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Investigating the feasibility of implementing microbially induced calcite precipitation to stabilize sand, clay and gold tailings

A research report submitted in partial fulfilment of the requirement for award of the degree of Master of Science in Civil Engineering specialising in Geotechnical Engineering at the University of Cape Town

(Breytenbach, 2016)

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Glossary

Agar:	a substance attained from red algae with a jelly consistency
Culture:	a microbiological culture is a method used to multiply microbial organisms by enabling their reproduction in a predetermined culture medium, the most common being nutrient broths and agar plates
Inoculate:	introducing bacteria to a substance and allowing for acclimatisation
Jack Bean urease enzyme:	a metalloenzyme, which catalyses the hydrolysis of urea to produce ammonia and carbon dioxide.
MIC:	minimum inhibitory concentration (MIC) is the lowest concentration of a chemical preventing the visible growth of a bacterium or bacteria. The MIC varies according to the microorganism and the chemical itself.
Propagate:	the act or action of increasing in numbers a pure culture of bacteria
Sporosarcina pasteurii:	a gram-positive facultative bacterium, formerly known as *Bacillus pasteurii*, with a highly active urease enzyme which consumes urea during metabolic processes. Given a calcium source and urea, during the consumption of urea, the bacterium can precipitate calcite and solidify soils through the process of microbiologically induced calcite precipitation (MICP) or biological cementation.
Triple bottom line:	the environment, society and the economy considered in sustainability
Tris buffer:	tris or tris(hydroxymethyl)aminomethane is an organic compound extensively used in biochemistry and molecular biology as a component of buffer solutions.
USCS:	unified soil classification system.

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Abstract

Microbially Induced Calcite Precipitation (MICP) is an emerging bio-mediated technology which has been successfully applied in soil improvement research. MICP uses the enzyme urease produced from bacteria to breakdown urea into carbonate ions. These carbonate ions combine with free calcium ions to form calcium carbonate, which acts as a bio-cement. MICP presents a unique, sustainable soil improvement solution to the pressing issues resulting from tailings impoundment failures. It has shown potential through increasing shear strength and decreasing porosity in soils.

However, MICP applications in soil improvement outside erosion mitigation in granular soils remain limited. This is similar to the limited use of injection treatment, in comparison to the more prevalent spraying and surface percolation in MICP applications.

This research focused on the efficacy of the developed injection procedure for administering the MICP treatment to increase shear strength and decrease porosity in sand, clay and gold tailings at greater depths and evaluating its feasibility. By determining the efficacy and significance of the treatment in improving the geotechnical characteristics of the soil samples, the methodology can be evaluated for its application as a soil improvement technique.

Results showed successful cementation of the particles of the soils tested with an increase in cohesion of 7.7% and 23.1% for clay, and tailings respectively and an infinite increase in the apparent cohesion of sand from 0 to 20kPa. The response to MICP treatment in terms of the angle of internal friction were inconclusive, where a decrease was observed across the board. This was attributed to complex stress-strain behaviour as well as the particle morphology. A decrease in porosity of approximately 26% in clay and 8% in tailings was observed, whilst sand had an increase of approximately 3%. The increase in porosity in sand was identified as a result of the erosion of the coarse uncemented particles during treatment. The results emphasised the greater success of MICP treatment in more granular soils, with sand achieving the greatest improvement with regard to the apparent cohesion and particle density. Characteristically, the particle sizes of the gold tailings fell between the fine clay and the coarse sand which was reflected in the response of the gold tailings to treatment. Overall, sand had the greatest increase in shear strength, followed by the gold tailings and lastly the clay. The gold tailings contained a higher percentage of fines than the sand, illustrating the limitation of MICP applications in fine grained soils. However, the predominant coarse fraction allowed for an overall increase in the shear strength parameters in the gold tailings.

An evaluation of the feasibility shows that the methods provide a scalable soil improvement technique in stabilisation applications in contrast to existing MICP surface treatments in sands. In clays and tailings however, interactions of heavy metals with the microbial community as well as the particle size limit the efficacy of MICP. In conclusion, MICP is found to be a feasible soil improvement technique in stabilising gold tailings with the consideration of the impact of heavy metals and the particle size on the efficacy of the treatment.

