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Ureolytic Bacteria and Calcium Carbonate Formation as a Mechanism of Strength Enhancement of Sand

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ABSTRACT

Ureolytic bacteria have a great involvement in calcium carbonate precipitation for different applications starting from removing contaminants and surface coating of monumental estuaries to strengthening and improving the mechanical properties of sandy materials. Ureolytic bacteria hydrolyze urea to generate ATP by the efflux of ammonium ions through ATP-synthase, producing carbonate. In the presence of calcium ions in excess, calcium carbonate will precipitate. In-situ calcium carbonate precipitation is known as biocementation or BioGrout which is superior to the chemical cemented sandy materials in terms of resistance of weathering factors, conservation of permeability and insignificant decrease in porosity. The presented review emphasizes on bacterial calcium carbonate precipitation for the purpose of strength formation.

***Keywords*: urease; Bacillus; calcium carbonate; BioGrout; biocementation**

1. Introduction

Calcium carbonate ($CaCO_3$) is one of the most common minerals on earth. Its precipitation is a common phenomenon, forms natural rocks and exists in environments such as marine water, fresh water, and soils (Ehrlich, 1998; Castanier et al., 1999). It forms oolitic, fossiliferous and considerable limestone in the sediments. The increase in the concentration or decrease in the solubility of the calcium or carbonate in solution causes the natural precipitation of $CaCO_3$. Abiotic change (e.g. evaporation or changes in temperature or pressure) or biotic action (microbial action) participates in the natural precipitation of $CaCO_3$. The biotic contribution in $CaCO_3$ precipitation exceeds the abiotic in most environments on earth (Castanier et al., 2000). The rate of microbiological $CaCO_3$ precipitation correlated with cell growth. Stocks-Fischer et al. (1999) have found that this microbial rate of precipitation was significantly faster than that of chemical precipitation.

Hammes and Verstraete (2002) suggested that the chemical $CaCO_3$ precipitation is controlled by the calcium ions concentration, carbonate concentration, pH and presence of nucleation sites.

In microbial $CaCO_3$ precipitation, the presence of nucleation site is not a key factor for $CaCO_3$ precipitation because the bacteria themselves behave as nucleation sites (Stocks-Fischer et al., 1999).

Bacterial $CaCO_3$ precipitation is a biomineralization process. Biomineralization is a biologically mediated process leading to mineral nucleation and growth of mineral products (Tang & Dove, 1997). Lowenstam and Weiner (1989) and Mann et al. (1989) have defined two modes of biomineralization: biologically induced biomineralization (BIB) and boundary organized biomineralization (BOB). In BIB, an organism changes its local microenvironment providing suitable conditions for the chemical precipitation of minerals thus biominerals are not directly associated with cellular structures. Whereas in BOB, an organism produces organic matrix within or on which inorganic particles are grown, thus a nucleation intracellularly or on the cell wall is observed during BOB.

2. Types and Morphology of Calcium Carbonate Crystals

Calcite, aragonite and vaterite are three crystal forms of $CaCO_3$ nucleation (same chemical formula, different structure). Calcite is considered as the most stable form of $CaCO_3$, with simple rhombohedral shape (De Yoreo & Vekilov, 2003). Its formation is favoured by the presence of magnesium, manganese ions and orthophosphate. Furthermore, crystal aging supports calcite precipitation (Wray & Daniels, 1957).

Aragonite is a metastable $CaCO_3$ form and changes into calcite over geologic time. It is an orthorhombic crystal and obtained at high temperature (Wray & Daniels, 1957; Tai & Chen 1998) or low temperature in the presence of magnesium ions and pH less than 11 (Tai & Chen, 1998). Its formation is favoured by the presence of magnesium and manganese ions but not orthophosphate (Tai & Chen, 1998).

Vaterite is rarely found in nature (Sanchez-Moral, 2003). It is produced in the pH range from 8.5 to 10 with the initial relative supersaturation between 6.5 and 8.5 (Kralj et al., 1990), low Ca^{2+} concentration (Yagi et al., 1984), or low temperature and high Ca^{2+} concentration (Roques & Girou, 1974). The vaterite morphology is influenced highly by the pH and temperature. Three vaterite crystal types are known: Spherulite (at pH < 9.3 and below room temp), hexagonal-plate (at pH 9.6), and lettuce (at pH 8.5 and temperature of 40^oC) (Kralj et al., 1990).

3. The Role of Urease Activity in Calcium Carbonate Precipitation

3.1 Urease

Urease was the first enzyme to be isolated in its crystalline form from *Canavalia ensiformis* (jack bean) (Sumner, 1926). The first three-dimensional structural model of urease was observed by X-ray of a bacterial source, Klebsiella aerogenes (Jabri, 1995). Kl. aerogenes urease consists of four domains, one of which contains an active site with a bimetallic Nickel center. These primary structures of Kl. aerogenes and Sporosarcina pasteurii (formerly known as Bacillus pasteurii) urease are identical (Mulrooney & Hausinger, 1990).

