APPLICATIONS OF MICROBIAL PROCESSES IN GEOTECHNICAL ENGINEERING

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Abstract

Over the last 10-15 years a new field of 'biogeotechnics' has emerged as geotechnical engineers seek to find ground improvement technologies which have the potential to be lower carbon, more ecologically friendly and more cost-effective than existing practices. This review summarizes the developments which have occurred in this new field, outlining in particular the microbial processes which have been shown to be most promising for altering the hydraulic and mechanical responses of soils and rocks. Much of the research effort in this new field has been focused on microbially induced carbonate precipitation via ureolysis (MICP); while a comprehensive review of MICP is presented here, the developments which have been made regarding other microbial processes, including microbially induced carbonate precipitation via denitrification and biogenic gas generation are also presented. Furthermore, this review outlines a new area of study: the potential deployment of fungi in geotechnical applications which has until now been unexplored.

Keywords:

Microbially induced carbonate precipitation, Ureolysis, Denitrification, Biogenic gas formation, Bacteria, Fungi, Geotechnical Engineering

1. INTRODUCTION

Geotechnical engineers are concerned with the engineering performance of the ground comprising soil, rock and the fluids (generally air or water) held within their pore space or voids. As such geotechnical engineers consider the behavior of the ground in terms of strength and stiffness in order to assess its performance in response to loading and unloading, which may be induced at the surface or at depth. Furthermore the ground acts as a source of material for use as fill, for example in the construction of embankments. Often there is a need to control the flow of water into or around structures to maintain stability and/or to ensure underground structures remain operational (e.g. tunnels); thus we are also concerned with the hydraulic behavior of the ground (e.g. permeability). In many instances the soil/rock available at a given site is not adequate in terms of engineering performance for the intended geotechnical application; ground improvement strategies are then employed to alter the hydraulic and/or mechanical behavior.

Conventional ground improvement techniques are highly invasive (e.g. jet grouting, permeation grouting, the formation of soil-cement/lime piles), are frequently energy intensive (e.g. compaction, vibration, heating, freezing, electro-osmosis) and often require the introduction of environmentally damaging chemicals or carbon-intensive materials into the subsurface (e.g. chemical grouts, cement). Cement production alone is estimated to contribute 5-7% of total global CO_2 emissions (Benhelal et al., 2013). Many countries worldwide have ambitious targets to reduce their carbon emissions, for example the UK has a target to reduce carbon emissions by 80% (against the 1990 baseline) by 2050. These targets present both challenges and tremendous opportunities for the construction sector in the transition towards low-carbon economies, as the use of cementitious materials is pervasive in

conventional ground improvement techniques. There is a clear need to widen our scope of ground improvement technologies to include lower carbon, less invasive, less energy demanding and more environmentally-friendly practices. One potential avenue for achieving this is to consider the role of microbial processes in soils and rocks.

Estimates suggest that there are 2 x 10^9 prokaryotes (archaea and bacteria) in a gram of soil sampled from surface (top 1m), decreasing to 1x10^8 prokaryotes at 1-8m depth in soil (Whitman et al., 1998, Gans et al., 2005). Even at greater depth prokaryotes are found in abundance, with 2.3x10^7 cells/cm^3 estimated to exist in subsurface sediments from 10-300m, reducing to 6x10^6 cells/cm^3 between 300-500m (Whitman et al., 1998). In terms of bacterial diversity, estimates range from 6,400-830,000 different bacterial species per gram of soil (Curtis et al., 2002, Gans et al., 2005). These estimates do not include the presence or diversity of eukarya (algae, fungi, protozoa). Despite the abundance and diversity of microorganisms in the ground and their ability to survive/thrive in extreme environments (e.g. Dong et al., 2008) geotechnical engineers until recently have largely ignored their presence, preferring to view the ground as a sterile engineering material.

In 2005, Mitchell and Santamarina published a seminal article outlining biological considerations in geotechnical engineering (Mitchell & Santamarina, 2005). This hailed the beginning of the emergence of a new sub-discipline of 'biogeotechnics'. Since then research in this area has proceeded at pace with the role of microbial processes in geotechnical engineering capturing the attention of many research groups across the world and regular symposia and conference sessions dedicated to the theme, e.g. Géotechnique Symposium in Print in 2013 on 'Bio- and chemo-mechanical processes in geotechnical engineering'. Further highlighting the

importance of this field, the National Science Foundation in the US awarded $18.5million in 2015 to establish the Center for Bio-mediated and Bio-inspired Geotechnics, led by Arizona State University.

This review seeks to present the developments which have occurred over the last 10-15 years, outlining in particular the processes which have been shown to be most promising for altering the hydraulic and mechanical responses of soils and rocks. Much of the research effort in this new field of biogeotechnics has been focused on microbially induced carbonate precipitation via ureolysis (MICP); while a comprehensive review of MICP is presented here, the developments which have been made regarding other microbial processes, including microbially induced carbonate precipitation via denitrification and biogenic gas generation are also presented. Furthermore, this review outlines a new area of study: the potential deployment of fungi in geotechnical applications which has until now been unexplored. The processes outlined herein underpin the development of nature-inspired ground improvement technologies, which have the potential to be more ecologically friendly and cost-effective, for the construction and maintenance of resilient infrastructure.

2. NATURAL MICROBIAL ACTIVITY

Although the main focus of this review is to present microbial applications which could be deployed in ground engineering, geotechnical engineers should also be aware of natural microbial activity. This section outlines (in brief) two main points: (i) the role of microorganisms in soil formation and structure and (ii) the negative impacts that have been attributed to microbial activity in a number of case histories.

2.1 Role in soil formation and structure

The perspective of soil as a sterile material not only ignores the presence of microrganisms in the ground but also the role that they play in soils and rocks. Indeed clay scientists and geomicrobiologists now widely acknowledge the important role microorganisms play in weathering processes and in the dissolution, transformation and formation of clay minerals (e.g. Barker & Banfield, 1996; Douglas & Beveridge, 1998; Konhauser & Urrutia, 1999; Konhauser, 2007; Gadd, 2007, 2010, 2017; Mueller, 2015; Cuadros, 2017). A typical pattern for microbially influenced mineralisation, (not considering metabolic processes), involves metal cations in solution interacting with charged groups on cell surfaces, with these sites lowering the interfacial energy required for heterogeneous nucleation to occur. If the local solution is supersaturated with respect to the metal cations then this results in nucleation and precipitation, with the available counterions (depending on the local geochemical environment) determining the final mineral phase (e.g. carbonate, phosphate, silicate etc., Douglas & Beveridge, 1998, Konhauser, 2007). Many studies have shown the close association or synthesis of low crystallinity or amorphous clay phases in the presence of microorganisms or microbial products (both bacterial and fungal species) (e.g. Barker & Banfield, 1996, 1998; Konhauser & Urrutia, 1999; Bontognali et al., 2014, Tazaki, 2006; 2013). Clay formation has been shown to occur even in low nutrient, high salinity experiments designed to simulate deep, subsurface hard rock environments (Tuck et al., 2006). Aside from their role in clay formation, microorganisms also interact with clay particles such that clay particles adhere to cell surfaces and bacterial exudates (e.g. polysaccharides) bind particles inducing aggregation, influencing clay fabric, they also intrude into clay pores affecting swelling and shrinkage behavior (Dorioz et al., 1993; Mueller, 2015). Fungi influence

101 soil aggregation via a number of different mechanisms, this is discussed in more detail
102 in section 3.4.2.
103
104 **2.2 Problematic effects of microbial activity**
105 Until the emergence of 'biogeotechnics' as a field of study, there was
106 relatively little mention of microbial processes within the geotechnical engineering
107 literature, except in rare cases where microbial activity was highlighted as a
108 contributing factor to problematic effects arising on site. Such case histories have
109 been reported by Mitchell & Soga (2005), Mitchell & Santamarina, (2005), Soga &
110 Jefferis, (2008) and Jefferis (2013). Negative impacts of microbial activity have been
111 related to the oxidizing or reducing behavior of bacteria, involved in for example, the
112 oxidation of soluble Fe^{2+} to Fe^{3+} resulting in precipitation termed as 'biofouling' or
113 'bioslime', this is known to contribute to clogging of groundwater wells (Jefferis,
114 2013).
115 In an extreme case, during the construction of the Carsington Dam in England
116 in the 1980s, the reaction of sulfuric acid (arising from pyrite oxidation) with
117 limestone contained in a drainage blanket, resulted in the precipitation of gypsum,
118 iron hydroxide and release of CO_2 (Mitchell & Soga, 2005; Mitchell & Santamarina,
119 2005). The former products resulted in clogging of the drainage blanket, whereas the
120 latter had a more catastrophic consequence; leading to the death of four men by
121 asphyxiation, where CO_2 had accumulated in an inspection chamber (Mitchell &
122 Soga, 2005; Mitchell & Santamarina, 2005). Cripps et al. (1993) hypothesized that
123 bacteria greatly accelerated the rate of pyrite oxidation (Mitchell & Soga, 2005;
124 Mitchell & Santamarina, 2005).

125 Furthermore, it has long been recognized that the accumulation of biomass and
126 growth of biofilms in the subsurface, often referred to as 'bioclogging' can lower soil
127 hydraulic conductivity (Slichter, 1905). Bioclogging can be problematic particularly
128 in filters, drains and geotextiles, for example in landfill barrier systems (e.g. Baveye
129 et al., 2008; Rowe, 2005; Ivanov & Chu, 2008), and efforts have typically focused on
130 minimizing microbial growth. More recently engineers are considering that
131 bioclogging could be beneficial in some applications and have attempted to reduce
132 hydraulic conductivity by enhancing microbial growth in the laboratory (Seki et al.,
133 1998, 2005) and in the field (e.g. McConkey, 1990; Blauw et al., 2009; Lambert et al.,
134 2010). Engineered bioclogging is not discussed in more detail in this article; readers
135 are referred to the review papers by Mitchell & Santamarina, (2005) Ivanov & Chu,
136 (2008) and DeJong et al., (2013).