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Dedication

To my beloved Joy, Fikile and Sharon. You have all shaped me into the woman that I am today. Joy, you told me that my dreams are valid and showed me that they are within my reach. Fikile, your lifelong sacrifices and dedication have led me here, and I thank you for everything. Sharon, your unwavering support, and faith in me has helped me to persevere in the most testing times. Thank you all for getting me here and pushing me even further, beyond my own expectations.

"*I stand on the sacrifices of a million women before me thinking what can I do to make this mountain taller so that the women after me can see farther*" – Rupi Kaur

1. Introduction

1.1 Topic of investigation

Bio-mediated soil improvement utilises biochemical reactions which produce calcite precipitates between soil particles, effectively modifying the engineering properties of the soils (Umar et al., 2016). Microbially induced calcium precipitation (MICP), a prime example, is a microbial geo-technological strategy which alters the soil structure through the formation of calcite crystals (Salifu et al., 2016). This research is concerned with determining the feasibility of applying MICP as a soil improvement technique for stabilising gold tailings.

The primary aim entailed determining the efficacy of the developed methodology for the implementation of MICP to improve the shear strength of gold tailings. Sand and clay soils were used to compare the response of the soils to treatment. The established methodology was evaluated according to its feasibility for practical implementation as a soil improvement technique in gold tailings.

1.2 Background

The magnitudes of waste generated by the mining industry reach the scale of natural geological processes, quantified as "several thousand tonnes", per annum (Kossoff et al., 2014). The primary contributor are tailings, which consist of "crushed rock and processing fluids". Tailings are a waste product generated following the extraction of economic materials in mining (Kossoff et al., 2014). These large volumes of tailings produced annually by the mining industry, reaching 14 billion tonnes in 2010, leave a significant environmental footprint spatially in terms of storage and temporally in terms of the design life and management (Adiansyah et al., 2015; Jones & Boger, 2012). Naturally, the vast quantities of materials contained within tailings dams result in, upon failure, catastrophic and costly consequences such as the destruction of property and the contamination of water bodies downstream. Therefore, the need for sustainable practices in the mining sector is highlighted by the growing interest as well as the concerns of the largely irreversible and severe consequences of tailings and other mining operations (Adiansyah et al., 2015; Braun et al., 2017).

In mineral rich African economies, the implementation of sustainable mining practices in the mineral resources sector is largely dependent on the contribution of the extractive industry (Sturman et al., 2020). The obstacle facing this sector, however, is the limited direction and availability of comprehensive formal structures in the daily mine operations and tailings management regarding this (Adiansyah et al., 2015; David Laurence, 2011). By targeting two additional areas, safety and resource efficiency, in addition to the triple bottom line, the issue of sustainability in mining can be addressed (David Laurence, 2011). Additionally, through the optimization of existing technologies and operational structures or alternatively their replacement with more energy efficient and low emission alternatives, significant strides can be made to achieve sustainability in the mining industry (Braun et al., 2017).

The hindrance to this shift in approach, often lies in the increased risk, practically and economically, as well as the limited availability of information or research in alternative technologies. In order to overcome these hurdles, the principal approach is geared towards the generation of a broader knowledge base and the exploration of potential practical applications (Braun et al., 2017). This highlights the relevance for research in this discourse and the need for contributions to the existing knowledge gap.

Worldwide, tailings impoundment failures have resulted in thousands of deaths, the destruction of property, the death of multitudes of aquatic life and the inundation and contamination of surrounding water sources (Kossoff et al., 2014). Evidently, TSF failure poses a significant threat to public health and safety, infrastructure as well as the environment. Hence, the stability of these structures is of paramount importance. The leading causes of failure in active impoundments can be broadly classified under: “foundation, slope instability, overtopping, mine subsidence, unusual rain, snow melt, piping or seepage, seismic liquefaction, structural, maintenance and unknown causes” (Kossoff et al., 2014; Lottermoser, 2010).