Urease is synthesized under condition of nitrogen starvation (Mobley et al., 1995). Mobley and his colleagues (1995) found that urease level is increased 20 to 25-fold when Bacillus subtilis cells were grown in nitrogen poor medium. There are contradictory statements regarding the location of urease in the bacterial cells. Reithel (1971) stated that urease is a cytoplasmic protein whereas Mclean et al. (1986) found that the urease is located in the membrane and periplasm of Staphylococcus sp. and Protus mirabilis.

3.1.1 Urease Reaction Mechanism

Urea is released into the environment due to biological action, for example, all mammals excrete urea as a detoxification product. Urease (urea amidohydrolase; EC 3.5.1.5) is widely distributed in soil and aquatic environments. Biotic urease activity is widespread in the environment and includes the action of bacteria, yeasts, filamentous fungi (Mobley & Hausinger, 1989), algae (Yates & Robbins, 1999), and a number of higher plants including jack beans (Canvalia ensiformis), soybean leaf and seed (Glycine max), pigweed (Chenopodium album) and mulberry leaf (Morus alba) (Sirko & Brodzik, 2000).

Urease hydrolyses the substrate urea generating ammonia and carbamate (Eq. 1). Carbamate spontaneously decomposes to produce another molecule of ammonia and carbonic acid (Eq. 2) (Mobley & Hausinger, 1989). The two ammonia molecules and carbonic acid subsequently equilibrate in water with their deprotonated and protonated forms, resulting in an increase in the pH (Eq. 3 and Eq. 4) (Mobley & Hausinger, 1989).

$$CO(NH_2)^2 + H_2O \xrightarrow{\text{Urease}} NH_3 + CO(NH_2)OH \quad (1)$$

$$CO(NH_2)OH + H_2O \longrightarrow NH_3 + H_2CO_3 \quad (2)$$

$$H_2CO_3 \longleftrightarrow HCO_3 + H^+ \quad (3)$$

$$2NH_3 + 2H_2O \longrightarrow 2NH_4^+ + 2OH \quad (4)$$

3.1.2 Effect of Minerals on Urease Activity

Different effects of minerals on urease activity have been proposed (Smith et al., 1993; Bachmeier et al., 2002; Hammes et al., 2003a). Some of the studies showed contradictory effect of these minerals on urease activity. The presence of Ni^{2+} ions in the active site of the urease is essential for the functional activity as well as the structural integrity of the enzyme. Bachmeier and her colleagues (2002) have shown an increase of $CaCO_3$ precipitation by the addition of Ni^{2+} ions in the growth medium of E. coli (pBU11). They claimed that the $CaCO_3$ precipitation rate was increased dramatically by the addition of Ni^{2+} ions (5–100 μM), exhibiting the highest rate in the presence of 5 μM. Similarly, urease production was optimized by the addition of 10 μM Ni^{2+} ions to the growth medium (Al-Thawadi, 2008). This role of Ni^{2+} ions on urease activity was confirmed by Mobley et al. (1995) who claimed all forms of purified urease required Ni^{2+}

ions. Contradictorily, the presence of additional Ni^{2+} ions proved not to increase $CaCO_3$ precipitation by S. pasteurii in another study (Bachmeier et al., 2002).

In addition to Ni^{2+} ions, activity of urease was enhanced by, but not dependent on— the presence of Na_2EDTA, DL-dithiothreitol (0.1-5 mM), Ca^{2+}, Ba^{2+} and citrate (2-20 mM) (Smith et al., 1993). The effect of Ca^{2+} on urease activity was confirmed by another study which showed that the urease activity increased up to 10-folds in the presence of 30 mM Ca^{2+} ions compared to the activity in the absence of Ca^{2+} for certain isolates (Hammes et al., 2003b). Contradictorily, the presence of Na^+, K^+, Cl^-, Br^-, acetate or nitrate (2-20 mM) did not affect the urease activity; however the presence of Li^+, Mg^{2+}, Zn^{2+} or I^- caused a decrease in urease activity (Smith et al., 1993).