137 As geotechnical engineers now begin to engage with, consider and explore a
138 wide range of microbial processes there are tremendous opportunities for: (a)
139 developing a better understanding of how microorganisms may contribute to soil
140 formation, structure and engineering behavior in a range of environments, (b)
141 investigating how microorganisms may influence the construction, operation and
142 maintenance of geotechnical structures taking into account site specific geology,
143 geochemical conditions and mineralogy and (c) understanding how particular
144 processes can be controlled and deployed to bring about hydro-mechanical alterations
145 in the ground. The following sections focus on the research conducted to-date for a
146 range of microbial processes being considered for deployment in geotechnical
147 engineering.

148

149

3. ENGINEERED MICROBIAL ACTIVITY

3.1 Microbially induced carbonate precipitation via ureolysis

3.1.1 Process

A significant proportion of carbonates found at the Earth's surface are thought to be of biogenic origin (Gadd, 2010). Microbially induced carbonate precipitation is a common biogeochemical process, which can occur via a number of different microbial pathways including photosynthesis, ureolysis, denitrification, ammonification, sulphate reduction and methane oxidation (Zhu & Dittrich, 2016). To date most of the studies investigating MICP for ground engineering applications have utilised ureolytic bacteria due to the relatively short times required to precipitate $CaCO_3$ and the large masses of $CaCO_3$ that can be precipitated due to the high solubility of the substrates in solution (urea and $CaCl_2$) (Van Paassen et al., 2010).

MICP via ureolysis relies on a bacterium hydrolyzing urea into ammonia and carbonic acid (Equation 1). This is followed by the production of ammonium ions and an increase in the pH surrounding the bacterial cell, due to the net production of OH^- ions (Equation 2). As the pH increases, carbonic acid (H_2CO_3) is converted to bicarbonate ions (HCO_3^-) (Equation 3), subsequently forming carbonate ions (CO_3^{2-}) (Equation 4). Calcium ions in solution interact with charged surfaces on the bacterial cell surface and the increase in pH promotes the subsequent precipitation of calcium carbonate ($CaCO_3$) (Equation 6) [*Ferris et al.*, 1992; 1996; *Mitchell et al*., 2010]. Figure 1. shows calcite crystals produced via ureolysis, with visible indentations indicating that *S. Pasteurii* cells are encapsulated by the precipitation of calcite.

$$CO(NH_2)_2 + 3H_2O \longrightarrow 2NH_4^+ + HCO_3^- + OH^- \quad (1)$$

$$HCO_3^- + H_2O + OH^- \longleftrightarrow CO_3^{2-} + 2H_2O \quad (2)$$

$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3\,(s) \quad (3)$$

MICP has been investigated for a wide range of applications including solid-phase capture of contaminants (e.g. Fujita et al., 2008), for building restoration (e.g. De Muynck et al., 2010) and concrete remediation (e.g. Bang et al., 2001; Van Tittelboom et al., 2010). The review presented here is only intended to cover its use in ground engineering applications. From this perspective it has been investigated, over the last two decades by numerous researchers from different backgrounds and with different objectives (in terms of end-state) leading to a substantial body of literature and a collection of varying experimental procedures.

Prior reviews have summarized the practical applications of MICP (often with a focus on soil stabilization), field scale testing that has been carried out to date, as well as the challenges and limitations of the technique (Anbu et al., 2016; DeJong et al., 2010; DeJong et al., 2013; Mujah et al., 2017; Philipps et al., 2013; Umar et al., 2016; Wang et al., 2017). This review aims to add to the body of knowledge by examining the experimental conditions, control parameters and injection strategies employed in MICP by urea hydrolysis; and comparing this to reported outcomes including for increases in compressive strength, decreases in permeability or erodibility, and uniformity of treatment.

3.1.2 Applications in geotechnical engineering

Soil stabilization

Investigation of the use of MICP via ureolysis has been widely studied for soil stabilisation, in particular for its ability to improve compressive strength, shear strength and stiffness, in particular in granular soils (i.e. sands and gravels) (e.g. DeJong et al., 2006; Whiffin et al., 2007, Van Paassen et al., 2010; Al Qabany &

200 Soga, 2013). Figure 2. shows that treatment with MICP via ureolysis transforms an
201 initially loose fine sand into a cemented sand/sandstone. Treatment of sands via MICP
202 has resulted in increases in unconfined compressive strength of greater than three
203 orders of magnitude (e.g. Al Qabany & Soga, 2013) and in some cases even over four
204 orders of magnitude (Van Paassen et al., 2010 and Terzis & Laloui, 2018). As a result
205 of the increase in strength and stiffness afforded by MICP it has also been proposed
206 for settlement reduction (Martinez & DeJong, 2009) and enhancing liquefaction
207 resistance (Montoya et al., 2013). Studies investigating soil stabilisation applications
208 have been widely reported in MICP review papers (Anbu et al., 2016; DeJong et al.,
209 2010; DeJong et al., 2013; Mujah et al., 2017; Philipps et al., 2013; Umar et al., 2016;
210 Wang et al., 2017).
211
212 *Erosion resistance*
213 MICP via ureolysis has been investigated as a method for reducing soil
214 erosion by creating a denser layer of $CaCO_3$ at the soil surface that is more resistant to
215 shear stresses imposed by wind or water, thereby protecting the underlying soil
216 (Figure 3). Both Gomez et al. (2015) Hamdan & Kavazanjian (2016) investigated
217 carbonate precipitation via urea hydrolysis as a means of suppressing dust generated
218 by wind erosion. Gomez et al. (2015) utilised *S. pasteurii,* whereas Hamdan &
219 Kavazanjian (2016) used the plant-based Jack bean urease enzyme. In both cases,
220 treated soils exhibited enhanced erosion determined either via jet impingement tests
221 (Gomez et al., 2015) or in wind tunnel tests, where the wind speed required to initiate
222 erosion in treated soils exceeded that of the control samples (Hamdan & Kavazanjian,
223 2016).

224 Studies have also demonstrated the potential of MICP via ureolysis to reduce
225 water-induced erosion, including for embankments and slopes in riverine and
226 coastal/estuarine environments and as a means of mitigating against scour around
227 bridge piers (Salifu et al., 2016; Amin et al., 2017; Bao et al., 2017). Results for all
228 studies showed increased erosion resistance of MICP treated soils, with MICP treated
229 slopes maintaining steep profiles (e.g. 53°) whereas untreated slopes exhibited
230 collapse when subjected to repeated raising and lowering of water levels (simulating
231 tidal cycles) (Salifu et al., 2016). In the case of scouring, although the treated sand
232 directly around the pier showed enhanced erosion resistance, the bridge pier was still
233 vulnerable to erosion due to undermining of the surrounding untreated sand (Bao et al.
234 2017).
235
236 *Permeability reduction in porous media*
237 The precipitation of microbially induced carbonate at particle contacts and on
238 grain surfaces reduces pore throat diameters and overall porosity, thus reducing
239 permeability. Sand columns treated with MICP have been shown to achieve as much
240 as 90-100% reduction in permeability from initial values (Gollapudi et al., 1995,
241 Tobler et al., 2012). Similarly MICP can be used to reduce permeability in porous
242 rock, e.g. sandstone (Tobler et al. in review). Although reduction in permeability may
243 be the target end-state, a homogeneous distribution of calcite is desirable, since a non-
244 homogenous distribution with more calcite precipitated close to the injection point
245 will result in a low permeability, but from a practical perspective will result in
246 clogging around the injection well cutting off further soil/rock volumes from potential
247 treatment (Tobler et al., 2012).
248

249 *Rock fracture sealing*

250 Rock fracture grouting using MICP has received considerably less attention
251 than soil stabilization. Initial work was carried out by Zhong & Islam (1995) with the
252 motivation of enhancing hydrocarbon production by plugging fractures. They found
253 that no plugging occurred in granite cores with artificially cut fractures unless the
254 fractures contained filling material such as sand, silica fume or limestone dust. Stoner
255 et al. (2005) used micromodels to investigate flow in fractures with realistic surface
256 roughness and found that, under constant flowing conditions, vein-like flow paths
257 formed due to MICP.

258 El Mountassir et al. (2014) sealed lab-scale artificial fractures consisting of
259 polycarbonate surfaces. In these experiments flocculation of the bacteria was induced
260 in order to aid settling and straining of the bacteria in the fractures. They found that,
261 for all flow velocities tested, preferential flow paths would form when MICP was
262 carried out with constant flow rate injections and no static periods. This was thought
263 to occur due to shear stresses on the fracture surfaces exceeding the bacterial
264 attachment threshold; they found that by reducing injection flow rates it was possible
265 to fill in the preferential flow paths. Using a similar injection strategy (although with
266 no induced flocculation), Minto et al. (2016) found that, in a large-scale artificial
267 granite fracture with radial injection, relatively uniform precipitation could be
268 obtained over an area at least 3.1m^2 and that high flow velocity could be used to limit
269 bacterial attachment and $CaCO_3$ precipitation in the vicinity of the well. Minto et al.
270 (2016) achieved a reduction in fracture transmissivity of three orders of magnitude in
271 3 treatment cycles.

272 Cuthbert et al. (2013) carried out a field trial in which a single fracture in
273 Dacite rock was sealed with eight MICP treatment cycles over four days. Two

adjacent monitoring boreholes were used for cross-hole conductance testing before and after MICP. To encourage flocculation of bacteria and attachment within the fracture, the bacteria was first mixed with 0.2M CaCl2 and then injected simultaneously with urea through a separate injection line. They inferred a reduction in fracture transmissivity of 99% close to the injection well, and 33% at a distance of 2m from the injection well via cross-hole conductance tests.

Only one study has to date been carried out on the mechanical behavior of MICP grouted fractures. Tobler et al., (in review) sealed four artificial fractures cut in 38 mm diameter granite cores. One core was thin sectioned for optical and SEM analysis whilst the remaining three were non-destructively scanned with X-ray computed tomography then shear strength was measured. Both SEM and X-CT revealed $CaCO_3$ covering most of both top and bottom fracture surfaces and, in places, entirely bridging the gap between surfaces. All sheared samples showed a higher residual resistance to shear than the uncemented rock surface and peak shear strength was found to correlate with the area of $CaCO_3$ bridging across the two fracture surfaces.

Fracture sealing with MICP appears to be viable, however, to date, all experiments have been carried out in single fractures that are horizontal and planar. MICP treatment in fracture networks with fractures of different aperture and orientation is likely to be more complex. Minto et al. (2016) hypothesized that hydrodynamic feedback between bacteria transport and $CaCO_3$ precipitation may lead to the sealing of large fractures first resulting in a progressive homogenization of fracture aperture within the network, however this remains to be tested.