Rapid urbanization and exponential population growth shed light on the depletion of resources, including suitable land for development which has become increasingly scarce. Therefore alternative technologies need to be examined for innovative possibilities in soil improvement (DeJong et al., 2006). Issues include eroding foreshores, wind erosion in addition to the need for suitable land for infrastructure pose pertinent geotechnical problems, affecting ecosystems, communities as well as development (Duo et al., 2018; Salifu et al., 2016; Zomorodian et al., 2019). Coastal degradation poses a significant threat to marine ecosystems as well as surrounding property and arable land within the floodplains of connected estuaries (Salifu et al., 2016). Wind erosion is a pressing concern in the agricultural sector in terms of the resulting environment degradation, air pollution and desertification which transforms agricultural regions into sterile lands (Maleki et al., 2016). Therefore, the need for effective soil improvement techniques has become more relevant with a reported increase in the worldwide failure of tailings storage facilities (TSF) alongside the aforementioned availability of land, wind, and coastal erosion.

Existing and widely utilised soil improvement techniques including compaction, displacement-replacement, fill preloading, anchors, soil nails, geosynthetics, mechanically stabilised earth, grouting, mixing, aggregate columns, and lime treatment, present effective and practical solutions to tackle these issues. However, in terms of environmental responsibility, a gap exists in these traditional methods. With growing pressure on the world’s non-renewable resources, the shift toward environmentally responsible practices is increasingly popular. One example is the use of bio-mediated soil improvement techniques, such as MICP.

MICP is an emerging bio-mediated technology which has been successfully applied in soil improvement research. The growing use of these treatments in soil improvement, has displayed “great promise” in terms of improving geotechnical characteristics such as increasing shear strength and decreasing porosity (Umar et al., 2016). MICP has been widely investigated for dust suppression as well as coastal erosion research and has grown in popularity as a surface treatment for granular materials. Therefore, MICP presents a potentially sustainable soil improvement solution to the pressing issues resulting from TSF failures.

1.3 Problem statement

Mine tailings are an abundant waste resource generated by the historically dominant mining sector in South Africa. Managing these structures requires finding solutions to various issues namely: erosion, dust pollution, slope instability and overall failure. Sustainable and effective soil improvement techniques are required in the mining sector for the management and maintenance of tailings facilities. In addition, tailings often contain potentially hazardous contaminants therefore the management and isolation of this material should be a key priority (Kossoff et al., 2014). The risk of contamination of ecologies and potable water sources is high, therefore sustainable preventative measures are needed.

1.4 Research objectives

The research objectives aimed to interrogate the viability of MICP as a soil improvement technique and develop a methodology for the implementation of the technique, specifically regarding clay and gold tailings. The literature review aided in the diagnosis of the research problem of the stabilisation of tailings, the need for environmentally responsible soil improvement techniques as well as the feasibility of MICP as a soil improvement technique. This informed the methodology developed to satisfy these research problems through the investigation of MICP in the stabilisation of the three soils. The methodology was then enhanced through the investigation of the role inoculation and saturation time had on the stability of the soils. This then allowed for the evaluation of the developed MICP technique as a soil improvement technique with respect to sustainability.

1.4.1 General objective

The general research objective entailed determining the feasibility of MICP as a soil improvement technique in gold tailings. The experimental approach intended to meet the general objective through the development of a suitable methodology for administering the MICP technique to the tailings. The technical feasibility of this process was evaluated. The efficacy and, repeatability and optimisation of the developed methodology was evaluated alongside the cost implications of the process to determine its overall technical feasibility as a sustainable soil improvement technique. The general objective was comprised of specific objectives, targeted to satisfy the corresponding research questions posed and accomplish the outlined research outcomes.

1.4.2 Specific objectives

a) Review literature to determine the issues resulting from TSF failure, the currently utilised soil improvement techniques, and the applications of MICP in fine grained soils and evaluate its effectiveness in order to inform the methodology.

b) Develop a methodology to implement MICP as a soil improvement technique in gold tailings, clay, and sand at laboratory scale.

c) Compare and evaluate the effect and efficacy of the MICP technique on the three soil types (sand, clay, and gold tailings) in terms of the impact on the porosity and shear strength.

d) Compare soil improvement techniques alongside MICP in terms of sustainability (environmental impacts, efficacy etc.).

