

Scale up of BioGrout: a biological ground reinforcement method

Agrandissement de BioGrout: méthode biologique pour la consolidation des sols

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ABSTRACT

BioGrout is a new soil improvement method based on microbiologically induced precipitation of calcium carbonate. Bacteria, which are able to convert urea into ammonium and carbonate, are injected in the soil, followed by a solution containing urea and calcium chloride. The produced carbonate precipitates with calcium. The calcium carbonate crystals form bridges between the sand grains, which increases the strength of the sand mass. The remaining ammonium chloride is extracted. In the laboratory, we proved the prospect of BioGrout as soil improvement method with sand column experiments. The next challenge was to establish homogeneous strengthening over larger soil volumes. We evaluated the new technology in a step-wise scale-up approach. First, biogrouting was applied in sand filled boxes of 1 cubic meter, simulating a single point injection. Recently, we used a large scale sand box (100 m³) in which the sand was treated over a distance of 5 m using screens of injection and extraction wells. The tests showed that it was possible to turn sand biologically into sandstone (UCS up to 12 MPa) using conditions and techniques as encountered in practice. Special attention is paid to the distribution of the calcium carbonate content and geomechanical parameters within the grouted sand bodies.

RÉSUMÉ

BioGrout est une méthode nouvelle pour la consolidation des sols basée sur la précipitation de carbonate de calcium causée par des bactéries. Ces bactéries sont capables de convertir l'urée en ammonium et carbonate. Quand sont injectées successivement dans le sol, ces bactéries et une solution contenant du chlorure de calcium et de l'urée, le carbonate produit précipite avec le calcium. Les cristaux de carbonate de calcium soudent les grains de sable entre eux et consolide ainsi les formations sableuses. Le chlorure d'ammonium est extrait au cours du traitement. En laboratoire, la faisabilité de BioGrout en tant que méthode d'aménagement de sols a été prouvée par des expériences sur colonne de sable. Pour déterminer la faisabilité de BioGrout dans des conditions et des techniques d'injection, réalisable à l'échelle, des expériences ont été effectuées dans des conteneurs de 1 m³ remplies de sable, simulant un point d'injection unique et dans un conteneur plus grand de 100 m³ dans lequel la solution était injectée en plusieurs points et extraite à 5 M de distance des points d'injection. Les essais ont prouvé qu'il était possible de transformer biologiquement le sable en grès (UCS jusqu'à 12 MPa) dans des conditions et des techniques semblables à celles utilisées en pratique. Une attention particulière est apportée à la distribution de la teneur en carbonate de calcium et aux paramètres géo mécaniques du sable consolidé.

Keywords: *Sporosarcina pasteurii*, urease, biocementation, ground improvement, permeation, soil stabilization, heterogeneity

1 INTRODUCTION

Soils often do not satisfy functional requirements: roads and railways undergo settlement. Dikes, dunes and slopes can become unstable or are subject to erosion. Earthquakes can cause liquefaction of loose sediments. Water and oil production wells in loosely cemented sediments often produce sand. In land reclamation projects the compaction of the recovered land is often a major concern. Soils stabilization can be desirable for many of these applications.

At the surface, soil stabilization can be achieved by using common constructive approaches like compaction, adding nails or sheets in a slope or mixing soil with lime or cement (Karol 2003). Lately, ecological approaches to prevent soil erosion are getting more attention, like planting trees, grasses and shrubs or stimulate mussel beds (Widdows & Brinsley 2002; Fan & Su 2008). When stabilization of a soil mass is required at depth, these superficial techniques are insufficient and strengthening techniques, like deep mixing, cement or chemical grouting are being used (Karol 2003). However, as the zone of influence of these methods is limited to the proximity of the mixing equipment or due to the high viscosity or short hardening time of the injected grouts, these traditional methods are not suitable for treating large volumes.

Chemical or cement grouting techniques are often costly and environmentally unfriendly and require heavy machinery, disturbing urban infrastructure. Finally, these methods significantly reduce the permeability of the strengthened soil, which hinders groundwater flow and limits the injection distance, making large-scale treatment unfeasible.

