/m^3)	Overall cost ($/m^3)
Chemical grouting	Lignosulphites	0.1-.03	20-60	2-18
	Phenoplasts	0.5-1.5	5-10	2.5-15
	Acrylates	1-3	5-10	5-30
	Acrylamides	1-3	5-10	5-30
	Polyurethanes	5-10	1-5	5-50
	Sodium Silicate	0.6-1.8	10-40	6-72
Microbial grouting	Organic waste and Microbes	0.05-0.1	10-20	0.5-2
	Food waste and Microbes	0.05-0.1	10-20	0.5-2
	Molasses and Microbes	0.1-0.2	5-20	0.5-4
	Iron Ore and Microbes	0.1-0.2	10-20	0.1-4
	$CaCl_2$, Urea and Microbes	0.2-0.3	20-30	4-9

3. MICP through Ureolysis

3.1 Process

MICP is a usual biogeo-chemical process, which can be achieved through various microbial methods including photosynthesis, urea hydrolysis, methane oxidation, removal of nitrogenous compounds, ammonification, and depletion of sulphate. Till date, most of the studies regarding MICP have made use of ureolytic bacteria because they require comparatively short time to precipitate CaCO3, large amount of calcium carbonate can be precipitation because of good solubility of particles [2]. MICP through ureolysis depends on bacteria that hydrolyze urea into ammonia and carbonic acid (Eq. 1). Then, ammonium ions are processed, due to the formation of hydroxyl ions, the pH surrounding the bacteria cell is increased (Eq. 2). Due to rise in pH, carbonic acid (H2CO3) is transformed to biocarbonate ions (HCO3-) (Eq. 3), eventually forming carbonate ions. Calcium ions present in the solution interact with the cells of bacteria and rises the pH, due to which calcium carbonate is precipitated more rapidly [3].

$$CO(NH_2)_2 + 3H_2O \rightarrow 2NH_4^+ + HCO_3^- + OH^- \quad (1)$$
$$HCO_3^- + H_2O + OH^- \leftrightarrow CO_3^{2-} + 2H_2O \quad (2)$$
$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3(\downarrow) \quad (3)$$

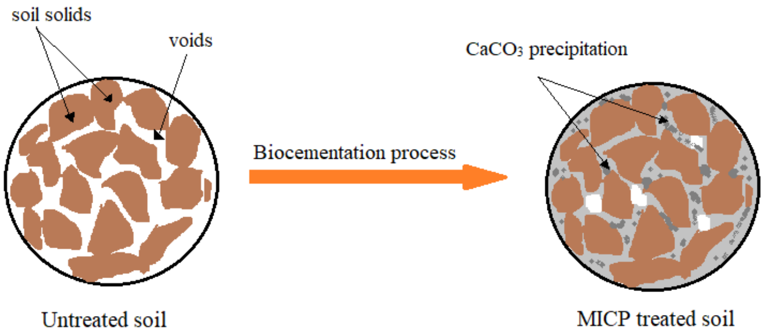

Figure 2: Biocementation process in soil

3.2 Application in Soil Mechanics and Geotechnics

3.2.1 Stabilization of soil

The use of microbially induced calcite precipitation through ureolysis has been extensively studied for stabilizing the soil, for its potential to ameliorate stiffness and strength mostly in porous soils. Loose fine sand has been transformed into a cemented sand/sandstone by treating it with MICP via ureolysis. The unconfined compressive strength (UCS) has been improved three times the initial magnitude after treating it with MICP [4] and some cases even four times [5]. It has also been proposed for reducing the settlement and improving liquefaction resistance. Improvement of sandstone through MICP has been shown in Fig. 2.

Figure 3: MICP treatment before and after

3.2.2 Erosion resistance

Soil erosion has been reduced by forming a dense coating of calcium carbonate at the surface of the soil which is against shear stress intruded by air or liquids such as water, thus it safeguards the soil beneath it. In Fig. 3, it can be seen that white CaCO3 is precipitated at the top surface which forms a impermeable erosion resistant layer which extends 10 millimeters (approx.) into the soil media. Many studies were carried out to test the application of MICP in slopes to observe soil erosion by wind and water, all the studies exhibited improved erosion resistance. In case of scouring, even though the soil treated with MICP near the pier exhibited improved erosion resistance, but due to scrubbing of neighboring untreated soil, the pier is yet in the risk of erosion [6].