3.2 Energy of Ureolytic Bacteria

Several microorganisms produced ATP through urea hydrolysis (Mobley & Hausinger, 1989). In S. pasteurii, for example, the generation of ATP is coupled with urea hydrolysis, and specifically NH_4^+ production (Fig.1). The proton motive force (or electrochemical potential) (Δp) controls the generation of ATP, according to Eq.5. The pH gradient (the difference between the pH inside and outside the cells) is symbolized by ΔpH while $\Delta\psi$ is the membrane potential or charge gradient.

$$\Delta p = \Delta pH + \Delta\psi \quad (5)$$

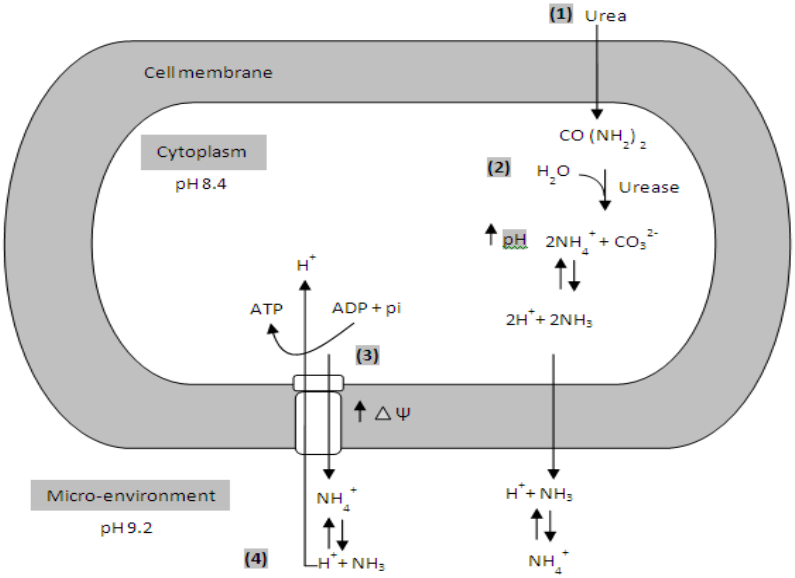

Fig.1. ATP-generating system coupled with ammonium during urea hydrolysis in S. pasteurii

Fig.1 shows the ATP-generating system coupled with ammonium during urea hydrolysis in S. pasteurii as discussed by Jahns (1996) and Whiffin (2004). The reactions are: (1) Urea diffusion into the bacterial cell according to the concentration gradient; (2) Hydrolysis of urea resulted in an increase in the cytoplasmic alkalinity (NH_3/NH_4^+) leading to the decrease in ΔpH;

(3) Ammonium diffusion outside the cells according to the concentration gradient; resulting in increased the membrane potential ($\Delta\psi$); (4) The increase in the membrane potential drives in the protons against the concentration gradient.

The generated ATP in neutrophilic organisms (organisms that prefer to grow in a neutral medium) depends on proton concentration gradient. The cells expel outside the protons from the electron transport chain, causing an increase in the concentration of protons (low inside/high outside). The protons then will be moved back into the cell according to the concentration gradient through the ATP-synthase; resulting in ATP production (Prescott et al., 1993).

For alkalophiles (organisms that grow optimally in high pH conditions), the condition is different than neutrophils. The pH outside the cell is high (low protons), so the protons suppose to diffuse from inside the cell down their concentration gradient (high inside/low outside) (Fig. 1). To solve this problem, the alkalophiles develop two mechanisms to drive the protons back into the cells as they need the protons to generate ATP. These mechanisms are increasing the pH inside the cells causing alkalinity of the cytoplasm (i.e. reducing ΔpH) and increasing the $\Delta\psi$ by the efflux of a cation (NH_4^+) via ATP synthase rather than H^+. Due to the increase in the charge separation across the membrane ($\Delta\psi$) the proton motive force drives back the protons into the cell against the concentration gradient.

3.3 Ureolytic Bacteria and Cementation Reaction

Bacterial $CaCO_3$ precipitation under appropriate conditions is a general phenomenon (Bouquet et al., 19973). There are a number of species of $CaCO_3$ minerals associated with bacteria, for example vaterite formation by Acinobacter sp. (Sanchez-Moral et al., 2003), aragonitic sherulites by Deleyahlophila (Rivadeneyra et al., 1996), calcite by E. coli (Bachmeier et al., 2002) and magnesium calcite spherulites and dumbbells by the slime-producing bacteria, Myxococcus xanthus (González-Muñoz et al., 2000; Holt et al.,1993). One of the most robust ureolytic bacteria is S. pasteurii. It is an aerobic, spore forming, rod shaped bacterium. It uses urea as an energy source and produces ammonia which increases the pH in the environment and generates carbonate, causing Ca^{2+} and CO_3^{2-} to be precipitated as $CaCO_3$ (Eq. 6- Eq. 8) (Stocks-Fischer, et al., 1999; Kroll, 1990). Alkaline pH is the primary means by which microbes promote calcite precipitation (Castanier et al., 2000a; Fujita et al., 2000). Based on various studies (Stocks-Fischer et al., 1999; Kroll, 1990; Castanier et al., 2000b; van Lith et al., 2003), a schematic model describing the role of ureolytic bacteria on calcium carbonate precipitation is illustrated in Fig.2.