Currently, the potential of biological techniques for ground reinforcement -biogrouting- is being investigated (DeJong et al. 2006; Whiffin et al. 2007; Ivanov & Chu 2008). When supplied with suitable substrates, micro-organisms can catalyze chemical reactions in the subsurface resulting in precipitation (or dissolution) of inorganic minerals, which change the mechanical soil properties. This study focuses on microbial induced calcium carbonate precipitation (MICP) by urea hydrolysis and its potential as ground reinforcement method.

2 MICROBIOLOGICALLY INDUCED CARBONATE PRECIPITATION FOR GROUND REINFORCEMENT?

There are many biological processes, which lead to precipitation of calcium carbonate (Castanier et al. 1999), but not all are suitable for ground reinforcement.

When ground reinforcement is required homogeneously distributed over a large volume in the underground, micro-organisms and substrates to induce calcium carbonate precipitation have to be injected and transported over a substantial distance into the porous material.

Transport of bacteria (and hence bacterial activity) is limited in fine grained soils. As bacteria have a typical size of 0.5 to 3 μm , they cannot be transported through silty or clayey 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.

High substrate concentrations (typically molar range) are preferred for biogrouting for economical reasons, i.e. to limit the number of flushes, to enable treatment over large distances (to limit the number of required injection wells) and enable ground improvement without disturbing the serviceability of any urban infrastructure present in the vicinity. For example, Whiffin et al. (2007) indicated that at least 60 kg CaCO_3/m^3 soil has to be precipitated, to obtain a threshold strength improvement in a loose granular material, which corresponds to approximately 2 mol CaCO_3 precipitate per litre of pore space. Substrate concentrations resulting in 100 mM CaCO_3 precipitation already require that the sand is flushed with 20 pore volumes to reach significant reinforcement. Lower concentrations require even more pore volumes and are therefore considered economically unfeasible. In other words, the combination of substrates should be sufficiently soluble in water before it is injected and converted into the poorly soluble calcium carbonate.

The rate of calcium carbonate precipitation is an important process variable. Compared to traditional ground reinforcement methods, which have gel times in the order of minutes (Karol 2003), microbial processes generally are slow. This is advantageous since the rate should be slow enough to avoid rapid precipitation and consequent clogging near the inlet since that would limit the injection distance. However, the conversion rate should not be too low either, as then it would take too much time to reach the required level of cementation.

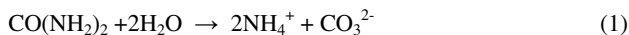
Another factor determining the feasibility of MICP for ground reinforcement is the required amount of substrates for CaCO_3 precipitation. This amount follows from the reaction stoichiometry and determines the process efficiency and costs. The choice of substrate can be based on the CaCO_3 yield. On the other hand, if cheap substrates, like waste streams, could be used, the required amount of substrate might become less important as an economical factor.

Finally the amount and nature of side products affect the process suitability. Apart from making a process less efficient, side products can affect the suitability of the process by disturbing the cementation process. For example, gas formation or microbial growth might lower the permeability and block the flow towards the desired location. In addition, some side products, but also substrates, might be toxic for the organisms, especially at high concentrations, and thereby leading to death and/or lowering the metabolic rates.

3 MICP BY UREA HYDROLYSIS

Most studies on biogrouting, use micro-organisms containing the enzyme urease and in particular the bacterium *Sporosarcina pasteurii* (DSM 33, renamed from *Bacillus pasteurii*) (DeJong et al. 2006; Whiffin et al. 2007). These micro-organisms are cultivated aerobically in the laboratory using a nutrient medium containing yeast extract (20 g/L), ammonium chloride (10 g/L) and a trace amount of nickel chloride (10 μM) and harvested after ca 24 hours. The suspension containing the bacteria is introduced in the soil and supplied with a solution of urea and

calcium chloride. The microbial urease catalyzes the hydrolysis of urea into ammonium and carbonate.



The produced carbonate ions precipitate in the presence of calcium ions as calcite crystals, which form cementing bridges between the existing sand grains (Figure 1).