1.5 Major research assumptions and hypotheses

1.5.1 Assumptions

The various concentrations for the treatment media were selected according to the formulas used in previous research which produced optimum results in terms of calcification (de Oliveira & Fahn, 2019; Henze & Randall, 2018; Lambert & Randall, 2019). This was done in order to investigate the impact of inoculation and saturation time on the soil treatment independent of the concentration. The key geotechnical parameters expected to change were the shear strength and the porosity following the MICP treatment which were established as the criteria for soil improvement in this study.

1.5.2 Research hypothesis

MICP was hypothesised to effectively cement the particles of the tailings, and in doing so, increase the shear strength of the material. With calcite precipitation, the porosity of the soil was hypothesised to decrease. In changing these geotechnical characteristics, the structural stability of the material would be improved, the margin of which was to be determined following experimentation. By optimising the methodology through the reduction in inoculation time and saturation, it was postulated that MICP was technically feasible for practical implementation as a soil improvement method for gold tailings.

1.6 Scope and limitations

A detailed literature review exploring the primary causes of TSF failure was required as well as existing soil improvement techniques. This would ensure the relevance of the proposed technique for application was identified. An experimental comparison between the unaltered and MICP strengthened tailings was to be made by interrogating the effect MICP has on the geotechnical properties of gold tailings, clay, and sand. The scope focused on South African gold tailings, sand and clay and the effect the MICP reinforcement had on the structural stability of the materials in terms of porosity and shear strength.

This research encompassed investigating the effect MICP had on the shear strength and porosity of gold tailings. The methodology was limited to laboratory scale application. In an upscaled set-up, the procedure for injection of the treatment media, as well as the depth of penetration would have to be investigated further. In addition, the efficacy of the treatment was limited to the method of application, the evaluation of which falls beyond the scope of this research. The research was limited to a short-term investigation of the behaviour and performance of the treated samples. Ideally, the variation in strength over time would be monitored to determine the durability of the treatment as well as the response of MICP treated clay to moisture in terms of swelling and heaving behaviour.

The efficacy of the treatment as well as the results of the investigation were constrained by the selected bacterial strain and inoculation methods. The selected bacterial strain, *Sporosarcina pasteurii* (formerly *Bacillus pasteurii*), was the only strain of ureolytic bacteria utilised for this research. Similarly, only one inoculation method, open inoculation, was explored. Only one type of tailings, clay and sand was investigated. The behaviour of other tailings and soil types such as copper tailings or silts may vary following MICP treatment. Furthermore, the research is limited in terms of the exploration of alternative calcite precipitation processes besides urea hydrolysis which include aerobic oxidation, denitrification, and sulphate reduction. In terms of the technical and economic feasibility, the study was limited to estimated values and various assumptions. A scaled experiment would be required to determine more accurate values.

This research targets sustainability development goal (SDG) number 9, which aims to build robust infrastructure, endorse sustainable industrialization, and promote innovation. Through research, innovative, environmentally responsible alternatives can be found and implemented for existing processes in industry including the maintenance of tailings.

2. Literature review

2.1 Introduction

Annually, the mining industry generates vast volumes of waste. The waste streams are primarily comprised of tailings, a by-product from the extraction of economic metals, minerals and fossil fuels during mining (Kossoff et al., 2014). The tailings, comprising of crushed rock and processing fluids, are pumped to various facilities where they are often stored as impoundments or dams (Kossoff et al., 2014).

Naturally, with magnitudes of tailings reaching up to several thousand million tonnes annually, the impoundments reach equally immense dimensions (Förstner, 1999; Fyfe, 1981). With at least 3500 of these structures worldwide, ranging between a few hectares to thousands in size, the environmental, social and economic footprint is evidently vast (Kossoff et al., 2014). In addition, recoveries of economic materials are never exact, resulting in small amounts of the minerals alongside the waste materials such as silicates, oxides, carbonates and sulphides remaining in the tailings residue highlighting the potential hazard of the tailings (Lottermoser, 2010). Therefore, failure of these structures can be and often is catastrophic resulting in tremendous consequences financially, environmentally as well as for the surrounding populations. Some of the key issues resulting from TSF failure include the inundation of surrounding areas, the pollution and sedimentation of surrounding water bodies, the contamination and destruction of ecosystems, the loss of human life and the extensive damage of property and infrastructure resulting in costly liability fees and business interruption (Kossoff et al., 2014; Lottermoser, 2010). Evidently, there is a need for innovative and sustainable practices concerning soil improvement in tailings management.