Much of the work on rock fracture sealing with MICP has been motivated by the context of deep geological disposal of spent nuclear fueld and higher activity

radioactive waste, where MICP could be an alternative grout capable of penetrating into fine aperture fractures with a sufficiently low pH (compared to cement grouts) to not negatively impact on the bentonite buffer performance.

Well sealing

MICP has been proposed for sealing leakage pathways around wells, particularly those that may be used for geological carbon sequestration (Cunningham et al., 2009). Phillips et al. (2013) demonstrated sealing of a large fracture in a 74 cm diameter sandstone core and of a fracture in the sandstone surrounding a real well at a depth of 341 m.

Linked to the potential of MICP for well sealing, are questions concerning how high pressure, high temperature, high salinity, groundwater constituents, anoxic conditions, wellbore cements, and the presence of residual oil, scale inhibitors, surfactants, and other fluids injected to enhance drilling and production, might affect bacterial ureolytic activity and precipitate properties. Of particular concern for CO_2 sequestration is longevity of the seal and the potential for acidic CO_2 saturated water to dissolve $CaCO_3$ and form wormholes (Minto et al., 2017).

The authors are not aware of any experiments that combine high pressure (>1.5MPa) with temperatures greater than 40ºC, however such test will be necessary in order to establish the maximum depth at which MICP may be used for well sealing at depth.

Other applications

The focus here has been on the use of MICP for geotechnical applications. However it should be noted that MICP is also being investigated for a range of other applications

including for bioremediation by co-precipitation of heavy metals and radionuclides (e.g. Mitchell & Ferris, 2005; Fujita et al., 2008; Fujita et al., 2010; Achal et al., 2011, 2012a, 2012b), for CO_2 sequestration (e.g. Cunningham et al., 2009; Mitchell et al., 2010; Phillips et al., 2013) and for the protection and restoration of concrete and stone (e.g. Bang et al., 2001; De Muynck et al., 2010; Van Tittleboom et al., 2010).

3.1.3 Control parameters and injection strategies

Soil and rock fracture grouting with MICP is fundamentally different to traditional grouting using cements and resins. Numerous methodologies have arisen, among the different research groups studying this process, for the delivery of bacteria, urea, and $CaCl_2$ so as to best control and optimize $CaCO_3$ precipitation for different target applications. Table 1. lists the different control parameters and the injection strategies that may influence MICP precipitation. The influence of these and the typical values/ranges that have been used in MICP treatments are discussed in detail in the following.

Reagents

Bacteria: For engineering applications, the bacterial concentrations used during bioaugmentation mostly fall within the range 0.1 OD_{600} to 1 OD_{600}, corresponding to $3.7x10^6$ to $8.6x10^7$ cells/mL following the relationship developed by Ramachandran et al. (2001) for *S. pasteurii*, although concentrations greater than 3 OD_{600} have also been used (Cheng et al., 2017).

Fixative: High ionic strength solutions have been used to "fix" bacteria onto media surfaces during bio-augmentation by reducing repulsive surface charges. Harkes et al. (2010) demonstrated that a 50 mM $CaCl_2$ solution injected after bacterial injection

would overtake the bacteria causing the bacteria to flocculate within the porous media and fix them to the media surface resulting in greater bacteria retention and greater precipitation within the desired area. Cuthbert et al. (2013) found that to get sufficient bacterial retention in a fast-flowing fracture, it was necessary to add 200 mM $CaCl_2$ directly to the bacterial suspension and mix with 400 mM urea resulting in the formation of strongly bound bacteria-$CaCO_3$ flocs at the point of injection and a 70% retention of injected bacteria within the fracture.

Urea and calcium concentrations: Hydrolysing 1 M of urea results in, at most, 1 M $CaCO_3$, hence, equimolar urea/calcium concentrations are often used for maximum efficiency. However, increasing calcium concentration shifts the saturation state of the system (and can increase pH if adjustment is not made) so excess calcium concentrations (i.e. above the urea concentration) may lead to more rapid precipitation.

Cheng & Shahin (2016) found the maximum amount of $CaCO_3$ was produced at equimolar urea/$CaCl_2$ concentrations of 0.4 M with both higher and lower concentrations reducing the total mass of precipitation. Following the same trend, Nemati et al. (2005) found that increasing $CaCl_2$ alone from 0.045 to 0.27 M resulted in increasing amounts of $CaCO_3$.

Al Qabany & Soga (2013) found no significant difference between the compressive strength of equimolar 0.1 M and 0.25 M solutions for a given $CaCO_3$ content. However, as the concentration increased to 0.5 M, slightly more $CaCO_3$ precipitation was required to achieve the same compressive strength and samples treated with 1 M urea/$CaCl_2$ frequently failed before testing. This was attributed to larger $CaCO_3$ crystals forming in the pore space at high concentrations of urea/$CaCl_2$

374 and a poor spatial distribution of $CaCO_3$ resulting in highly heterogeneous samples.
375 Shahronkhi-Shahraki et al. (2014) on the other hand found unconfined compressive
376 strength was greater when urea or $CaCl_2$ concentrations exceeded 0.5 M, although at
377 these concentrations they did not use equimolar concentrations of urea and $CaCl_2$.
378 They observed greater unconfined compressive strength when the urea concentration
379 exceeded that of $CaCl_2$ (based on a limited number of specimens).
380
381 pH adjustment: $CaCO_3$ saturation is dependent on pH hence, by decreasing initial
382 solution pH, a delay in $CaCO_3$ precipitation can be introduced (Dupraz et al., 2009;
383 Mitchell and Ferris, 2005). Decreasing the cementing solution pH to 6.5 with the
384 addition of hydrochloric acid has been used by Minto et al. (2016), El Mountassir et
385 al. (2014), Tobler et al. (2011) and others, to delay precipitation around the injection
386 point and to allow a greater number of injection cycles before clogging occurs.
387 Gomez et al. (2015) used the same procedure so that bacteria, urea and $CaCl_2$ could
388 be pre-mixed on the surface and applied without precipitation occurring in the
389 injection tubing.
390
391 Urease activity: The rate of urea hydrolysis is governed by urease activity (measured
392 in mM urea hydrolysed/min), which is determined by the amount of enzyme present
393 in the solution. Given that the bacteria are the source of the enzyme, this is often
394 reported as the specific urease activity K_{urea}, (mM urea/min/OD_{600}). K_{urea} is
395 commonly measured using the change in electrical conductivity over a period of five
396 minutes, based on the premise that non-ionic urea is hydrolysed to ionic ammonium.
397 The calibration relationship often used, is that developed by Whiffin (2004), where
398 Urea hydrolysed (mM) = 11.11 x Change in Conductivity (mS/cm). Urease activity

values in the range of 0.5 to 60mM urea hydrolysed/min have been reported with specific urease activity values typically in the range of 0.8 to 29mM urea hydrolysed/min/OD (Minto et al., 2016; Whiffin, 2004; Harkes et al., 2010; Van Paassen et al., 2010; Terzis & Laloui, 2018).

Whiffin (2004) investigated the influence of bacterial concentration on ureolytic activity for different cultivations of *S. pasteurii*, and there was observed to be no correlation with biomass; for a given OD_{600}, urease activity varied by more than one order of magnitude. By contrast, Cheng et al. (2017) prepared different bacterial concentrations starting from initial OD_{600} values in the range of 2-2.5 and achieved suspensions with low, medium and high urease activities of 5, 10 and 50 μM urea hydrolysed/min, respectively. It should be noted that these levels of urease activity are considerably lower than those reported in other studies using *S.Pasteurii* (see above). During MICP treatment they kept all other variables constant and found that specimens treated with a lower urease activity suspension resulted in improved treatment, achieving a given unconfined compressive strength at a lower $CaCO_3$ content (Figure 4). Many researchers have related $CaCO_3$ content with unconfined compressive strength (UCS), under different experimental conditions (Al Qabany and Soga, 2013; Cheng et al., 2017, 2014, 2013; Choi et al., 2016; Rowshanbakht et al., 2016; Terzis and Laloui, 2018; van Paassen et al., 2010), data from these studies are also included in Figure 4 in order to understand the scale of variation. It should also be noted that differences in experimental procedure regarding carrying out UCS tests, can also lead to variability; some researchers use end caps to prepare perfectly flat ends, which can result in higher strengths being achieved than for specimens tested without the use of end caps.

423 The results presented in Figure 4 with respect to urease activity reflect a
424 general trend in the data in the literature in which parameters that act to decrease the
425 rate of ureolysis (low temperature, low urea concentration) or slow $CaCO_3$
426 precipitation (low $CaCl_2$ concentration) results in marginally greater UCS for a given
427 $CaCO_3$ content. This may be due to the influence of the rate of ureolysis on the
428 amount, size and distribution of crystals. Van Paassen (2009) demonstrated that high
429 rates of ureolysis (>0.3mM urea hydrolysed/min) resulted in the formation of large in
430 (spherical) crystals, whereas intermediate ureolysis rates resulted in smaller calcite
431 crystals and very low rates in a small number of very large calcite crystals.

432 *Flow conditions*

433 Fluid velocity: Bacterial attachment occurs when cells become physically wedged
434 between grains and trapped in pore throats (straining), or when cells are transported
435 close enough to a surface that electro-static attractive forces overcome repulsive
436 forces. Shear forces imparted by the flow velocity play a role in limiting attachment
437 and can also cause detachment of bacteria (Bakker et al., 2002).

438 In fractures, El Mountassir et al. (2014) and Stoner et al. (2005) have shown
439 that preferential flow paths form when MICP is applied under constantly flowing
440 conditions. El Mountassir et al. (2014) showed that hydrodynamic feedback
441 reinforced preferential flow paths at the fluid velocities tested (7.2 to 119 m/hr) and
442 that they remained stable until the injection rate was decreased. This is presumably
443 because at constant flow rates, as permeability decreases due to calcite precipitation,
444 the velocity increases within the remaining open channels, until the shear forces
445 become too high for the bacteria to attach. Minto et al. (2016) proposed that flow
446 velocity could be used to control where bacteria attach (and hence where $CaCO_3$
447 precipitates) within a fracture due to the radial flow drop-off in fluid velocity that

448 occurs around a single injection point. It follows that for multiple injection cycles in
449 radial flow systems, maintaining a constant pressure rather than a constant flow rate,
450 or sequentially decreasing the flow rate for consecutive cycles, may act to distribute
451 bacteria over a large area and progressively seal the fracture towards the injection
452 point.