$$Ca^{2+} + Cell \rightarrow Cell\text{-}Ca^{2+} \quad (6)$$

$$Cl^- + HCO_3^- + NH_3 \leftrightarrow NH_4Cl + CO_3^{2-} \quad (7)$$

$$Cell\text{-}Ca^{2+} + CO_3^{2-} \rightarrow Cell\text{-}CaCO_3 \downarrow \quad (8)$$

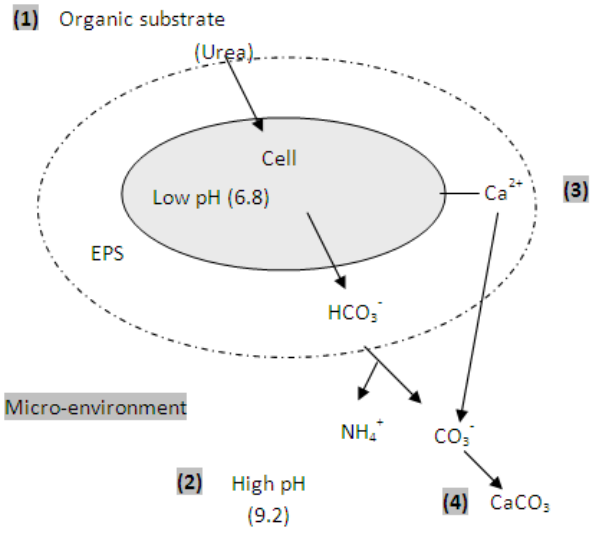

Fig.2. Schematic model

Fig.2. Schematic model based on the literature (stocks-fischer et al., 1999; kroll, 1999; castanier et al., 2000; Van lith et al., 2003) summarizing the role of ureolytic bacteria in $caco_3$ precipitation in the presence of ca^{2+} ions. The processes involved in $caco_3$ precipitation are: (1) hydrolysis of urea (Eq.1- Eq.3), (2) increasing the alkalinity of the micro-environment (Eq. 4), (3) surface adsorption of ca^{2+} ions (Eq.7) and (4) nucleation and crystal growth (Eq.8-Eq.9). Eps stands for extra-polysaccharide in the case of the presence of eps surrounding the ureolytic cells.

There are two metabolic pathways for bacterial carbonate formation. These pathways are autotrophic and heterotrophic pathways (Castanier et al., 2000b). Regarding autotrophic pathway, CO_2 is used as a carbon source causing its depletion in the bacterial environment. In the presence of Ca^{2+} ions, such depletion enhances the production of $CaCO_3$.

Regarding heterotrophic pathways, bacteria can precipitate $CaCO_3$ through active or passive precipitation. In active precipitation, the production of CO_3^{2-} is due to ionic exchange through the cell membrane by calcium and/or magnesium ionic pump. During passive precipitation, the production of CO_3^{2-} is due to ammonification of amino acids, dissimilatory reduction of nitrate, or degradation of urea or uric acid. In all cases, ammonia as a metabolic end product is produced which induces a pH increase.

$CaCO_3$ precipitation rate in general is a linear function of the ion concentration product of (Ca^{2+}) and (CO_3^{2-}) (Longdon et al., 2000) hence obeying 2nd order kinetics or 1st order kinetics if one of the reactants (e.g. calcium) is in excess. Different rate constants have been obtained for bacterial $CaCO_3$ precipitation (Table 1). The microorganisms can influence the attainable saturation and the rate of $CaCO_3$ precipitation, controlling the polymorph of the produced $CaCO_3$ crystals (Bosak et al., 2004; Bosak et al., 2005). When the concentration of Ca^{2+} and CO_3^{2-} exceeds the solubility product (Ksp), supersaturation of solution is reached (Eq. 9). The higher the supersaturation (S) is the more likely precipitation of $CaCO_3$ is to take place. The saturation level (S) of a solution with respect to $CaCO_3$ is defined by:

$$S = \frac{\left[Ca^{2+} \right] \left[CO_3^{2-} \right]}{K_{sp}} \quad (9)$$

Where (Ca^{2+}) and (CO_3^{2-}) represent the concentration of the dissolved Ca^{2+} and CO_3^{2-} respectively and K_{sp} is the calcite solubility product (Table 2).