Figure 4: Surface treatment using MICP

3.2.3 Sealing Fractures in Rock

MICP treatment in sealing the fractures found in rock was not that effective as it was in soil stabilization. El Mountassir et al. (2014) studied the path taken by the microbes for various velocities in a rock fracture, they found that different path was preferred for various velocities; they thought it is because of the shear stress on the surface of the fracture which is more than the electrostatic attraction. Therefore, they altered (decreased) the discharge rate to seal entire fracture [7].

3.2.4 Hydraulic Conductivity Depletion in Porous Media

Precipitation of CaCO3 takes place at the junctions where the soil grain touch one another, due to precipitation the pore size in soil media is decreased, thus the permeability is decreased. Studies say that treating sand with MICP has reduced the permeability around 90-100% of the original value [8]. Likewise, to decrease the hydraulic conductivity in permeable rocks, it is treated with microbially induced calcite precipitation. Even though permeability reduction is the main objective, uniform dispersion of calcite crystals are more preferred, because non uniform dispersion of calcite may form precipitations here and there, which may lead to blockage especially near the insertion point.

3.3 Management Guidelines and Insertion technique

Filling porous soil and cracked rocks using MICP is much complicated and far different from ordinary cement grouting. Many studies have been underwent to study the best possible and suitable way of application of MICP in order to achieve the requirements such as strength enhancement, reduction in permeability of the soil and various other treatment based on the condition of the proposed medium. Various management limits and insertion techniques that may alter the precipitation process are discussed below.

3.3.1 Reagents

Sometimes due to high permeability, the bacterial solution penetrates beyond the required area. To overcome this, fixatives such as Calcium chloride is introduced after bacteria insertion, this makes the bacteria to form small clumps within the required portion of the ground, so that more number of bacteria settle there and higher precipitation is carried out. If more amount of calcium is inserted then the precipitation is much quicker.

pH has great influence on the duration of calcium carbonate precipitation, to delay the process pH of the solution is reduced. Precipitation occurs very close to the place where insertion of the microbes takes place; therefore the pH is reduced so that more amounts of bacteria is introduced before blockage due to precipitation takes place. Thus, the entire area can be treated properly [7], [9].

The rate of ureolysis is handled by urease activity, which relies on the quantity of enzymes present in the solution. During the treatment of MICP, many researchers kept all other parameters fixed and determined that specimen handled with a slower urease activity showed better result. Studies have been made correlating strength and calcium carbonate content in various conditions [4], [5]. The rate of urea hydrolysis can be slowed by decreasing the temperature and reducing the urea content. Fig. 4 shows the result with respect to urease activity, it is clear that for lower activity the performance have been good enough. When all the variables are kept constant and for given calcium carbonate content, more strength is achieved at slow urease activity.

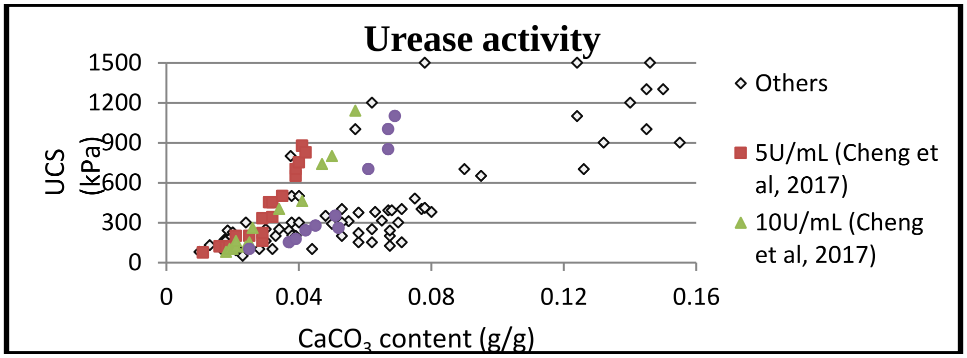

Figure 5: Relationship between $CaCO_3$ content and UCS based on urease activity [5], [10]–[14]