453 In porous media, the effect of bacterial attachment due to straining and
454 filtration becomes more significant, particularly as the pore throat sizes approach that
455 of the bacterial cells (Tobler et al., 2014)). Tobler et al. (2014) found greater bacteria
456 penetration through a Bentheimer sandstone core as velocities increased (superficial
457 velocity from 0.06 to 0.18 m/hr) and Van Paassen et al. (2009) found little to no
458 $CaCO_3$ within approximately a 100 mm radius around a spherical injection point in
459 Itterbeck fine sand, corresponding to a superficial flow velocity in the region of 0.4
460 m/hr. This indicates that, even in porous media, velocity can be used to control where
461 $CaCO_3$ precipitates.

462

463 Static periods: Periods of no flow are often used in lab-scale experiments to allow
464 bacteria to attach to the porous media. Typically, between 0.5 and 1.5 pore volumes of
465 bacteria are injected followed by a static period ranging from 2 to 4 hours (Alvarado
466 and DeJong, 2008; Bernardi et al., 2014; Sham et al., 2013), 12 hours (Shahronkhi-
467 Shahraki et al., 2014) or even up to 24 hours (Amin et al., 2017; Cheng et al., 2017).
468 This is followed by the injection of cementing solution which is also often left static
469 for a duration of 24 hours (Amin et al., 2017; Cheng et al., 2017, 2014; Cunningham
470 et al., 2011; Shahronkhi-Shahraki et al., 2014; Sham et al., 2013). Using this
471 approach, each point in the porous media becomes like a batch reactor in which
472 bacteria, urea and $CaCl_2$ are present with only limited transport due to diffusion. The

473 24-hour duration of the static cementation period appears to be motivated by
474 experimental convenience rather than consideration for the amount of bacteria, urease
475 activity, and urea concentration.

476 Ideally during cementation, adequate urea, $CaCl_2$ and time would be provided
477 for sufficient $CaCO_3$ precipitation that the bacteria become encased, at which point
478 the reaction ceases. However, due to the Michaelis-Menten kinetics of urea hydrolysis
479 (e.g. Shashank et al., 2018), reaction rates decrease and urea starts to become a
480 limiting factor before it is fully exhausted, hence an unfeasibly long time is required
481 to fully encase the bacteria. To overcome this, some researchers (Bernardi et al.,
482 2014; Harkes et al., 2010) inject subsequent volumes of fresh cementing solution,
483 which may not be fully utilised, but may prove more cost effective as the bacteria is
484 are more expensive to grow, process and transport to site than the cementing solution.

485

486 Single vs cyclic injection: A single injection of ureolytically active bacteria followed
487 by cementing solution has been shown to be effective for increasing strength in sands
488 e.g. Whiffin et al. (2007) and Van Paassen et al. (2010) whilst maintaining porosity.
489 Additional injections of bacteria further increase strength and may result in a more
490 uniform treatment volume (Cheng and Cord-Ruwisch, 2014; Minto et al., 2017a).
491 However, they also decrease porosity and thus permeability, which may or may not be
492 desirable depending upon the application.

493 When grouting rock fractures, it is necessary to inject multiple cycles of
494 bacteria followed by cementing solution. Each cycle progressively precipitates $CaCO_3$
495 on the exposed fracture surface in multiple layers, which are necessary to completely
496 bridge the fracture aperture so as to substantially reduce fracture transmissivity
497 (Cuthbert et al., 2013; El Mountassir et al., 2014; Minto et al., 2016; Tobler et al., in

498 review). When multiple injection cycles are used, it is possible to deliver the same
499 total amount of bacteria, whilst still keeping concentrations close to their optimum
500 values by using an increased number of injections at a lower concentration. An added
501 advantage of this may be more uniform precipitation as preferential flow paths block
502 first, re-directing reagents in subsequent injections (Cheng and Cord-Ruwisch, 2014;
503 Minto et al., in review; van Paassen, 2009).

504 An alternative approach is a single bacterial injection followed by cementing
505 solution that either contains nutrients or is interspersed with injections of nutrients
506 (Bernardi et al., 2014; Cunningham et al., 2014; Phillips et al., 2013). The aim of the
507 nutrient addition is to stimulate bacteria growth whilst simultaneously precipitating
508 $CaCO_3$. For this approach to be effective, the relative rate of growth must be an
509 appreciable fraction of the rate of cell death and cell encapsulation within the
510 precipitating $CaCO_3$; hence it favours slower precipitation rates.

511

512 *Medium*

513 Mineralogy: MICP has been successfully applied in silica sands, gravel (van Paassen
514 et al., 2012) and organic soil such as peat (Canakci et al., 2015); in porous rock such
515 as Berea sandstone (Cunningham et al., 2014; Minto et al., 2017a; Nemati and
516 Voordouw, 2003); and for fractured rock including dolerite (MacLachlan, 2017),
517 dacite (Cuthbert et al., 2013), granite (Minto et al., 2016), fractured sandstone
518 (Phillips et al., 2016, 2013) and fractured limestone (Ross et al., 2001).

519 Mineralogy has been shown to influence $CaCO_3$. Studies have reported
520 increased rates of ureolysis and precipitation after initial calcite deposition, suggesting
521 that *S. pasteurii* preferentially attach to these surfaces over silica, glass or
522 polycarbonate (Tobler et al., 2012; Schultz et al., 2011; El Mountassir et al., 2014).

523 Furthermore, the activation energy required for nucleation is typically greater than for
524 crystal growth (e.g. Rodriguez-Blanco et al., 2011) such that $CaCO_3$ precipitation
525 proceeds more rapidly once calcium carbonate is already present within the system,
526 i.e. arising from an initial MICP treatment or in limestone or marble media.
527
528 Degree of saturation: Lab scale MICP tests are typically performed under fully water-
529 saturated conditions, particularly when permeability change is of interest. However,
530 tests that incorporate a drainage step after bacteria injection and cementation (Amin et
531 al., 2017), or were carried out under unsaturated conditions (Cheng et al., 2013), or
532 took place in the field where saturation state could not be controlled (Cheng and
533 Cord-Ruwisch, 2014; Gomez et al., 2015) often report more uniform $CaCO_3$
534 distribution and greater depth of treatment.
535 Of all the variables explicitly studied, saturation state has the greatest effect on
536 the $CaCO_3$/UCS relationship, with lower degrees of saturation during treatment
537 resulting in greater strength for the same amount of $CaCO_3$ (Figure 5). Cheng et al.
538 (2013) reason that lower saturation concentrates bacteria and reagents at the
539 interparticle contact points. This is likely to be because unsaturated conditions result
540 in a film of liquid occurring at soil particle contact points hence precipitation is
541 concentrated at these contact points where it contributes to strength increase.
542 Furthermore, unsaturated conditions will result in the presence of menisci; bacteria
543 have been observed to preferentially attach at air-water interfaces rather than solid-
544 water interfaces (Schäfer et al., 1998), therefore menisci will promote bacterial
545 attachment.
546

When applying MICP reagents, whether by percolation under gravity or a pressurised injection, for a given flow rate the interstitial (or seepage) velocity will increase as saturation decreases. In a similar manner to increasing the fluid velocity, this ought to have the effect of delivering bacteria and urea further into the media before attachment and hydrolysis occur. This may explain the more uniform $CaCO_3$ distribution and greater depth of treatment observed in samples treated in unsaturated conditions or with unsaturated stages.

Soil structure: Van Paassen et al (2009b) demonstrated that initial dry density influences the relationship between $CaCO_3$ and UCS. In order to achieve the same strength (UCS), a specimen with a lower initial dry density required a greater content of $CaCO_3$ to be precipitated compared to the same material compacted to a higher initial dry density. While for specimens with the same $CaCO_3$ content , that compacted to a higher initial dry density exhibited a higher UCS value (Van Paassen et al., (2009b).

All studies presented in Figure 6 were conducted in sands of differing particle size and grading and all were treated at the core scale (35-100 mm diameter) with the exception of van Paassen et al. (2010) who cut samples out of a large block of treated sand in a 100 m^3 experiment. Terzis & Laloui, (2018) tested a medium and fine sand, and showed that the medium sand achieved considerably higher UCS values (and stiffness) for a given $CaCO_3$ content than the fine sand. This is despite the medium sand being initially more porous (Terzis & Laloui, 2018). They determined via micro-CT scanning that in the medium sand the diameter of the $CaCO_3$ bonds (where $CaCO_3$ bridges particles) created were larger than in the fine sand, reducing inter-particle stresses at contact points, and thus enhancing resistance to shearing. The difference in

behavior for the two specimens may also arise from differences in the sand properties, including for example angularity of the grains, roughness and initial pore structure, all of which could influence bacterial attachment and precipitation.

Recent studies at Arizona State University on Enzymatic Induced Calcium carbonate precipitation have shown an optimum strength for Ottawa 20/30 sand (with a d50 of 400 μm) reaching 1 MPa at just 1% of $CaCO_3$, which would fall to the left even of the trendline plotted for Terzis & Laloui (2018) data presented in Figure 6. These studies indicate that initial porosity, the distribution of contact points and area of contact points, in conjunction with the size and distribution of calcite crystals precipitated influences the strength achievable via MICP treatment.

Environmental conditions

Influence of oxygen concentration: *S. pasteurii* is an obligate aerobe yet conflicting results have been found as to the influence of oxygen on the rate of ureolysis. Mortensen et al. (2011) report higher rates of conductivity change (a proxy measure for ureolysis) for anoxic conditions, as compared with oxic conditions. Tobler et al. (2011) found no significant difference in ammonium production (measured by Nessler assay) when aerobically cultured *S. pasteurii* were injected into oxic and anoxic groundwater.

Parks (2009) found lower growth rates for *S. pasteurii* grown under anaerobic conditions but comparable rates of pH change were observed suggesting comparable rates of ureolysis for aerobic and anaerobic media. When exposed to oxygen, bacterial population growth in the anaerobic media increased, indicating viable cells had survived, but the author notes that growth without oxygen could not be conclusively shown. Whereas Martin et al. (2012) found that S. pasteurii would not actively grow under anaerobic conditions, but that there was still urease activity. These studies

indicate that bio-stimulation (i.e. growth of indigenous ureolytic bacteria) may be problematic in subsurface conditions with limited oxygen supply.