Table 1: Different rate constant of bacterial urea hydrolysis

Source	Rate constant (d^{-1})	Reference
In agriculture Topsoil(mixed micro-organisms)	0.01 - 0.11	(Nielsen et al., 1998)
In Vadese Zone subsoils (mixed micro-organisms)	0.09 - 1.68	(Swensen & Bakken, 1998)
Sporosarcina pasteurii	0.09 - 0.91	(Ferris et al., 2004)

Table 2 shows the biocementation conditions which are reported in the literature for the production of $CaCO_3$. The purpose of this $CaCO_3$ precipitation was stone formation via CIPS (Calcite *In-situ* Precipitation System), bacterial or plant urease. The loose materials (sand granules) were packed and then injected by combining calcium/urea with the bacterial or plant enzyme. The components of the CIPS solutions were not mentioned.

Table 2: biocementation conditions which are reported in the literature for the production of $CaCO_3$.

Type of reaction	Urea (mM)	Ca (mM)	Urease activity	Maximum Strength(MPa)	Depth of Penetration(mm)	Reference
Bacterial Urease	100-1250	100-1000	11-28 mM urea.min^{-1}	0.6-30	1000	(Al-Thawadi, 2008)
Bacterial & plant urease	1500	1500	4-18 mM urea.min^{-1}	1.8 (3 applications)	170	(Whiffin, 2004)
CIPS	ND	ND	ND	0.679-3.8 (1 application)	179	(Harkes et al., 2010)
Plant urease	200	600	0.3 $g.L^{-1}$	NM	300	(Van Meurs et al., 2006)

ND: Not Defined, Property to Calcite Technology Pty Ltd (Perth, Australia)
NM: Not Measured.

3.4 Applications of Calcium Carbonate Precipitation via Bacterial Urea Hydrolysis

Bacterial $CaCO_3$ formation through urea hydrolysis is known as bacterial calcite precipitation (BCP). BCP is highly desirable because it is natural and pollutant free. There are several applications for BCP, most of which considered for purposes other than strength development. Some of these applications are: (1) Removal of contaminants (e.g. radio-active pollutants) and calcium ions from groundwater and wastewaters; (2) Protection and restoration of limestone monuments and statuary; (3) Creation of sacrificial patinas on limestone and production of biological mortars or cements; (4) Plugging the pores of the oil-recover reservoir rock and (5) Stone formation. Below are more details of these applications.

3.4.1 Removal of Contaminants from Groundwater and Calcium Ions from Wastewaters

The capturing of divalent radionucleotide Strontium90 ($90Sr^{2+}$) in the groundwater, was investigated after the addition of high concentration of urea and very low concentration of Ca^{2+} ions (Fujita et al., 2000; Warren et al., 2001). Strontium carbonate ($90SrCO_3$) was precipitated; in such a way that ($90Sr^{2+}$) replaces Ca^{2+} ions in the calcite crystal preventing the spread of radio-nucleotide contamination.

The potential of removing Ca^{2+} ions from industrial wastewaters facilitated by BCP instead of chemical precipitation was studied (Hamames et al., 2003). The presence of a high concentration of calcium ions (500-1500 $mg.L^{-1}$) in the wastewater causes severe scaling in the pipelines and reactors due to calcium formation as carbonate, phosphate and/or gypsum. By the addition of a low concentration of urea (0-16 $g.L^{-1}$), up to 90% of the calcium ions were removed from the examined wastewater.

3.4.2 Protection, Restoration of Limestone Monuments and Statuary and Creation of Biological Mortars

Physical, biological and chemical factors may cause the weathering of monumental stones. Construction materials, For example, are exposed to Cl^{-}, SO_4^{2-}, CO_2 and atmospheric moisture which react with the surface layer or penetrate inside the materials (Chunxiang et al., 2009). Consequently, a loss of cohesion of stone material, progressive mineral matrix dissolution and micro-cracks formation will be enhanced (Chunxiang et al., 2009; Tiano et al., 1999). In the case of calcareous stones, the porosity will increase due to $CaCO_3$ leaching and weakening of the superficial structure of the stone (Tiano et al., 1999). The attempt which was done by Tiano (1999) in stopping or slowing down the deterioration of monumental statuary by ureolytic bacteria was successful in surface coating but not strength production, as no significant difference in strength was recorded after $CaCO_3$ precipitation. In a recent study, a new layer of $CaCO_3$ was precipitated on the surface of an old concrete layer by S. pasteurii (Ramakrishnan et al., 2005). It was concluded that cracks remediation may enhance the strength and the durability of the concrete.

Chunxiang and his colleagues (2009) have successfully precipitated calcium carbonate layer (138-289 μm) on cement-based material using S. pasteurii. They have drawn a method to improve the water penetration resistance and resist the attack of the acid (pH >1.5) of the construction material surface. Their method depends on applying calcium first and adding urea one hour later. The researchers recommended working with low urea conversion rate to obtain a compact and hard calcium carbonate layer on the surface of concrete.