In addition to tailings management, there is a pressing need for innovative and sustainable practices concerning stability in the general soil improvement sphere. The existing conventional methods of soil improvement have major limitations including high energy and natural resource depletion, contribution to greenhouse gas emissions and the use of toxic or hazardous synthetic materials which may be harmful to people as well as the environment (Achal & Mukherjee, 2015; DeJong et al., 2010; Haouzi & Courcelles, 2018). As a result, innovative and novel approaches to soil stabilization, utilizing natural processes with minimal reported environmental impacts are growing in popularity (DeJong et al., 2010).

Biological mediation is a prominent soil improvement system in the sphere of alternative soil improvement methods which promises potential solutions to the challenges posed by TSF stability and conventional soil improvement. This soil improvement approach utilises a manipulated system of chemical reactions mediated by biological processes within a soil which alter the engineering properties through the production of various by-products (DeJong et al., 2010). MICP is a noteworthy example of bio-mediated soil improvement which has been distinguished for its potential for adapting soil properties to fit the required land-uses (Whiffin et al., 2007). During MICP, facultative bacteria such as *Sporosarcina pasteurii* (formerly

Bacillus pasteurii) generate the urease enzyme following metabolic processes which hydrolyses urea and utilises calcium to form bio-cement which bonds the soil particles together creating a solidified mass (Achal & Mukherjee, 2015; Lambert & Randall, 2019; Okwadha & Li, 2010). This process is ideal for application in soil improvement as the densification of the soil mass resulting from the bio-cementation improves the strength and stability of the treated soil (Montoya & De Jong, 2015).

Stabilising soil is the aim of soil improvement and this is achieved by altering the mechanical properties of the soil mass through two primary processes: the mechanical modification which alters the arrangement of the soil particles and the chemical modification through the addition of various synthetic chemicals or materials which alter the soil structure through the formation or deposition of new materials in the soils matrix (Achal & Mukherjee, 2015; Haouzi & Courcelles, 2018). This includes compaction, preloading, consolidation, admixtures or inclusions and unconventional methods such as the use of timber (Briaud, 2014). The efficacy of these methods is evaluated based on the extent to which various mechanical properties are altered or improved. From a geotechnical engineering approach, the strength and porosity are the key properties monitored in order to quantify the improvement of the soil as well as the impact of the MICP treatment in these parameters (Whiffin et al., 2007).

Hence, by developing an experimental methodology of implementing MICP as a soil improvement technique in clay, sand and gold tailings, the changes in the strength, stiffness, and porosity can be quantified. In doing so, the efficacy of MICP as a stabilisation technique can be evaluated based on the margin to which these geotechnical parameters are altered. The technique can be evaluated in terms of its technical feasibility for various soil materials as a general soil improvement technique as well as specifically for its application in the stabilisation of gold tailings which are the two challenges addressed by this research. Additionally, the economic feasibility of MICP can be contrasted with conventional methods following experimentation for its application in South Africa as well as in large scale operations. These include tailings management and soil improvement for infrastructure and development.

2.2 TSF Failure

2.2.1 Overview

Mining is a core component of the world's economy with numerous enterprises such as the aircraft, ceramic, computer, construction, metal and paint industries which are reliant on the extracted commodities (Kossoff et al., 2014). This is highlighted by the job creation proliferated by the mining industry, providing employment to over 40 million people directly and 200 – 250 million indirectly (Azapagic, 2004). This is particularly relevant in Africa, which possesses over 30% of the global mineral reserves and mining is vital to the livelihood of millions of people continent wide (Sturman et al., 2020). Evidently, this crucial industry will likely endure well into the future therefore finding environmentally responsible solutions to aid some of the processes and key issues is an achievable goal for implementing sustainability. The optimization of current technologies and procedures or their substitution with energy-efficient and low-emission alternatives can make a significant contribution to achieving this goal (Brewer, 2012; Dubiński, 2013).