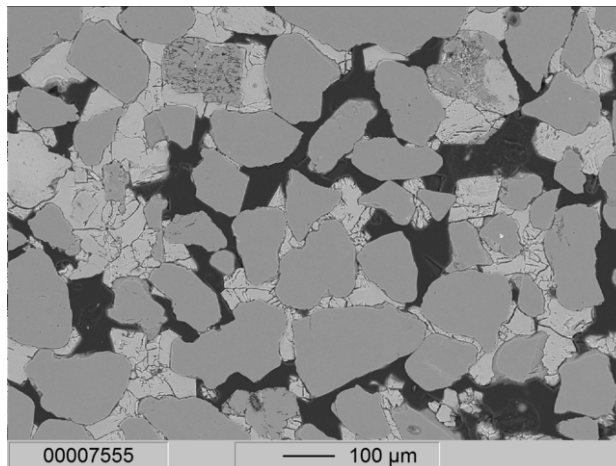


Figure 1 Electron microprobe image of a thin section of biocemented sandstone. Calcium carbonate (calcite) crystals (light gray) have precipitated between the sand grains (dark gray) induced by microbially catalyzed hydrolysis of urea. The sandstone remains porous (black).

The remaining ammonium chloride solution is removed by physical extraction and rinsing with water. Once precipitated, the calcium carbonate will only dissolve very slowly (at a geological time scale), either when continuously flushed by buffered acidic groundwater or as a result of acidifying processes in the pores (e.g. degradation of biomass). When sufficient calcium carbonate is precipitated, durable soil stabilization can be achieved.

First sand column experiments were performed by mixing bacteria with urea and calcium chloride and injecting the suspension simultaneously. However, as mixing bacteria and reagents, results in flocculation of the bacteria and immediate precipitation, the sand columns containing medium grained sand clogged close to the inlet and significant cementation was established only over a few centimeters. The injection procedure was adjusted by injecting bacteria and reagents sequentially, with an optional pulse in between to stimulate a homogeneous distribution of adsorbed bacteria to the sand grains and prevent clogging near the inlet (Van Paassen et al. 2007; Harkes et al. 2008). Using this bacterial placement procedure, sand column experiments were performed in which 20 cm sand packed were homogeneously cemented to a desired strength varying from loosely cemented sand to moderately strong rock with unconfined compressive strengths of 0.2 – 20 MPa (Harkes et al. 2008). The corresponding amount of precipitated calcium carbonate varied from 30 to 600 kg/m^3 and a clear correlation was established between CaCO_3 content and unconfined compressive strength.

4 SCALE UP EXPERIMENTS

4.1 5 m column experiment

First step in the scale up process was to prove that cementation could be induced over a long distance from an injection well (Whiffin et al. 2007).

A PVC column (66 mm in diameter and five meter in length) was placed vertically and carefully packed with sand to get a homogeneous density with a total pore volume of 6L. The sand was from a quarry in Itterbeck, Germany (Smals IKW, SZI 0032, also referred to as Itterbeck fine). This sand was uniform, fine to medium grained (d_{50} : 0.166 mm; d_{60}/d_{10} : 1.64; (BSI 1999)) and mainly siliceous (97%). Fluids were flushed through from top to bottom. First 6L of bacterial suspension was flushed through, followed by 6L of 0.05 M calcium chloride solution, which was used to distribute and fix the bacteria in the sand column and finally with 9L of reagent solution, containing 1M urea and calcium chloride (analytical grade), until unreacted urea was observed in the effluent. After that, the column was left to react for 24 hours. Monitoring of several process variables (optical density (a turbidity measurement which is indicative for bacterial concentration), urease activity, ammonium concentration and pore pressure) indicated that most of the bacteria were retained in the column. The urease activity was well distributed over the column, but decreased in time, mainly close to the inlet.

Both bacteria and reagents could be injected over the full column length at low pressures (hydraulic gradient < 1 ; a flow rate of approximately 7 m/day) without resulting in clogging of the material. Although the pressure drop over the top half meter of the column indicated the permeability had dropped 10 fold during the injection of bacteria, the remaining permeability after cementation over the full column was reduced by a factor 0.7.

After treatment the column was cut in 20 cm sections from which cores were extracted for further analysis. Permeability tests on the cores showed no significant difference between cemented and non-cemented parts of the column. Calcium carbonate had precipitated over the entire 5 m treatment length. Within the first meter, CaCO_3 content varied significantly. Confined compressive strength tests indicated a significant improvement of strength and stiffness over several meters (Whiffin et al. 2007). The maximum treatment distance did not appear to be limited to 5 meters and it seemed to be possible to extend this distance further.