Pressure: *S. pasteurii* has been shown to continue to grow and hydrolyse urea at pressures from 7.5 to 10 MPa and at temperatures between 30 and 40ºC (Mitchell et al., 2013; Verba et al., 2016). Cunningham et al. (2014) reduced the permeability of a 25.4 mm diameter Berea sandstone core at 7.6 MPa whilst Phillips et al. (2016) decreased injectivity into a fractured sandstone around a 341 m deep well where pressure reached 8.3 MPa and downhole fluid temperature was 24.5ºC. Mitchell et al. (2013) slowly increased pressure to 7.6 MPa over 20 days so as to allow the bacteria to acclimatize whilst the other researchers do not appear to have taken this precaution.

Temperature: Increasing temperature acts to increase the rate of ureolysis, for example Van Paassen (2009) found that between 5°C and 70°C the rate of ureolysis doubled approximately every 8°C. However as the ureolysis is driven by the urease enzyme, increasing temperatures leads to denaturation of the enzyme. Illeová et al., (2003) demonstrated using Jack bean urease that all enzyme activity was lost after 40mins exposure to a temperature of 87.5°. Zhong and Islam (1995) found *S. pasteurii* cultivated at room temperature required five days to adapt to a temperature of 50ºC but ultimately more $CaCO_3$ was precipitated at 50ºC. Cheng et al. (2017) also found increased $CaCO_3$ precipitation at higher temperatures, but noted that strength increase was less efficient. Conversely, Wu et al., (2017), investigated urea hydrolysis in the absence of a calcium source, and found decreasing rates of ammonium production at temperatures above 30ºC with no ammonium production at 50ºC.

623 Combination of environmental factors: Environmental factors, including e.g.
624 temperature, pressure, salinity, which may influence MICP are numerous and are
625 interlinked. Furthermore they are also impacted by the injection strategy used. As
626 such at this point it remains unclear from the limited studies presented in the literature
627 on environmental factors as to the individual influence of these parameters on the
628 resulting behavior of MICP treated soil/rock.

629 Indeed, when reviewing data from the literature, it was often clear that there
630 were many combined variables influencing the differences in mechanical behavior
631 observed. Figure 7 presents the UCS vs $CaCO_3$ for all studies (in grey) and the
632 outliers of all the datasets are highlighted (Van Paassen et al., 2010 and Terzis &
633 Laloui, 2018). Terzis & Laloui (2018) were able to achieve a given unconfined
634 compressive strength at lower calcite contents indicating a more efficient process.
635 Some of the main differences listed between these two studies are highlighted: (i) the
636 urease activity used by Terzis & Laloui (2018) was an order of magnitude lower at
637 1.7mM/min compared to the 18.3mM/min used by Van Paassen et al. (2010), (ii)
638 Terzis & Laloui injected multiple cycles building up layers of calcite precipitation
639 (Terzis et al., 2016), whereas Van Paassen used a single injection sequence (bacteria,
640 followed by fixative, followed by cementing solution), (iii) Van Paassen used whole
641 cells, whereas Terzis & Laloui used lyophilized cells, which may also influence
642 enzyme kinetics (Lauchnor et al., 2015; Graddy et al., 2018; Fidaleo and Lavecchia,
643 2003; Stocks-Fischer et al., 1999) . This illustration demonstrates that many different
644 variables play a role in selecting suitable strategies for the deployment of MICP in
645 geotechnical engineering applications.

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647
648
649

650 **3.1.4 Challenges and limitations**

651 *Uniformity*

652 Uniformity of treatment remains a challenge for MICP. Due to the transport
653 and retention of bacteria and consumption of reagents, it is possible to end up with a
654 greater concentration of cells close to the injection point and a gradient in $CaCO_3$
655 precipitation from inlet to outlet. Due to the low viscosity of the MICP solutions,
656 injected material first follows existing preferential flow paths which can lead to
657 inhomogeneous treatment and potentially, pockets of untreated media.

658 However, MICP has been demonstrated to be effective in columns of 5 m
659 length (Whiffin et al., 2007) and in 100 m^3 radial injection experiments (van Paassen
660 et al., 2010). Methods to improve treatment uniformity are 1) radial injection (which
661 is common in field trials, as opposed to linear injection most often used in lab scale
662 experiments) which increases velocity in the vicinity of the well thus decreasing
663 bacterial attachment, 2) lower the pH of the urea/$CaCl_2$ cementing solution (typically
664 to 6.5) to introduce a delay between urea hydrolysis and $CaCO_3$ precipitation, and 3)
665 multiple injection cycles of bacteria followed by cementing solution, possibly with
666 lower reagent concentrations, as each cycle will distribute additional bacteria the
667 soil/rock and, hence, treat a different region of the porous/fractured media as flow
668 paths evolve in response to clogging of the pore space with $CaCO_3$.

669

670 *Monitoring*

671 For ground improvement by MICP, monitoring of where, and to what extent,
672 treatment has occurred will be critical. This is also true for ground improvement with
673 traditional cement grouts, however, an empirical body of knowledge has accumulated

674 for cement grouts through their use over hundreds of years which will not initially be
675 available for MICP.

676 At the lab scale, measurement of properties such as changes in mass,
677 permeability, shear-wave velocity and X-ray attenuation are effective at establishing
678 treatment effectiveness (DeJong et al., 2006; Minto et al., 2017). At field scale,
679 traditional geophysical monitoring techniques such as ground penetrating radar,
680 electrical resistivity tomography, soil self-potential, ultrasound and seismic surveys
681 may prove effective, together with monitoring injection pressures, cross-hole
682 conductance testing (Cuthbert et al., 2013) and NMR well monitoring (Kirkland et al.,
683 2017).

684

685 *Modelling and predicting*

686 Several models have been produced to fit lab-scale and field experimental
687 data. These mostly use simplified geochemistry in 1D (Ebigbo et al., 2012; Fauriel
688 and Laloui, 2012; Hommel et al., 2016; Martinez et al., 2014) or 2D (Cuthbert et al.,
689 2013; van Wijngaarden et al., 2016). Those that use more complete geochemical
690 models such as PHREEQC are limited to 1D (Barkouki et al., 2011; Dupraz et al.,
691 2009; Wu et al., 2011) or 2D with between four (Qin et al., 2016) and 17 (Zhang and
692 Klapper, 2010) reactive species.

693 Published 3D models are limited to Nassar et al. (2018) which, together with
694 van Wijngaarden et al. (2016) and the authors' own as yet unpublished model (Figure
695 8) may be the only models with sufficiently complex reactive transport and flexible
696 boundary conditions together with simplified and tractable geochemistry to be of use
697 at field scale.

698 Given the complex nature of the MICP process, reliable predictive models for
699 field-scale do not currently exist. These engineering models allow us to explore the
700 consequences of a range of possible injection strategies in silico, with the aim of
701 narrowing them down to those worth testing experimentally.
702
703 *By-products*
704 The main by-product of MICP is ammonia/ammonium (often in the odourless
705 form ammonium chloride) which is considered a groundwater pollutant that is toxic to
706 aquatic organisms and can cause algal blooms at high concentrations. In order to gain
707 regulatory approval, Cuthbert et al. (2013) had to extract from a separate borehole at
708 five times the rate of injection so as to collect the majority of ammonium produced in
709 their field trial. Esnault-Filet et al. (2012) collected ammonium chloride and paid for
710 treatment of it at a local wastewater treatment works. Other field tests do not report
711 any regulatory requirement to collect, treat, or limit the production of ammonium
712 (Gomez et al., 2015; Phillips et al., 2016) and this is likely to reflect whether or not
713 MICP is being carried out in a sensitive environment or close to drinking water
714 supplies.
715
716 *Upscaling*
717 For MICP to make the jump from field trials to a practical engineering ground
718 improvement method, it will be necessary to massively upscale the process.
719 Preparation of the cementing solution should pose no issue as $CaCl_2$ is available in
720 large quantities either as food grade or industrial grade (e.g. road de-icing salt) and
721 urea is mass produced as fertiliser. Both could be transported dry and mixed to the
722 desired concentration on site.

723 Growth of bacteria may be more challenging to upscale, however two
724 promising methods have been tested in the field: stimulation of naturally occurring
725 ureolytically active bacteria in the ground (biostimulation) which requires no special
726 bacteria culturing equipment nor transport and handling of bacteria (Gomez et al.,
727 2018); or the approach demonstrated by (Van der Star et al., 2009) who started from a
728 moderately large volume (100 L) of pure-strain *S. pasteurii* grown under sterile
729 conditions in the lab which was used as a seed culture to inoculate a 5 m^3 on-site bio-
730 reactor (bioaugmentation). In this case, less than sterile growth conditions were
731 acceptable because ureolytically active bacteria tend to out-compete other strains
732 when ammonia is present or urea is available (Graddy et al., 2018) and the initial
733 concentration of *S. pasteurii* added to the bio-reactor would likely be orders of
734 magnitude greater than that of any competing strains.
735
736
737 **3.2 Microbially induced carbonate precipitation via denitrification**
738 **3.2.1 Process**
739 Whilst MICP by urea hydrolysis is the process most widely studied, for a
740 range of engineering applications (Phillips et al. 2013), there are various other
741 processes which may result in precipitation of calcium carbonate, among which
742 denitrification based MICP is considered the most promising (Van Paassen et al,
743 2010b). As part of the nitrogen cycle, denitrification (also known as dissimilatory
744 reduction of nitrate) is a process naturally occurring in the subsurface, in which
745 organic matter is oxidized to inorganic carbon and nitrate is reduced to nitrogen gas.
746 The reduction of nitrate (NO_3^-) to nitrogen gas (N_2) goes through several
747 intermediate reactions, which involves specific enzymes and the formation of

748 intermediate nitrogen compounds: nitrite (NO_2^-), nitrous oxide (N_2O), and nitric oxide
749 (NO) (Rebata-Landa and Santamarina, 2012). Accumulation of these intermediates
750 should be avoided as nitrite and nitric oxide are toxic and inhibit microbial growth
751 and nitrous oxide is a very strong greenhouse gas (Almeida, Julio et al. 1995; Chung
752 and Chung 2000; Zumft, 1997; Madigan et al. 2012, Pham et al, 2016). In order to
753 enable the efficient and full reduction of nitrate to nitrogen gas, selecting the right
754 substrate composition is essential (O'Donnell 2016, Pham et al. 2016). Too much
755 nitrate may lead to accumulation of intermediate compounds, whilst leaving a large
756 excess of organic substrate would be inefficient.