One of the key issues facing the mining industry worldwide is the failure of tailings impoundments (Kossoff et al., 2014). With 17 cases of tailings accidents occurring worldwide since 2000, the figure may seem minor considering the volumes of tailings generated annually, reaching approximately 14 billion tonnes in 2010 alone (International Commission on Large Dams, 2001; Jones & Boger, 2012; Lottermoser, 2010; Rico et al., 2008). However, due to the severe and often irreversible consequences of poor tailings management and the catastrophic failures, the stability and upkeep of tailings facilities is of paramount importance and will likely continue to be in the future (Adiansyah et al., 2015).

For adequate tailings management, the material and its properties need to be sufficiently understood in order to anticipate its behaviour and potential failure. As described in section 2.1, tailings are a residue non-economic waste generated following the processing of mined minerals or ore (Adiansyah et al., 2015; Lottermoser, 2010). Tailings often contain processing fluids and concentrators from mills and washeries in addition to the primary component of crushed rock (Kossoff et al., 2014). In addition, small trace elements of the economic commodity being extracted remain in the tailings as the recovery process is not completely stringent (Lottermoser, 2010). This particulate suspension, essentially a fine-grained sediment water slurry, is then pumped from mining site and disposed of directly or indirectly. The particulate suspension is water intensive yet cost effective and efficient for transporting large volumes of tailings (Adiansyah et al., 2015).

The physical and chemical properties of tailings vary based on the processing implemented at the mine facility as well as the composition of the materials being extracted. Physically, tailings grain sizes typically vary between coarse (625 µm to 2 mm) and fine (3.9–625 µm), with coarse grains being more prevalent (Sarsby, 2000). According to Sarsby (2000), hard rock tailings are typically gravel (>2 mm) and clay (<3.9 µm) free, however due to the high variability resulting from process requirements, tailings have been variably characterised. For instance, Lottermoser et al (2010) state that the grain size of tailings varies between sand and clay (2 mm to 2 µm). Typically, dry tailings primarily consist of between 70 to 80% sand particles and 20 to 30% clay particles (Lottermoser, 2010). **Figure 2-1** below displays the particles typical size distribution curve for tailings as well as other waste streams generated by mining proposed by Robertson and Younger (Robertson, 1994; Younger, 2002).

Based on the curvature and the span of the tailings curve, the tailings appear to fall between well graded with a high clay content and uniform particle size distribution (Chebet, 2017). Based on these descriptions, sand and clay can be selected as representative soils for the range of particle size distribution which can be observed in tailings.