3.3.2 Properties of Flow

The flow property of the fluid is highly affected due to the precipitation calcium carbonate. When the precipitation starts, the pore size of the porous media is reduced due to which the bacteria injected is forced into a narrow space. As a result, the bacteria stick to the soil grain surface. It may also if the attractive force between the bacteria and soil grain is higher than repulsive force. Preferential flow paths are formed when the rate of application is not varied [7]. When the flow rate is steady and due to precipitation at some area, the permeability within the media decreases, as a result the velocity within the remaining area increases. The flow velocity is high till the shear force becomes very large for the bacteria to get trapped in. It suggested that in order to achieve bacterial attachment at desired location (i.e., where precipitation can take place) the velocity of the flow is adjusted [9]. In radial flow method, numerous injections are performed with maintaining constant pressure on the contrary to constant flow or reducing the rate of flow for successive injections. This is proved to supply bacteria over a wide area so that it can gradually block the fracture or voids in the medium.

3.3.3 Medium

Studies have described that if the rate of urea hydrolysis is increased, the precipitation of $CaCO_3$ takes place faster after the calcite accumulation, thus the bacteria stick to the surface of soil grain [7], [15]. Moreover, the energy needed for the formation of crystal from the solution is generally more than the energy needed for the growth of the crystal.

Table 3: Concentration of Chemical used for Bacterial growth and Cementation

	Chemicals used	Concentration
Bacterial Growth Medium	Diazanium sulphate	10 g/L
	Tris Buffer	0.13 M/L
	Yeast	20 g/L
Cementation Medium	Ammonium chloride	10 g/L
	Calcium chloride	111 g/L
	Urea	90 g/L
	Sodium hydrogen carbonate	2.12 g/L
	Nutrient Broth	3 g/L
	Polyvinyl acetate	7.5% w/w

Degree of Saturation: In lab, when study of modification of permeability is of regard during MICP tests then the tests are usually conducted under a state where water is fully saturated. Anyhow, tests that or were performed under unsaturated condition [11], or carried out in situ where saturation conditions cannot be managed [16] frequently reveal more calcium carbonate conveyance and higher depth of treatment. Saturation state has a big impact on the CaCO3. Lower saturation state gathers bacteria and reagents at where the inter-particle touches, thus it improves the strength. Fig. 6 clearly shows that when the saturation rate is lower, then the UCS is higher for a given amount of CaCO3 content.

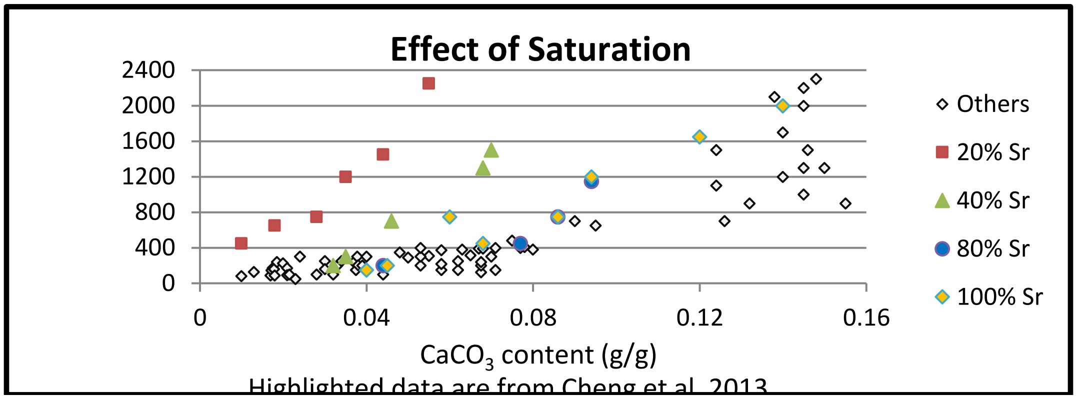

Figure 6: Relationship between $CaCO_3$ content and UCS with respect to Saturation [10]–[14]