4.2 Cubic meter experiments

In practice, conventional ground reinforcement methods are not one dimensional processes. Cement and chemical grouts are mostly injected in the ground by wells, either radially when injected through a linear tube well or spherically when injected from a single point.

To evaluate the potential of BioGrout for field applications, experiments were performed in controlled 3D environments, using conditions and injection techniques resembling those envisioned in practice. To mimic a spherical point two experiments were performed in a multibox container set-up ($0.9 \times 1.1 \times 1 \text{ m}^3$), having drainage filters on the sides. The box was filled with sand, which was packed by dropping the filled box a few times using a forklift to a homogeneous density. Two types of sand were used. In the first container a sand from river Maas was used, which was uniform, medium grained (d_{50} : 0.367 mm; d_{60}/d_{10} : 1.6; (BSI 1999)) and mainly siliceous (93%). In the second, Itterbeck fine sand was used similar to the reported column experiments (Whiffin et al. 2007). 100 L bacterial suspension was cultivated under sterile conditions. And reagent solutions were prepared containing 0.5 M urea and calcium chloride (industrial grade). Fluids were injected sequentially with constant flow rate (50 L/hr) at the centre of the box and drained along the sides, maintaining a constant flow rate and a constant water level at the sides.

The first test, using sand from Meuse river, showed that the majority of bacteria flushed out and the remaining urease activity decreased exponentially in time. After 50 days, in which 3500 L of reagent solution was flushed through in 8 batches, about 200 mol CaCO_3 (20 kg/m^3) had been precipitated inside the sand with 12% of the reagents being converted.

Excavation of the sand body showed that CaCO_3 was only present along the sides mostly in the corners and at the bottom.

In the second experiment with Itterbeck fine sand the injection pressure rose gradually during treatment to a maximum 1 bar. Several times while flushing a pressure drop was observed, indicating the likely occurrence of fracturing events. After three of such events, sand transporting wells appeared at the sand surface, indicating preferential flow paths upwards, diverging from the ideal spherical injection. After these events, flow was stopped and a clay/cement plug installed (or enlarged) to prevent further leakage. Again, the urease activity dropped gradually in time. During day 20 (2500 L) the ammonium concentration in the effluent, which was produced in 48 hours, reached only 0.4 M, indicating that the average urease activity had dropped below 5 mM urea/hr. At day 25 (2650 L) 50 L of additional bacterial suspension was injected increasing the maximum ammonium production rate up to 0.7 M/day, or 15 mM urea/hr, which dropped only slightly during the further duration of the experiment. After 40 days, in which 4000 L of reagent solution was flushed through in 16 batches, about 1000 mol CaCO_3 (100 kg/m^3) was formed inside the sand box with 50% efficiency. Manual cone penetration tests indicated cone resistances higher than 5 MPa at shallow depth. Removing the loose sand with a water hose from the top revealed a strongly cemented surface. At one side ridges with high CaCO_3 content were observed in a regular pattern parallel to the induced flow direction (Figure 2).



Figure 2 The surface of the cemented sand body of the second cubic meter test. Ridges with high CaCO_3 content are visible in a regular pattern on the left.



Figure 3 The spherically shaped cemented sand body.

Demobilizing the box showed that the cemented sand body had a spherical shape, which could be expected from a single

point injection (Figure 3). Further excavation indicated that the areas close to the injection point and along the principal flow axes (horizontally towards the drained sides and vertically upwards as result of the leakage events) contained less CaCO_3 , which was attributed to a lower retention of bacteria in those areas due to a higher velocity and lower hydraulic residence time. In the lower half towards the bottom corners of the sand body the highest amount of CaCO_3 was found (up to 250 kg/m^3 or 17% of dry weight). The unconfined compressive strength (BSI 1999) varied between 0 (loose sand which could not be tested) and 9 MPa.