Le Metayer-Levrel et al. (1999) have successfully studied bacterial cementation aiming at creation of biological mortars (i.e. mortar is a mixture of cement, water and fine sand forming a paste to fill the gaps between construction blocks and bind them together) and patinas (Anticorrosive Protection of Materials in the Atmosphere) on limestone. Their method depends on spraying the entire surface of limestone with bacteria followed by a nutritional medium containing calcium and urea. A protective surface coating of $CaCO_3$ (several micrometers deep) was formed. They have claimed that calcite particles were precipitated within the bacterial cells and then expelled afterwards. A relatively low penetration depth of 500 μm was reported by immersing the limestone sample in the cementation media (Rodriguez-Navarro et al., 2003). The use of Myxocccus xanthus (a slow growing bacterium) resulted in $CaCO_3$ precipitation at the wall of the porous materials without plugging them. The resistance of mortar specimens and surface deposition to degradation process was improved by bacterial $CaCO_3$ formation (De Muynck et al., 2007).

3.4.3 Plugging the Pores of the Oil-Recover Reservoir Rock

Bacterial cells were used to plug the highly permeable rocks of the oil-reservoir. Between 8 and 30% of the total oil present in oil-reservoir was recovered from ordinary oil production method (Leonard et al., 1986). Oil-recovery depends on primary and secondary treatments (water flood) to recover the crude oil in the pores of the reservoir rock (Bryant et al., 1987). To improve the recovery method after primary and secondary treatments, conventional methods depend on chemical or thermal energy is used. These conventional methods are considered inefficient as they led to 67% retention of the total oil within the pores of the reservoir rock (Bryant et al., 1987). Therefore, there was an interest in the use of microbes to enhance the oil-recovery. This use of microbes can be through microbial production of bio-surfactants and biopolymers at the surface; microbial growth in the pores of the oil-reservoir rocks producing gasses, surfactants and other chemicals; or microbial plugging of the pores in the oil-reservoir channels, which may resulted in increasing the sweeping effectiveness of the recovery process.

The rocks of the oil-reservoir contain high permeability zones. When the water is injected to displace oil, it will move through the pores of the highest permeable zone, bypassing much of the oil. Because of the small size of the bacteria, they will move to high permeable areas, plugging the pores, and as a consequence the sweep efficiency and oil recovery will be enhanced up to 100% (Leonard, 1986; Bryant, 1987; Behlülgil & Mehmetoğlu, 2002; Knapp et al., 1983)

In the ordinary method of bacterial enhancement of oil-recovery, plugging of the pores was due to bacterial multiplication (MacLeod et al., 1988), production of gasses that increases the

pressure (Jack et al., 1982), production of organic acids, surfactants (Zobell, C.E., U.S. Patent 2,413,278, 1946) and polymer (MacLeod et al., 1988). Much attention is devoted to the plugging of highly permeable zone via bacterial urea hydrolysis. This type of plugging probably offers a feasible alternative to block the rock pores; improving the residual oil recovery. Complete plugging within days was achieved by mixing bacteria with sand before packing into cores followed by application of calcium, urea and carbonate (Gollapudi et al., 1995). Moreover, it was found that the bacteria plug the sand granules by providing a nucleation site at which $CaCO_3$ was precipitated through alkaline environment (Stocks-Fischer et al., 1999).

3.4.4 Stone Formation by Bacterial Calcite Precipitation (BioGrout)

In geotechnical engineering, the sandstone is produced by filling the sand voids with chemical grouts in a process called chemical cementation or chemical grouting (Indraratna & Chu, 2005). Chemical cementation depends on chemicals such as sodium silicate, calcium chloride, calcium hydroxide (lime), cement, acrylates, acrylamides and polyurethanes to bind the sand granules (Karol, 2003). Construction materials cemented chemically are subjected to weathering which leads to increase the porosity changing the mechanical features of the cemented materials (Tiano et al., 1999).

Biocementation or BioGrout is a sand consolidation technology, in which ureolytic bacteria release carbonate from urea hydrolysis in the presence of an excess of calcium ions to form calcite ($CaCO_3$) in-situ. Under the proper conditions, this calcite can result in soil solidification and has found significant commercial interest (Whiffin, 2004; Al-Thawadi, 2008).

Biocementation could be greatly enhanced by using microorganisms with high urease enzyme activities indirectly involved in $CaCO_3$ consolidation (Stocks-Fischer et al., 1999). Besides the high urease activity, a high tolerance to urea, calcium, ammonium and either nitrate or chloride (depending on the calcium salt used) enhance the biocementation (Whiffin, 2004). There is a lack of knowledge regarding the high strength production of the biocemented products (sand stone formation). Most of the $CaCO_3$ precipitation studies achieved a consolidation or patching treatments for existing material as described previously. On another hand, there are few studies aimed at the production of sand stone (Table 2). Sandstone production depends on how strong is the binding between the sand particles, which affects the cementation quality of the precipitated calcite.