Soil structure: Initial dry density plays an important role in the correlation between UCS and calcium carbonate content. Fig. 7 demonstrates that a sample with higher initial dry density requires less amount of $CaCO_3$ content to achieve a specified strength (UCS) when compared to the same material at lesser initial dry density. Whereas for sample with same amount calcium carbonate content is precipitated, then higher sample (UCS) is obtained when the sample is compacted to have greater dry density. Both fine sand and medium sand has been tested to find the changes in property due to variation in density of the sample. It was observed that the UCS value was comparatively higher for particular calcium carbonate content in medium sand when compared to that of fine sand [13].

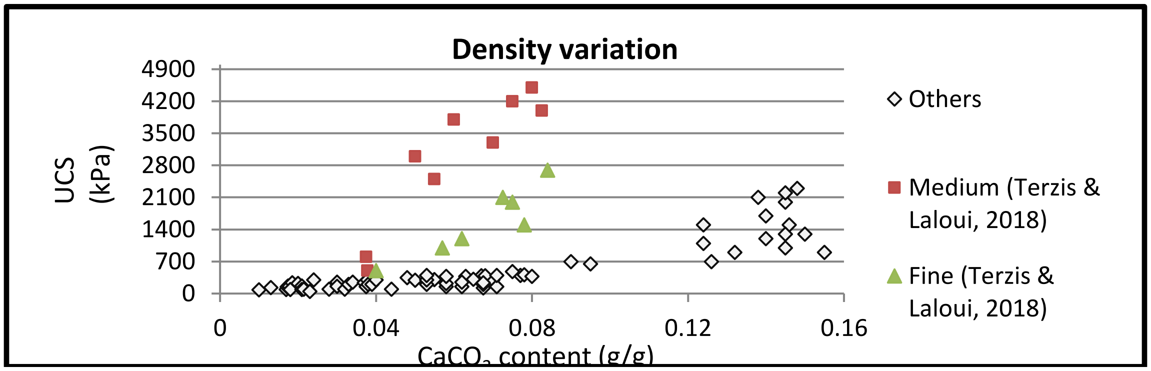

Figure 7: Relationship between $CaCO_3$ content and UCS based on initial dry density [5], [11]–[13]

3.3.4 Environmental Conditions

S. pasteurii is a bacterium which grows in the presence of air and requires oxygen, hence, when this bacterial is used for treatment of ground, availability of oxygen in the site hugely affects the progress. Tobler et al (2011) found that the bacteria grown in the presence of oxygen when inserted into the groundwater with or without oxygen did not produce in ammonia accumulation [17]. Martin et al. (2012) described that when oxygen availability is limited or there is no oxygen supply then the bacteria may not grow but urease activity may undergo [18]. This states that it might be difficult for the growth of local ureolytic bacteria in ground when oxygen is not sufficient.

Increase in temperature increases the rate of urea hydrolysis, Van Paassen (2009) observed that the rate of urea hydrolysis has multiplied twice for every 8°C increase in temperature, between the ranges of 5°C and 79°C[19]. Urea hydrolysis is carried out by urease enzyme, however increase in temperature may deprive the activity of the enzymes.

3.4 Challenges and Disadvantages

By-products: Ammonia which is an odorless gas generally derived from NH_4Cl is a chief by-product of MICP via urea hydrolysis. It pollutes groundwater, causes algal blooms at high concentration and it is toxic to aquatic organism. To attain maintenance acceptance, the by-products (produced during ureolysis) had to be removed from a different hole which was bored, the removal was carried out to extract ammonia produced in the ground. The rate of removal was five times that of injection.

Homogeneity: In treatment using MICP, consistency or uniformity of the treatment is always a problem. Since bacteria is being absorbedby the soil grains and possibility of restriction in its movement, the concentration of cells are more near the injection point. Due to less viscosity of the solution, bacteria flow in their favored direction which causes non-uniformity in the process. Some methods to enhance the homogeneity of the treatment are listed below

- To delay the process of ureolysis and calcium carbonate precipitation, the pH of urea or calcium chloride solution is lowered (generally 6-6.5).
- Bacteria can be injected in multiple cycles with reduced concentration of the reagent, so that more bacteria is dispersed in every cycle leading to the treatment of various areas of the subject media.
- Using radial injection the velocity of solution in the locality has been increased, thus reducing the possibility of bacterial attachment.