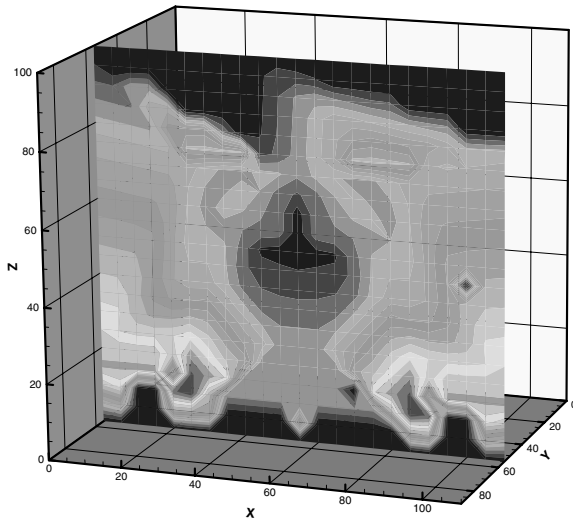


Figure 4 CaCO_3 measurements on samples taken from a quarter of the second cubic meter enabled the construction of cross-sections through the cemented sand body (Contours are mirrored over the mid cross-section). Colors indicate CaCO_3 content from <1 (blue) to >15 (red) [% of dry weight].

4.3 Large scale (100m^3) experiment

The last scale up step before commercial application was an experiment at large scale (100 m^3 , Figure 5). A concrete box ($8 \times 5.6 \times 2.5 \text{ m}$) set-up was filled (under wet conditions) with sand (Itterbeck fine) with an average dry density of 1560 kg/m^3 . A bioreactor was built on site, in which bacteria were cultivated in 5 m^3 nutrient broth under non-sterile conditions, but using a sterile cultivated 100 L inoculum.



Figure 5 set-up for large scale Biogrout experiment (100m^3). Three injection wells (left) and three extraction wells (right) were used to flush liquids through the sand body.

Fluids were injected sequentially in batches through three injection wells and transported over a distance of 5 m along the length of the sand box towards three extraction wells. Total flow rate was approximately $1 \text{ m}^3/\text{h}$ with a hydraulic gradient of about 0.3 m/m.

Geophysical measurements (shear wave transmission from top to bottom) indicated a significant increase in average stiffness around the injection points, after 1 day of flushing. Manual cone penetration tests showed that after several days of flushing the cone resistance around the phreatic surface became higher than 5 MPa.

After 12 days in which 100m^3 of reagent solution containing 1M urea and calcium chloride was flushed through in 10 batches, the sand was rinsed with water and excavated. The cemented sand body, about 40 m^3 became clearly visible and was limited by the induced hydrological flow field. Flow lines could be distinguished, especially close to the extraction wells (Figure 6).



Figure 6 Detail of the cemented sand body close to the extraction point shows that the cemented patterns are clearly related to the flow paths through the sand body.

Cementation along the flow lines was reasonably homogeneous, while perpendicular to the flow lines CaCO_3 content varied significantly. Excavation of a vertical cross-section along the centre flow line (figure 8) showed that below the cemented phreatic surface lenses were formed with CaCO_3 content varying from 0.8 to 24 % of the dry weight. Lenses had a thickness in the order of centimeters and extended horizontally at a scale of meters. Several cemented blocks were sampled, from which cores were drilled to test geotechnical parameters.

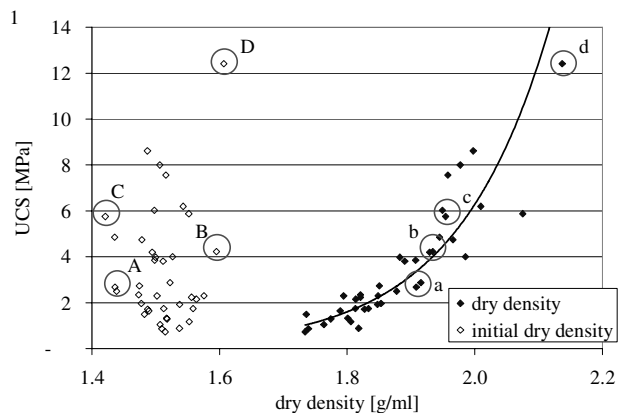


Figure 7 Correlation between dry density and unconfined compressive strength for biocemented sand from the 100m^3 experiment, and initial dry density for the same samples, which is derived by subtracting the CaCO_3 content from the dry density.

Unconfined compressive strength varied considerably from 0 to 12 MPa. UCS of the large scale test correlated much better to dry density than to CaCO₃ content. Assuming CaCO₃ content is the difference between initial and final dry density, the initial dry weight was obtained, which varied between 1420 and 1620 kg/m³. Encircled points in figure 7 show that although some samples have similar CaCO₃ content, they differ in strength and dry density (c and d) due to a difference in initial density (C and D). Other samples have comparable strength and dry density (a and b), while one has low initial density (A) and high CaCO₃ content and the other having high initial density (B) with low CaCO₃ content.