757 Although various organic substrates can be used to stimulate denitrification in
758 the subsurface, most studies have used a solution containing calcium acetate and
759 calcium nitrate (Van Paassen 2009; Van Paassen et al. 2010; Van der Star et al., 2012;
760 Kavazanjian et al., 2015, Hamdan et al. 2017; Pham et al. 2016), for which the
761 catabolic reaction is written as:

762

763 $$C_2O_3H_2^- + 1.6NO_3^- + 0.4H_2O \rightarrow 0.8N_2 + 2HCO_3^- + 0.6OH^- \quad (4)$$

764

765 This catabolic reaction provides the energy for indigenous denitrifying micro-
766 organisms to grow. At maximum growth, a significant amount of substrates will be
767 converted to biomass. The resulting metabolic reaction at maximum growth can be
768 written as (van Paassen et al., 2017, Pham 2017):

769

770 $$1.21C_2H_3O_2^- + 0.97NO_3^- + 0.17H_2O \rightarrow CH_{1.8}O_{0.5}N_{0.2} + 0.39N_2 + \quad (5)$$

771 $$1.41HCO_3^- + 0.76OH^-$$

772

773 The actual growth rate is often limited due to limited availability of substrates,
774 nutrients or trace elements, or due to accumulation of intermediate compounds. As a
775 result the actual metabolic reaction stoichiometry varies between conditions of
776 maximum growth (5) and zero growth, which corresponds to the catabolic reaction
777 (4).

778 By using soluble calcium salts as substrates, the produced inorganic carbon
779 precipitates as calcium carbonate:

780

781 $Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+$ (6)

782

783 Calcium carbonate ($CaCO_3$) precipitation buffers the pH as it consumes the alkalinity
784 produced by reduction of the nitrate. Maintaining a stable pH helps to prevent the
785 accumulation of toxic intermediate nitrogen compounds and stimulates microbial
786 growth (Pham et al., 2016). O'Donnell (2016) showed that a mixed microbial
787 community developed by bio-stimulation in a natural soil was more efficient at
788 denitrification than a pure culture of a well-known denitrifying bacteria, pseudomonas
789 denitrificans.

790

791

792 **3.2.2 Hydro-mechanical behavior and applications**

793 Similar to biomineralization by urea hydrolysis, $CaCO_3$ precipitation by
794 denitrification can reduce soil permeability by filling up the pore space or increase
795 soil strength, stiffness and dilatancy by coating and roughening the soil particles or
796 creating cementitious bonds at the particle contacts (Figure 9). O'Donnell et al. (2017)
797 reported that $CaCO_3$ precipitation of 1 to 2% (by mass) was sufficient to increase

798 cyclic shear strength in cyclic direct simple shear tests by 40% on both natural and
799 laboratory standard sands. Pham et al. (2018) found that treatment resulting in a
800 $CaCO_3$ content of 0.65% more-than-doubled the small strain stiffness under static
801 compressive loading conditions. Through shear wave velocity measurements
802 O'Donnell (2016) observed that sands treated by denitrification showed a greater
803 improvement in the shear stiffness of the soil when compared to ureolysis-treated
804 specimens at the same carbonate content. This was attributed to bigger calcite crystals
805 due to the slow rate of precipitation via denitrification. Precipitation was also more
806 dominant at inter-particle contacts due to interaction between gas bubbles and
807 precipitation. O'Donnell (2016) also showed that after failure, when samples treated
808 by MICP via denitrification were de-aggregated and reconstituted they retained some
809 increase in static and cyclic strength and stiffness (compared to untreated soils),
810 which was attributed to particle surface roughening.
811
812 **3.2.3 Challenges and limitations**
813 While recent results for urea hydrolysis have shown that ureolytic bacteria can be
814 stimulated in situ, in most cases MICP through urea hydrolysis still requires ex situ
815 cultivation and injection of (specific) ureolytic bacteria. The main advantage of MICP
816 by denitrification is that the process does not require ex situ cultivation. The substrate
817 solution will stimulate indigenous denitrifying bacteria. Secondly, if nitrate is
818 completely reduced to nitrogen gas the process does not leave any toxic by-products.
819 The absence of a harmful by-product (e.g., ammonium chloride) is another potential
820 advantage of denitrification over ureolysis. However, compared to urea hydrolysis,
821 MICP via denitrification is a relatively slow process (Martin et al. 2013; Van Paassen
822 et al. 2010) Van Paassen et al. (2010b). For continuously cycled substrate solutions,

823 over a period of 100 days, Van Paassen et al. (2010b) reported precipitation ranging
824 from 1 to 9.5% $CaCO_3$ (by mass). O'Donnell et al (2017) required 30 flushes over a
825 period of 400 days to precipitate approx. 2.5% $CaCO_3$. Pham et al. (2018) aimed to
826 optimize treatment protocol and showed that using a large number of flushes with low
827 concentrated substrate solution resulted in a more efficient conversion than a low
828 number of flushes with high concentrated substrate solution, they obtained 0.65%
829 $CaCO_3$ in 15 flushes in 35 days. The low rate at high concentrations may be the result
830 of inhibition by toxic intermediates or limited substrate availability. This implies that
831 a lower initial nitrate concentration provides a more efficient environment for MICP
832 via denitrification (Hamdan et al. 2017). The consequence of the low reaction rate and
833 the preferred use of low concentrations is that a larger volume of solution needs to be
834 injected and a long treatment time is required. Another result of the low reaction rate
835 is that the precipitation process generates a relatively low number of large crystals.
836 The effect of crystal size and distribution on the mechanical performance still requires
837 further investigation. Another challenge to be solved is the interaction between the
838 different product, $CaCO_3$ minerals, nitrogen gas and biomass. Although by-products
839 of the denitrification reaction are not toxic, they do affect the hydro-mechanical
840 behavior of soils and may affect the crystallization process. For example, during the
841 experiments reported by Pham et al. (2018), hydraulic conductivity reduced
842 significantly, which was mainly attributed to the combined formation and entrapment
843 of nitrogen gas and biomass.
844
845
846
847

848 **3.3 Biogenic gas formation via denitrification**

849 **3.3.1 Process**

850 Although biogenic nitrogen gas may be considered as a by-product of MICP via
851 denitrification, as described in the previous section, several recent studies have
852 investigated the potential use of biogenic nitrogen gas alone for ground improvement
853 (He et al. 2013; He and Chu 2014; Kavazanjian et al. 2015, Pham et al. 2016,
854 O'Donnell, 2017a). The most common biogenic gases that are formed in the
855 subsurface are methane (CH_4), nitrogen (N_2), hydrogen sulphide (H_2S), and carbon
856 dioxide (CO_2). These gases are the product of metabolic processes of microorganisms.
857 As nitrogen gas has a low solubility and is neither toxic nor a greenhouse gas,
858 biogenic production of nitrogen gas is considered to be the most appropriate candidate
859 for ground improvement via biogenic gas generation (Van Paassen et al. 2017). As
860 shown in the previous section, the amount of nitrogen gas produced depends on the
861 metabolic conversion. Depending on the growth rate of the bacteria the yield of
862 nitrogen gas over nitrate (N_2/NO_3^-) ranges from 0.4 to 0.5. However, the volume of
863 produced gas depends on the solubility, bubble size, pore pressure and partial pressure
864 of the gas phase. Van Paassen et al. (2017) presents a theoretical framework for
865 estimating the volume of gas produced by a biogenic process and the resulting degree
866 of saturation, combining Henry's law and the ideal gas law. The results show that for
867 a given amount of produced substrate consumption the resulting gas saturation
868 decreases with depth, due to an increase in pressure and gas solubility.

869

870 **3.3.2 Hydro-mechanical behavior and applications**

871 The presence of entrapped biogenic nitrogen gas in the pore volume may significantly
872 affect the hydro-mechanical behavior of the soil. The presence of gas can significantly

873 reduce the hydraulic conductivity of soils, even if it fills up a small fraction of the
874 pore space (Ronen et al., 1989; Baird & Waldron, 2003; Mahabadi and Jang, 2014;
875 Mahabadi et al. 2016). Biogenic nitrogen gas production may also mitigate both static
876 liquefaction (He and Chu 2014; Pham et al. 2016) and earthquake-induced
877 liquefaction (Rebata-Landa and Santamarina 2012; He et al. 2013; Kavazanjian et al.
878 2015). The gas phase increases the compressibility of the pore fluid (Biot, 1941;
879 Tsukamoto et al. 2002; Ishihara et al., 2004), which dampens pore pressure build up
880 during monotonic and cyclic undrained loading (Yang et al. 2004; Yegian et al. 2007,
881 He and Chu 2014) It has been shown that small levels of desaturation can increase
882 liquefaction resistance significantly (Ishihara and Tsukamoto, 2004; Okamura and
883 Soga, 2006). For example He et al. (2013) demonstrated that by desaturating a clean
884 coarse sand through denitrification, to a degree of saturation of 80 to 95%, they could
885 significantly dampen pore pressure build up, prevent loss of bearing capacity and
886 significantly reduce settlements arising from surface loading. O'Donnell (2016)
887 reported reaching a degree of saturation of approximately 94% via biogenic gas
888 formation within 1 to 3 days in laboratory columns using a clean, uniform medium
889 fine sand and demonstrated that a 40% increase in cyclic shear strength was obtained
890 upon cyclic simple shear testing of specimens at this degree of saturation.
891
892 **3.3.3 Challenges and limitations**
893 The potential of using biogenic nitrogen gas to reduce hydraulic conductivity or to
894 increase liquefaction resistance seems promising. Particularly because the amount of
895 substrates required to generate a significant amount of desaturation is very low. A
896 single flush containing 50 mM dissolved nitrate is sufficient to fill up 48 to 60% of
897 the pore volume with nitrogen gas close to the surface or 14 to 16% of the pore