Tracking and Monitoring: Keeping an eye of where and what range the process has taken place in difficult in ground treatment using MICP. Even in ground enhancement with cement grouting, it is difficult to supervise the progression. Since, cement grouting is done over a decade there is sufficient empirical knowledge in supervising, unlike MICP which is a new method. At laboratory monitoring can be done by measuring some of its characteristics such as shear wave velocity, alteration in weight, X-ray depletion and hydraulic conductivity. At field level monitoring, some of the old techniques like ultra sound, seismic survey and electrical resistivity are carried out.

Upscaling: Huge upscaling should be done for MICP to move on from field and lab test to an engineering application. Since calcium chloride salt is available in large quantity and urea is produced in huge quantity as fertilizer, production of binding solution should not cause issue. Raw materials (urea and $CaCl_2$) can be conveyed in desiccated form and can be blended to required concentration at the work place.

4. MICP through Denitrification

4.1 Process

While MICP through ureolysis is mostly used for a scale of engineering exercises, calcium carbonate can be precipitated using several other methods, of which MICP through denitrification is pondered to be hopeful [2]. Denitrification is directly taken place in the ground due to nitrogen cycle, which oxidized the organic substances to inorganic carbon and nitrate is converted to nitrogen gas (N_2). The conversion of nitrate (NO_3^-) to nitrogen gas (N_2) faces many chemical processes in-between, in which with the help of certain enzymes the intermediate nitrogen compounds such as nitrite (NO_2^-), nitrous oxide (N_2O), and nitric oxide (NO) are evolved. These intermediate gases and by-products must be evaded as they are harmful and a source of greenhouse gases. In order to reduce these intermediates proper layer of composition is essential. For MICP via denitrification, calcium acetate and calcium nitrate were used in substrate in many researches.

$$C_2O_3H_2^- + 1.6NO_3^- + 0.4H_20 \rightarrow 0.8N_2 + 2HCO_3^- + 0.6OH^- \qquad (4)$$
$$1.21C_2H_3O_2^- + 0.97NO_3^- + 0.17H_2O \rightarrow CH_{1.8}O_{0.5}N_{0.2} + 1.41HCO_3^- + 0.76OH^- \qquad (5)$$
$$Ca_2^+ + HCO_3^- \rightarrow CaCO_3(\downarrow) + H^+ \qquad (6)$$

Due to reduction of nitrate, alkalinity is built up, which is utilized by $CaCO_3$ and it restricts the change in pH. Microbial growth is enhanced by maintaining a constant pH and the intermediates which contain toxic substances are not gathered [20].

O’Donnell (2016) described that more than a familiar denitrifying bacteria, a group of several microorganisms in the ground was much fruitful [21].

4.2 Mechanical Behavior and Applications

Alike MICP by ureolysis, here the permeability of soil can be reduced by sealing the voids (pores) by $CaCO_3$ precipitation. As a result of this the soil is strengthened and the stiffness is improved. Calcite crystals grow at the contact points of the soil particles (as shown in Fig. 11). O’Donnell et al. (2017) stated that 1%-2% (by mass) of $CaCO_3$ precipitation is enough to improve CSS (cyclic shear strength) by 40% in cyclic direct shear tests on laboratory and field sands [21]. O’Donnell (2016) found that shear stiffness of the soil is found to be improved in sand dealt with denitrification when compared to ureolysis. In MICP via denitrification, the $CaCO_3$ is precipitated in slow rate, thus bigger calcite crystals are formed.