5 DISTRIBUTION OF RESULTING PROPERTIES

The scale up experiments showed significant cementation at large distance from the injection points, proving the technical feasibility of BioGrout for ground reinforcement. However, the CaCO₃ content and consequent geotechnical parameters were not as homogeneously distributed as desired, both on a macro as well as on a micro-scale. Although initial conditions, like layers in the sand packing, could explain part of the observed variability, it is assumed that a large part of the observed heterogeneity is process induced. Several mechanisms, which might explain the observed heterogeneities, are discussed below:

First of all, the amount of CaCO₃ formed at a specific location depends on the amount available catalyzing bacteria. The distribution of bacteria and bacterial activity, both in time and space, is hard to assess. During injection of bacteria, the distribution of bacteria varied over the distance and between different flow paths. 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). After placement, the attached bacteria could have lysed (and excreting their enzymes), flushed out, or become encapsulated in the crystals, all resulting in a decrease in activity in time.

Secondly, the CaCO₃ content depends on the amount of supplied reagents and the way of supplying it. During the injection of urea and calcium chloride, the reagents are converted, while being transported within the soil. Consequently, close to the injection points more CaCO₃ will form than towards the extraction points as the downstream areas have received less reagents, which is observed in both the 5 m column and the large scale test. Decay of bacterial activity by encapsulation or flush out might lessen this effect.

Process induced preferential flow paths can explain part of the heterogeneity. Limited overburden pressure in the cubic meter and large scale tests, caused close to inlet preferential flow upward towards the surface, resulting in an increased CaCO₃ content along the phreatic surface.

The variation in CaCO₃ content in the first meter of the 5 m column experiment, at the top surface of the 2nd cubic meter test and close to the extraction wells in the large scale test, could be a result of locally clogged areas (by bacteria or crystals). These clogs will cause the development of preferential flow paths with a faster flow velocity around the clog and stagnant zones in the areas downstream of the clog where supply of bacteria and nutrients can become limited. Normally, such flow diversions converge shortly after a clogged zone, but in this case precipitation in the preferential flow paths might hinder reconvergence. The difference in CaCO₃ content revealing the flow lines close to the extraction wells in the large scale test, might also be explained alternatively: as the rate of crystal growth is directly related to the available crystalline surface, large crystals tend to consume more CaCO₃. A difference in number and size of crystals between two flow paths might be self-augmenting.