The growth of $CaCO_3$ crystals for the purposes of artificially cementing sediments proved difficult because of the low yields obtained from a number of different reactions at room temperature. However, the successful bonding of calcareous sediments with derivatives of aluminium alkoide indicates that $CaCO_3$ is a promising route to stabilise loose particles (Koplick, , 1989). Superior to this sediment cementation attempt, the loose particles were well cemented by chemical precipitation of $CaCO_3$ through Calcite In-situ Precipitation System (CIPS), producing high degrees of calcite cementation similar to the natural sediments within less than 24 hours. This CIPS technology (a non-microbial cementation process) is similar to the natural process that forms the rocks (Ismail et al., 2002). This successful rock formation by chemical $CaCO_3$

precipitation indicates a great deal of scope for further work on the strength of microbiological $CaCO_3$ precipitation in a porous medium.

Nemati and Voordouw (2003) have described the use of urease to cement a porous medium. In this study, reducing the permeability of a porous medium by enzymatic $CaCO_3$ precipitation through Canvalia ensiformis was achieved. Nemati and Voordouw used between 0.1 and 1.0 M (>33 $g.L^{-1}$) calcite together with high urease activity for a successful plugging of the sand core. Unfortunately, the strength build-up was not monitored.

The study of Whiffin (2004) was the first published study in bacterial plugging of loose sandy material through urea hydrolysis (BioGrout) for the purpose of strength production. She used P. vulgaris and S. pasteurii during her study. This study was successful in producing strength of 1.8 MPa which was achieved through three applications of bacterial cells and cementation solution. Whiffin (PhD. thesis, Murdoch University, Perth, 2004) has established a biocementation method which depends on a fast flow rate to inject the biocementation mix (bacteria, calcium and urea). The fast flow rate is not recommended to consolidate fine sands. The used cementation solution (calcium/urea) ranged between 0.75 and 1.5 M. In some cases Whiffin has recorded a full precipitation of calcite within 18 hours. Due to the calcite precipitation, which blocks the pores, a low penetration depth (maximum of 170 mm, i.e. penetration depth is the distance along a packed sand column which can be penetrated by bacterial cells and cementation solution to cement the sand granules) was achieved. Beside this low penetration depth, the inconsistency of urease production by P. vulgaris and S. pasteurii are considered to be another problem which has arisen throughout her study.

To our knowledge, a PhD study (Al-Thawadi, S.M., PhD. Thesis, Murdoch University, Perth, 2008) was the first study to use biological cementation to produce high strength comparable to that of the traditional cemented construction materials such as sandstone and concrete with high penetration depth. This study developed a method of producing high strength cemented sandy materials (up to 30 MPa, equivalent to construction cement) through bacterial hydrolysis. This high strength production was achieved without a significant decrease in the permeability. Moreover, high penetration depth up to 1 m was achieved. This study suggested that the mechanical strength enhancement of cemented sandy materials was caused mostly due to the point-to-point contacts of rhombohedral $CaCO_3$ crystals and adjacent sand grains (Fig.3). These bridges will bind the sand granules together and increase strength and stiffness (Al-Thawadi, 2008; Harkes et al., 2010).

4. Advantages of Sand Stone Formation by Ureolytic Bacteria

For consolidation of loose material, it is vital to conserve the permeability of cemented samples so that the water moves through the voids in the stone hindering the deterioration due to water logging by bacterial cementation. Retention of the permeability was evident by the absorbance of water recorded in the biocemented surfaces (Tiano et al., 1999).

The bacterial cells were evident to be reused 2-3 times (Whiffin, 2004) and over 20 times (Al-Thawadi, 2008) in subsequent applications of calcium and urease only. Therefore, reusing

the cells in-situ is a cost saving process as the cost of culturing the cells is not considered. Moreover, consolidation of porous media can be achieved by the direct use of bacterial culture without the need to concentrate the cells or extract urease from the bacteria. Thus, there is no need for additional processing for the bacterial culture to produce sand-stone.