898 volume at 25 m below the groundwater level. Another advantage of microbially
899 induced desaturation through denitrification is that desaturation can be achieved over
900 large areas through bio-stimulation of indigenous soil bacteria, which can reduce
901 some of the challenges encountered when using bioaugmentation, enhancing gas
902 distribution compared to abiotic gas injections. However, in order to rely on the gas
903 phase to improve liquefaction resistance, long-term persistence of the gas phase must
904 be ensured. Although Okamura et al. (2006) and Eseller-Bayat et al. (2013) reported
905 that abiotically induced desaturation can persist for periods of several years, the gas
906 may escape through upward migration and/or dissolution or through convective and
907 diffusive transport through groundwater. The amount of gas which can be trapped in
908 the pore space depends on the pore size distribution and connectivity between the
909 pores. When gas bubbles are smaller than the pore throats between the grains, they
910 may easily migrate upwards due to buoyancy. Once the bubbles increase in size they
911 may get trapped at pore throats. If additional gas is being produced the bubble can
912 only migrate further if pressure in the bubble exceeds the capillary pressure or air
913 entry pressure required to squeeze through the pore throat. In this way the gas phase
914 gradually forms a network of gas filled pores, until it finds a zone of higher
915 permeability, which allows the gas network to vent and rapidly migrate upward. If
916 upward migration is restricted by a low permeability layer (e.g. clay), gas pockets
917 may form, and if the gas pressure exceeds the overburden pressure then cracks may
918 form in the soil as the soil above the gas pocket may be lifted up (Sobkowicz and
919 Morgenstern, 1984; Grozic et al., 1999; Leroueil et al. 2015). An excess amount or
920 sudden rapid venting of trapped gas may reduce bearing capacity and is considered a
921 major hazard for offshore foundations. Considering the durability of the gas phase and
922 that its potential to mitigate liquefaction may be limited, a number of authors suggest

923 the use of biogenic gas formation as the first step in a combined two-stage process of
924 desaturation and carbonate precipitation via denitrification (O'Donnell, 2017a,b). In
925 particular, this has been considered for mitigating liquefaction, where gas formation
926 provides enhanced resistance in the short term and calcium carbonate precipitation
927 provides enhanced resistance in the long term (Kavazanjian et al. 2015; Khodadadi et
928 al. 2017; O'Donnell 2016).
929
930
931 **3.4 Fungal hyphal networks**
932 **3.4.1 Introduction**
933 The benefits of harnessing bacterial processes in soils are now being widely
934 investigated within the geotechnical engineering community. Fungi, however, despite
935 accounting for up to 25% of the biomass on earth (Miller, 1992) are rarely considered,
936 and only in a problematic context (e.g. human exposure to molds, Geostrata, 2003).
937 However, of the 99,000 known fungal species, less than 0.3% are pathogenic to
938 humans and animals and less than 10% are capable of colonising plants; an even
939 smaller fraction of these are plant pathogens (Carris et al., 2012).
940 The classification of fungi into phyla, historically considered to include
941 Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota (e.g. Webster and
942 Weber, 2007) is continuing to change as research provides more evidence for further
943 differentiation and exapansion of the kingdom (introduction of Glomeromycota and
944 Microsporidia phyla). Regardless of their classification, soil fungi can generally be
945 considered as falling into the following main categories: (i) saprotrophic (i.e.
946 decomposers) that digest dead organic matter (dead wood, leaf litter producing fungal
947 biomass, carbon dioxide and other compounds such as organic acids, which are of

948 critical importance for nutrient cycling in soils, (ii) pathogenic or parasitic fungi that
949 colonise hosts (e.g. plants or other organisms) causing disease and (iii) fungi that exist
950 in symbiotic relationships these include mycorrhizal fungi (ectomycorrhizal and
951 arbuscular mycorrhizal) which live in a mutually beneficial symbiotic relationship
952 with plants increasing their uptake of nutrients and water (e.g. nitrogen and
953 phosphorus) and protecting against soil pathogens, and lichens which live in
954 symbiotic relationships with algae and cyanobacteria (Jeffries et al., 2003; Konhauser,
955 2007; Hoorman, 2011).

956 Fungi have widely ranging morphologies from single-celled yeasts to multi-
957 cellular fungi, that is, fungi that predominantly grow through the development of
958 hyphae. Hyphae are multi-cellular tube-like structures, consisting mainly of chitin (a
959 polysaccharide containing nitrogen), typically with diameters in the range of 1 – 30
960 μm and lengths from several microns to several metres (Islam et al., 2017). Hyphae
961 can branch into multiple hyphae, and, anastomose creating complex three-
962 dimensional networks. The mass of branching hyphae is known as the mycelium. A
963 densely packed mass of hyphae can form into sclerotia, consisting of a hardened
964 aggregated mass of hyphae containing food reserves. Sclerotia may form when
965 nutrients are scarce, although other stimuli can also trigger their formation (Money,
966 2016).

967

968 **3.4.2 Fungi-soil interactions**

969 Fungi are known to play an important role in soil aggregation, both in the
970 formation of aggregates and in maintaining aggregate stability (Lynch and Bragg,
971 1985, Rillig, and Mummey, 2006). From an agricultural perspective soil aggregate
972 stability is important for maintaining transport of air, water and nutrients within the

973 soil. From a geotechnical engineering perspective the aggregation of soils influences
974 their hydraulic behavior (i.e. permeability and water retention capability) (e.g. Juang
975 & Holtz, 1986, Barbour, 1998, Vanapalli et al., 1999) and their mechanical behavior
976 (Barden & Sides, 1970; Alonso et al., 1987). Although it is widely acknowledged that
977 aggregated soils are encountered within geotechnical engineering (e.g. Collins &
978 McGown, 1974, Alonso et al., 1987) little, if any, consideration has been given to the
979 role of microorganisms in the formation or stability of aggregates in this context.
980 Studies by soil and agricultural scientists have observed increased size of
981 aggregates formed in soils inoculated with fungi and enhanced resistance to
982 breakdown upon wetting, for a range of different fungal species including mycorrhizal
983 and saprotrophic species (e.g. Tisdall and Oades, 1979; Tisdall and Oades, 1982;
984 Degens et al., 1996; Caesar-TonThat and Cochran, 2000, Caesar-ThonThat, 2002,
985 Peng et al., 2013). Rillig & Mummey (2006) outline three categories of mechanisms
986 by which fungi (focused on arbuscular mycorrhizal fungi, AMF) can contribute to soil
987 aggregate stability: (i) Biophysical, (ii) Biochemical and (iii) Biological mechanisms.
988 The biophysical influence of fungal hyphae is similar to the action of plant
989 roots (although at a smaller scale) where hyphae act to enmesh and entangle soil
990 particles, binding micro-aggregates together (Tisdall & Oades, 1982). The effects of
991 plant roots are well-studied, they bind soil particles and aggregates together providing
992 an additional apparent cohesion against shearing (Stokes et al., 2009). The level of
993 reinforcement provided is dependent on root tensile strength and root architecture
994 (e.g. root diameter, root length density). Greater shearing resistance is provided by
995 many smaller diameter roots than by a smaller number of larger diameter roots, where
996 the fraction of the soil plane occupied by the plant roots is the same. (Stokes et al.,
997 2009). By drawing similarities with plant root reinforcement literature, the mechanism

998 by which fungal hyphae bind particles and aggregates might also be expected to
999 depend on the morphological properties of the fungal networks (e.g. hyphae diameter,
1000 density, and interconnectivity) and the tensile strength of the different strains of
1001 fungal hyphae (Rillig & Mummey, 2006). However, little is known of how these
1002 properties vary between different species and strains. Hyphae may also be
1003 hypothesised to contribute to water transport and retention in soils, ultimately
1004 inducing wetting and drying cycles on a localised-scale (Rillig & Mummey, 2006)
1005 which may influence binding of soil particles to hyphae and influence mechanical
1006 behavior of micro-aggregates; these effects remain largely unexplored. Additionally,
1007 the growth of fungal hyphae have been observed to influence soil structure by
1008 aligning clay particles along hyphae, due to the stress exerted on soil particles during
1009 growth, possibly even forming micro-aggregates (Rillig & Mummey, 2006).

1010 In terms of synthetic fibers, it has been widely reported in geotechnical
1011 engineering that the addition of fibers increases soil strength (i.e. compressive, shear
1012 or tensile strength at failure) and increases strain to failure (i.e. increased ductile
1013 behavior) (e.g. Ranjan et al., 1996; Santoni et al., 2001, Michalowski & Čermák,
1014 2003). The reinforcing effect increases with increasing fiber content (up to a limit)
1015 and increasing aspect ratio (length/diameter) (e.g. Michalowski & Čermák, 2003).
1016 Fungal hyphae can be considered to be micro-scale roots with a very high aspect ratio.
1017 Furthermore, unlike synthetic fibers fungal hyphae may also exhibit anastomosis
1018 forming complex interconnected three-dimensional networks with further potential for
1019 entanglement and enmeshment of soil particles and aggregates.

1020 Soil aggregate formation and stability are also influenced by biochemical
1021 processes. Fungal hyphae are known to secrete biochemical products into their
1022 surroundings (exudates), as well as containing products in their hyphal walls, that may

after decomposition persist in the soil (Rillig and Mummey, 2006). Chenu (1989) demonstrated that scleroglucan (a fungal polysaccharide) improved the stability of kaolinite and montmorillonite aggregates, and increased clay porosity. Glomalin-related soil protein has been correlated with soil aggregate stability for AMF amended soils (e.g. Wright and Upadhyaya, 1996, 1998; Rillig 2004) and is thought to act as a 'glue-like' substance. Studies by Caesar-TonThat & Cochran, (2000) and Caesar-ThonThat, (2002) on a saprotrophic species highlighted the importance of insoluble extracellular compounds polysaccharides on the water stability of aggregates amended with a saprotrophic fungus. Comparing aggregate stability for soils inoculated with fungi with those inoculated with liquid media in which the microorganisms were grown, demonstrated that the binding agents remain in close association with the hyphae and are not excreted into the liquid/soil media (Aspiras et al., 1971).