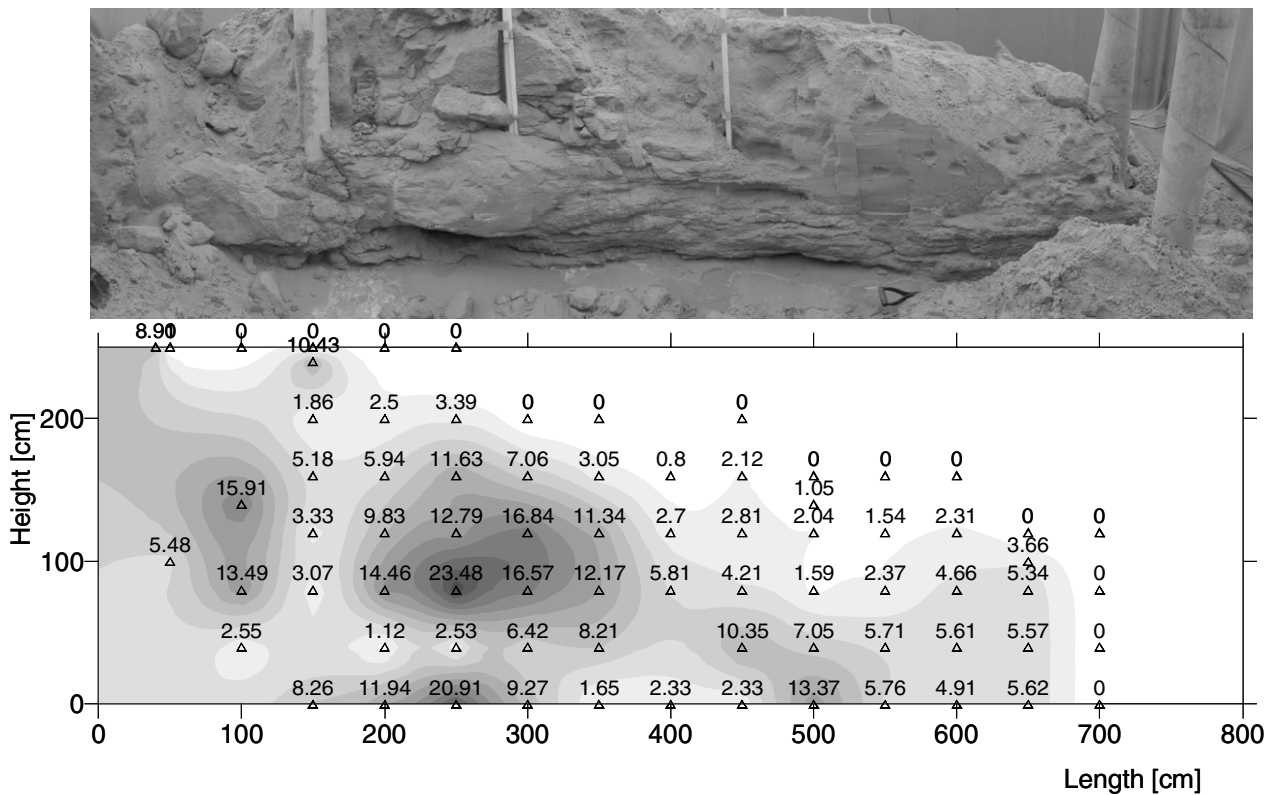


Figure 8 Cross-section along the longitudinal centre line through the centre injection and extraction well of the large scale BioGrout experiment showing CaCO₃ content in [% of total dry weight]. The injection well is located at 150 cm and the extraction well at 650 cm.

The lack of CaCO₃ close to the injection points in all three scale up experiments, could be the result of a higher flow velocity, causing more bacterial flush out and hence lower activity and less CaCO₃. This was shown especially in the cubic meter test with sand from Meuse river, where the bacterial attachment was lower due to the coarser grain size. Another explanation for the lack of CaCO₃ around the injection points, considers the kinetics of CaCO₃ 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.

The high amount of CaCO₃ in the lower half 2nd cubic meter test might be a result of buoyancy-driven flow induced by the density differences between the injected fluids. The fluid density of the reagent solution containing 0.5 M urea and CaCl₂ being 1046 kg/m³, while of the solution containing the product 1 M NH₄Cl being 1013 kg/m³. At such a density difference buoyancy driven flow can be expected, especially if the distance between injection distance increases or the flow rate decreases (Post & Prommer 2007). Viscosity and density vary during different phases in the process. Initial in situ pore fluid, bacterial suspension, fixation solution, reagent solution, product solution and rinsing fluid all have different viscosity and density. Apart from buoyancy driven flow these differences can lead to instabilities and fingering patterns (Rosen et al. 2001), especially at the interface between two liquids.

Understanding and control of this (process induced) heterogeneity is considered essential for practical applications. Not only does it affect the mechanical behavior of the treated sand body, but also it might affect the extraction of resulting ammonium chloride. On the other hand, traditional ground reinforcement methods, like jet-grouting or soil mixing, are often characterized by a comparable level of heterogeneity. Also for many applications the heterogeneity might be less important. For example to lower the amount of eroded sand in river beds, increase the stiffness under a railway track, or increase the slope stability of an embankment, homogeneity might be less important. Even models exist, which take (random) heterogeneity into account (Hicks & Samy 2002). 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.

6 CONCLUSIONS

Scale up experiments have shown the technical feasibility of BioGrout as ground improvement method under conditions and techniques as used in practice, both as single point injection or over a horizontal distance using screens of injection and extraction wells. Engineering parameters correlate well with CaCO₃ content or better with dry density. Control of the in situ distribution of bacterial activity and reagents and the resulting distribution of CaCO₃ and related engineering properties in the subsurface are the greatest challenge for further optimization, especially if BioGrout is applied in an open system. Further research should demonstrate which of the suggested mechanisms are responsible for the observed heterogeneity in deposition of carbonate and consequent geotechnical parameters. Further optimization of the process performance should enable commercial application.

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