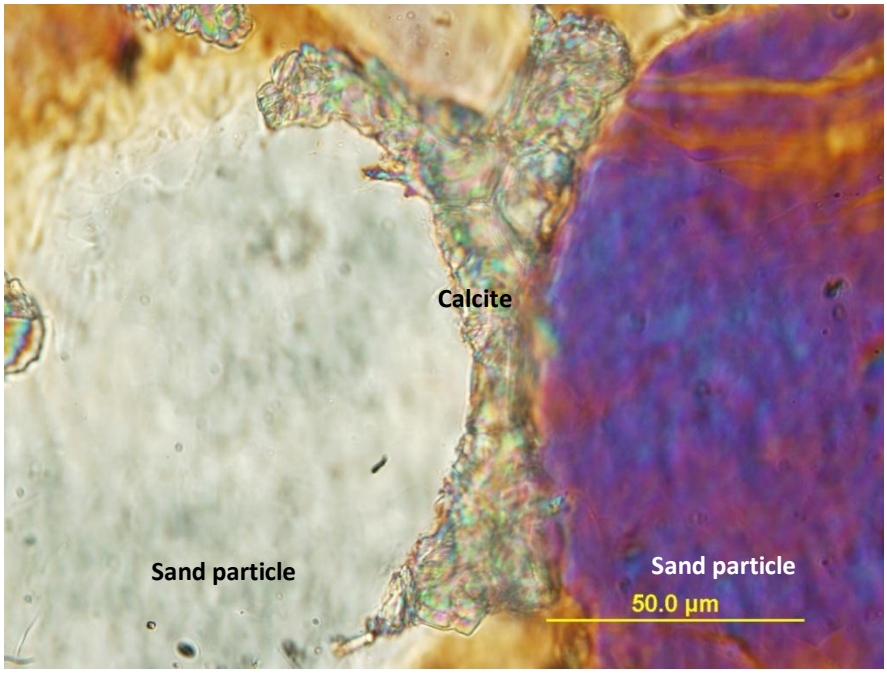

Fig.3. Light microscopic image for Calcite crystals produced by ureolytic bacteria binding two sand particles (Al-Thawadi, 2008)

5. Future Perspective

Calcium carbonate precipitation by ureolytic bacteria has attracted great attention recently. Several patents have been filed in biodeposition (Europe), remediation of concrete (USA), biomineralization (China) and high strength production (Australia). The field application is considered to be one of the main challenges for calcium carbonate precipitation technology; therefore converting the controlled laboratory work into field application is a necessity to get a considerable benefit from those patents.

The biocementation research group at Murdoch University, Australia has patented biocementation technology jointly with CSIRO's Calcite Technology (Kucharski et al., 2006). Deltares (GeoDelft formerly) in the Netherlands showed the interest to apply this technology for soil improvement market. Deltares major interest was to protect their natural dykes. Consequently, Murdoch University, Calcite Technology and Deltares have worked together to apply biocementation technology in so called BioGrout. BioGrout is a process to adapt soil properties (stiffness and strength improvement), together with biosealing (reduction in

permeability by the use of bacteria) form what so called SmartSoils (Van Meurs et al., 2006). This collaboration has unleashed in producing $40m^3$ sandstone within 12 days, reaching unconfined compression strength of 12 MPa (Van Paassen et al., 2009).

Biocementation can form the foundation of a new technology of approaching geotechnical problems. Injecting the bacterial solutions into the soil, ground and porous rocks can build up calcite which binds particles together. So it is a promising technology in ground improving, tunneling, underpinning of houses and so on. Researchers in Deltares, Netherlands are investigating other uses for ureolytic bacteria such as road construction, and hardening the ground beneath buildings that might be exposed to liquefaction during earthquakes.

A geotechnical revolution is unleashed with the help of biocementation. To enable the commercial application of biocementation, a penetration depth of several meters is required. In addition, the possibility of controlling the degree of strength development, low flow rate in injecting the different required solutions and bacteria in situ, low cost especially by enriching the bacteria from local environment, reuse of bacterial cells, relatively homogeneous cementation and retention of permeability are required. All of those criteria are proven to be possible through biocementation technology (Al-Thawadi, 2008).

Although biocementation technology is a promising technology due to its suitability to field application, it also results in an environmental problem due to its high production of high concentration of ammonium. Ammonium which is an end product of urea degradation might leak into the ground water causing environmental problems. To solve this problem, biocementation using other carbon sources such as organic acids instead of urea is recommended as this would avoid the production of ammonium. To avoid the formation of ammonium ions during biocementation reaction, van Paassen and his colleagues (Van Paassen et al., 2008) have used organic acids (acetate, lactate, succinate and malate) as carbon sources, an electron acceptor and a counter ion. To reach 40 Kg Ca^{2+}/m^3, the conversion rate of the desired organic acid was between 38 and 113 days. This conversion rate is too low as compared to the conversion rate of urea (minutes to several hours depending on bacterial urease activity). Therefore, researchers in future should investigate how to increase the conversion rate of the organic acids in order to precipitate calcium carbonate in situ.

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