Filamentous or mycelia-forming fungi such as those belonging to the Ascomycota Basidiomycota phyla are also known to secrete proteins called hydrophobins (Wessels et al., 1991; Wessels, 1996). Hydrophobins play varied roles in the functional processes that occur throughout the growth and life cycle of fungi including, modification of environmental conditions to allow sporulation and aerial hyphae formation (Wessels, 1996; Wösten et al., 1999; van Wetter et al., 2000), mediation of hyphal attachment to surfaces, substrate colonisation (Wösten et al., 1994; Temple et al., 1997) and involvement in the production of fruiting bodies (Lugones et al., 1999). Hydrophobins self-assemble at surficial interfaces forming amphipathic (or amphiphilic) layers capable of altering surface wettability. Given the role of hydrophobins in aiding fungal hyphae attachment to surfaces, and the role in

altering surface properties, it is envisaged that these proteins also play a role in soil aggregation (Rillig & Mummey, 2006).

Finally, in terms of biological mechanisms, fungi may influence the location and density of microbial populations in the soil, for example exudates may act as substrates for bacterial growth, which could also impact on the formation or stability of soil aggregates (Rillig & Mummey, 2006).

The extent of the role played by each mechanism within a given soil will be highly dependent on the fungal type and species (or indeed community as a whole) and the soil composition, grain size and pore size distribution. For example, Aspiras et al., (1971) demonstrated by sonicating fungal inoculated aggregates, that aggregate stability was not greatly reduced, despite the hyphal network being disrupted, concluding that the role of binding substances, (mainly polysaccharides) is more important than the physical entangling effect of the hyphae for clayey soils (where clay content was >25%). Whereas Degens et al., (1996) demonstrated for sandy soils that aggregation could be attributed to increases in hyphal length, with hyphae observed via Scanning Electron Microscopy to cross-link sand grains together via short hyphal lengths. Furthermore Degens et al., (1996) observed no difference between the hot-water extractable carbohydrate carbon content of aggregated and non-aggregated soils, indicating that microbial polysaccharides were not in this case the dominant mechanism controlling aggregation. What is not yet clear is how aggregations on a local scale, formed or maintained stable via fungal activity, may influence the bulk hydraulic and mechanical behavior of soil.

3.4.3 Hydro-mechanical behavior and applications

Fungi are ubiquitous in soils and the observations of fungi soil-interactions outlined above support the proposal that fungal growth could indeed be engineered for geotechnical engineering applications. To date, the use of fungi for soil improvement applications has been largely limited to the combined study of plant-mycorrhizal systems (e.g. Mardhiah et al., 2016; Graf & Frei, 2013, Jeffries et al., 2003), in eco-engineering studies. The introduction of mycorrhizal fungi has mainly been considered as a means to enhance plant growth for successful re-vegetation of degraded soil systems following erosion, landslide or desertification (e.g. Requena et al., 2001, Caravaca et al., 2003). The presence of mycorrhizal fungi promotes the formation and stability of aggregates acting as stores for nutrients and water for plant growth (Tisdall & Oades, 1982), thus accelerating and aiding plant colonisation (Graf & Frei, 2013, Jeffries et al., 2003, Peng et al., 2013). Furthermore, mycorrhizal have been shown to increase root production, root length density and for some species even enhance plant root tensile strength (Stokes et al., 2009). Peng et al., (2013) demonstrated that independent of the involvement of plant roots, hyphal networks have a positive impact on the stability of soil aggregates. The mechanisms by which arbuscular mycorrhizal fungi may influence soil aggregations are expected to be similar for other types of fungi (Rillig & Mummey, 2006). Furthermore, considering that binding substances are known to be closely associated with hyphal surfaces for a range of fungal types (Aspiras et al., 1971), it is proposed that other fungal species could by themselves also be considered for soil improvement applications, for example to enhance resistance against water or wind-induced erosion (Tisdall et al., 2012; Mardhiah et al., 2016;).

Researchers at the University of Strathclyde (El Mountassir and Salifu) have been investigating the hydro-mechanical behavior of fungal inoculated soils over the past two years. Early results based on engineering the growth of *Pleurotus ostreatus* demonstrate that fungal hyphae can result in the enmeshment and entanglement of sand particles (Figure 10A), with hyphae and sclerotia turning loose sand into a cohesive mass (Figure 10C). Water drop penetration tests conducted on fine sands 6 days after inoculation with *Pleurotus ostreatus*, indicate that the fungal treated sand exhibits extreme hydrophobicity; 10μL water droplets did not penetrate the sand where mycelium growth was visible even after 24hrs (Figure 10B), whereas penetration was immediate (within several seconds) in the non-inocculated control samples. These results are promising for the deployment of fungi in a range of ground engineering applications where enhanced cohesion, or the ability to control surface wettability is desirable.

Finally, for geotechnical applications where greater soil strength may be desirable, than that which can be achieved by hyphae and its associated products alone, fungal biomineralisation processes could be triggered. Fungi are known to play a significant role in mineral formation and transformations in the natural environment (e.g. Gadd 2007, Gadd, 2017) and can induce biomineralisation by nucleating and precipitating minerals, most commonly carbonates and oxalates, on or within cell walls (Gadd, 2007; Gadd, 2017). Some fungi are known to precipitate calcium carbonate extra-cellularly and urease positive fungal strains can also break down urea resulting in the formation of calcium carbonate in a calcium rich environment (Li et al., 2014; Kumari et al., 2016; Li and Gadd., 2017).

Given the vast number of different fungal species and variations in their behavior there is huge scope for their deployment in geotechnical engineering. It is

envisaged that ground improvement technologies incorporating fungi could be relatively cheap given that treatment of soil surfaces could be conducted in a relatively easy manner over potentially large areas.

3.4.4 Summary

The use of fungal hyphal networks in ground improvement is a new avenue of research within biogeotechnics, with many open questions. To begin to investigate the feasibility and limitations of their deployment from an engineering perspective, a better understanding of the possible changes to soil behavior that can be induced by fungal inoculation is needed for a range of fungal species.

4. CONCLUSIONS

During the last 10-15 years, geotechnical engineers have started to consider the use of microbial processes in the development of novel nature-inspired ground improvement technologies. MICP via ureolysis, is the process which has gained the most attention within the geotechnical community to-date, with many research groups worldwide investigating the process and injection strategies for its deployment. It is evident that there are numerous control parameters and variables related to the reagents, flow conditions, the medium in which it is to be deployed and environmental conditions, which all influence the hydro-mechanical behavior of the resulting treated soil or rock volume. These all need to be considered in order to design suitable strategies for its use in geotechnical engineering applications. Other microbial processes also being considered for the manipulation of the hydraulic and mechanical behavior of the ground include MICP via denitrification and biogenic gas formation. Although, it is clear that there remain a whole host of microbial processes

1146 that could be explored by geotechnical engineers. This review outlined one such area
1147 for investigation: the potential engineered growth of fungi in soils.

1148 Aside from the development of new technologies, there is an additional
1149 opportunity for geotechnical engineers to enhance their understanding of existing soil
1150 behavior by considering the role that microorganisms play in the formation of soil
1151 particles and soil structure. In order to achieve this aim and that of novel ground
1152 improvement technologies, increased collaboration between geotechnical engineers
1153 and geomicrobiologists will be required in order to explore more fully a wider range
1154 of microbial processes under both natural and engineered conditions.

1155

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1893 **TABLES**

1894 *Table 1. Control parameters and variables in MICP treatments*

Reagents	Bacteria concentration ± use of fixative Urea and calcium concentrations pH adjustment Urease activity
Injection strategy	Fluid velocity Static treatment periods Single/Cyclic injection
Medium	Porous/Fractured Mineralogy Degree of saturation Soil structure (Grain size & pore size distribution, density) Particle shape & roughness
Environmental Conditions	Temperature Pressure Salinity of pore fluid Anoxic/Oxic

FIGURE CAPTIONS

Figure 1. SEM image of CaCO3 precipitate resulting from urea hydrolysis. Indentations within the CaCO3 are a result of S. pasteurii cells in the process of being encapsulated.

Figure 2. Loose sand before and after treatment with MICP.

Figure 3. Surficial treatment of sand for erosion reduction. White $CaCO_3$ concentrated at the top of the sample forms a low permeability erosion resistant layer that extends approximately 10 mm into the silica sand.

Figure 4. Relationship between $CaCO_3$ content and unconfined compressive strength for all studies (grey circle outlines: data from Al Qabany and Soga, 2013; Cheng et al., 2014, 2013; Choi et al., 2016; Rowshanbakht et al., 2016; Terzis and Laloui, 2018; van Paassen et al., 2010) with comparable urease activity highlighted (Cheng et al., 2017).

Figure 5. Relationship between $CaCO_3$ content and unconfined compressive strength for studies in which saturation was either fully saturated or not recorded (grey circle outlines: (Al Qabany and Soga, 2013; Cheng et al., 2017, 2014; Choi et al., 2016; Rowshanbakht et al., 2016; Terzis and Laloui, 2018; van Paassen et al., 2010) with controlled saturation states highlighted (Cheng et al., 2013).

Figure 6. Relationship between $CaCO_3$ content and unconfined compressive strength for all studies (grey circles) (Al Qabany and Soga, 2013; Cheng et al., 2017, 2014; Choi et al., 2016; Rowshanbakht et al., 2016) with datasets highlighted (Terzis and Laloui, 2018) comparing medium and fine sand.

Figure 7. Relationship between $CaCO_3$ content and unconfined compressive strength for all studies (grey circles) (Al Qabany and Soga, 2013; Cheng et al., 2017, 2014; Choi et al., 2016; Rowshanbakht et al., 2016) with outlier datasets highlighted (Terzis and Laloui, 2018; Van Paassen et al., 2010).

Figure 8A. Schematic representation of the coupled 3D model of MICP treatment processes developed at the University of Strathclyde. B. Predicted $CaCO_3$ precipitation, using the University of Strathclyde model, for MICP treatment using a single injection well within a heterogeneous sand.

Figure 9. Calcite crystals formed via microbial denitrification bridging silica sand grains.

Figure 10A. Hyphae of Pleurotus ostreatus enmeshing sand grains imaged under an optical microscope, B. Growth of mycelium of Pluerotus ostreatus in fine sand 6 days after inoculation with P.ostreatus. Water drop penetration tests showed that water droplets of 10μL did not penetrate even after 24hrs. C. Hyphae and sclerotia of Pleurotus ostreatus binding originally loose sand grains together.