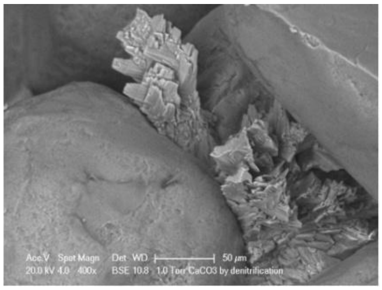

Figure 8: Calcite crystals precipitated between soil grains

4.3 Advantages and Disadvantages

In many conditions, MICP through ureolysis requires laboratory cultivation and only then bacteria can be injected. MICP via denitrification does not require any laboratory cultivation, unlike ureolysis process. The solution will act with the native bacteria which can process denitrification. Also, if nitrate is totally processed to nitrogen gas, then no by-product is left in this process. Precipitation of $CaCO_3$ delays in denitrification process. 1%-9% $CaCO_3$ precipitation by mass takes place in a 100 day period of time (approximately) if the solution is cycled continuously [2]. When, high concentration of solution is injected at low rate then the process may be obstructed by the intermediates which are toxic. Therefore, the process of denitrification can be ameliorated by injecting low concentration solution at low rate, thus less amount of bigger crystals are formed.

Table 4: A summary of advantages and limitations for various microbial pathways

Microbial Pathway	Relative Advantage	Limitation
Urea Hydrolysis	1) Efficiency of chemical transformation is very high (upto 90%). 2) Comparatively faster and easy process.	1) High amount of Ammonium ion is released. 2) Bacterial growth requires ex-situ cultivation.
Denitrification	1) If nitrate is completely reduced to nitrogen gas, then no by-product is produced. 2) Ex-situ cultivation of bacteria is not required. 3) Lower concentration of reagents are preferred.	1) Rate of $CaCO_3$ precipitation is slow. 2) Initially nitrate ion is produced which hinders the growth of bacteria.
Sulphate Reduction	1) It is an aerobic process.	1) Hydrogen Sulfide gas is produced which is extremely toxic.

		2) Large amount of bacteria is required.

5. Future Considerations and Conclusion

Biogeotechnics is one of the most recent branches in geotechnical engineering. Many researches are being done in this topic since a decade. In MICP process, there are many gaps since it is a very new method in biogeotechnics. Though so many researches is being carried out in recent days, still there is a lot of gaps which has to be studied in future to obtain a best suitable method for every requirement.

As we have discussed above, there are many limitations and challenges, most of the MICP processes are not uniform while treating, and hence it has to be studied such that uniformity is achieved. Although some adjustments have been made to improve the uniformity, still proper uniformity was not achieved. Modeling the processes should be done using data such that the action of MICP can be predicted. Studies should be made to monitor the process after application of MICP. However, studies can be made for several parameters and variables which can be adjusted based on the field requirement.

MICP process through ureolysis produces many by-products which are serious environmental pollutants, its treatment require high cost and sometimes untreatable, and hence studies should be made to minimize the formation of by-products. However, in MICP through denitrification, the by-products are avoided. Most of the experiments are conducted in laboratory in a controlled environment, but in-situ analyses must also be done to understand the behavior in field. In laboratory, small scale treatment is done, so studies must be done on upscaling. Despite being advantageous, denitrification process is generally not opted for large scale applications since nitrate used as a substrate is costly, and hence alternate methods must be determined for easy availability of nitrates at cheaper price. Flow and movement of medium in the soil must be studied further, as they are affected by various factor in a particular environmental condition, which affects the efficiency of the process.

Future scope must be carried on various applications of MICP, not only enhancing the strength and stability of the soil, but also in various other processes such as self healing property of the soil. Further studies must be carried on usage of alternative fixates such as cuttlebone, eggshells, etc. which are rich in calcium ions. Also, treatment of MICP should also be conducted on materials like bricks and concrete, not just soil alone, to determine the effectiveness of the treatment, so that this field of study can be further extended.

Till date, MICP through ureolysis is the process which attracts the engineers and more widely used. Worldwide many researches are being carried out to improve the method of application and injection strategy. MICP through denitrification has shown promising results while being a slow process. The main motive of MICP process is to treat the soil in a sustainable manner and to make it a cost-effective process. More studies must be concentrated on both laboratory and in situ levels. In order to fully explore this topic and gain more knowledge collaboration between geotechnical engineers and microbiologists is essential.

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