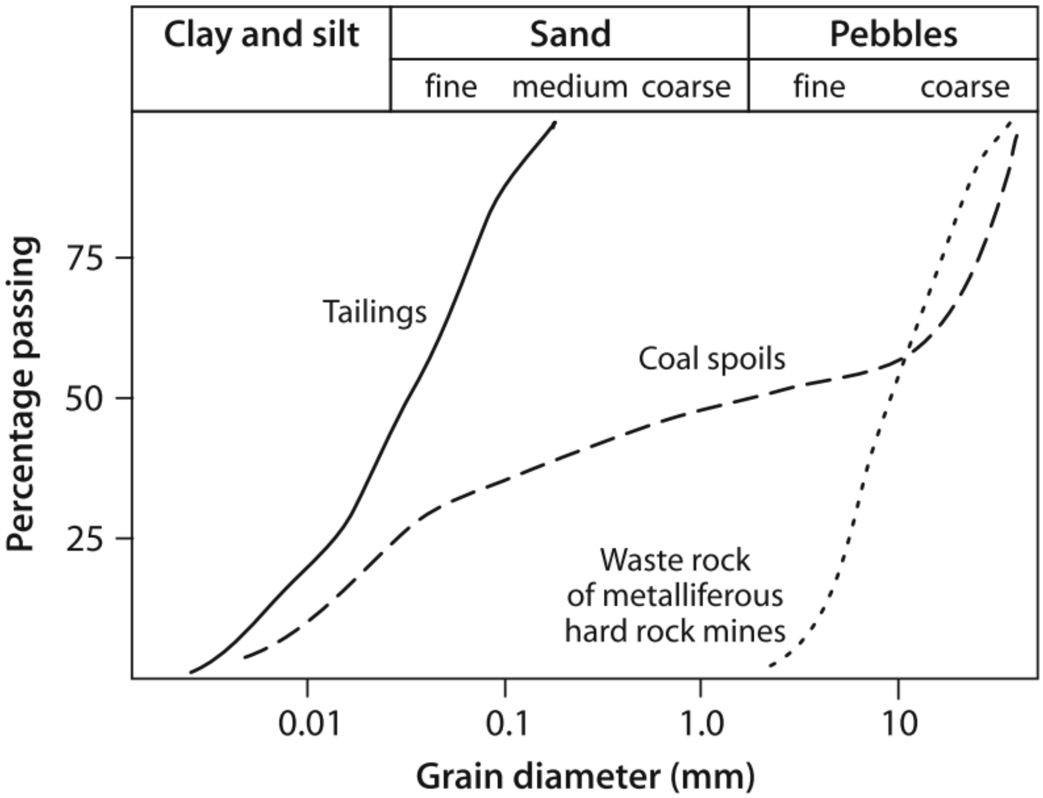

Figure 2-1: Schematic particle size distribution curves for waste rock, coal spoils and tailings which are waste products produced as a result of mining and its associated processes. Tailings are encompassed in clays and silts as well as fine to medium sands with either well graded particles with a high clay content or uniform particle sizes. (Robertson, 1994; Younger et al. 2002).

Sand is a commonly used soil material in MICP research, which allows for a comparative analysis. However, clay and tailing in particular, are much less often encountered allowing for novel observations to be made.

Like the particle size distribution, bulk density and specific gravity vary widely in tailings, based on the characteristics of the parent rock from which the commodities are extracted (Kossoff et al., 2014). Generally, the range for the bulk density is reported as 1.8–1.9 t m^{-3} with a specific gravity of 2.6–2.8 (Bjelkevik, 2005; Sarsby, 2000). These physical properties are noteworthy as they largely impact the applied mineral processing to modify or liberate the minerals from the unwanted solids, the porosity as well as the deposition and settling characteristics of the tailings. This includes the class of chemicals for the selected hydrometallurgical processing such as froth flotation, the resistance to wind and water erosion, the settling and consolidation characteristics in the impoundments, the leaching properties and the location of the material deposition relative to the outlet slurry (Lottermoser, 2010; Younger & Wolkersdorfer, 2004).

The physical properties may also impact on the chemical behaviour of tailings where smaller sized particles have been observed to be kinetically predisposed to atmospheric oxidation and an increased susceptibility to the release of absorbed contaminant elements (Kossoff et al., 2012). The chemical make-up of tailings is determined by the minerology of the parent ore, the processing fluids utilised in mineral extraction, the efficiency of the extraction process as well as the exposure during storage. The efficiency of the extraction process is another factor as remnants of various elements remain in the railings as no extraction process is ever 100% exact. This efficiency is often dependant on the economic interaction between the efficiency, the value of the commodity and the investment of the plant (Dixon-Hardy & Engels, 2007). Trace elements of organic chemicals, cyanide and sulfuric acid have also been reported (Lottermoser, 2010).

As a result of the mineral processing, chemical, physical and biological reactions as well as degradation following deposition , tailings often contain potentially hazardous contaminants such as toxic trace metal and metalloids (Kossoff et al., 2014; Praharaj & Fortin, 2008). Atmospheric oxidation is a primary contributor as previously contained contaminants are mobilised and reactions between the solids and liquids as the system attempts to reach equilibrium result in composition changes and the generation of various species in and out of solution. Biologically mediated processes such as mineral dissolution and formation as well as physical and chemical processes such as compaction, cementation and recrystallisation further enable this form of diagenesis (Praharaj & Fortin, 2008). Therefore, in a reasonable and accountable mining establishment, the isolation of the tailings is prioritised to prevent entry and subsequent contamination of groundwater, rivers, lakes as well as the air (Kossoff et al., 2014). Accidents in tailings impoundments pose a serious threat to the health of humans, the surrounding flora and fauna due to the various contaminants they contain as described above. The means of storage may also have a role to play in the overall safety of tailings in terms of the risk of contamination as well as stability, which brings to light tailings repositories and tailings disposal.

The most common mode of tailings management is the use of a tailings dam or pond, which is a large surface impoundment or sedimentation lagoon which captures and houses the mine waste residues and process water (Lottermoser, 2010). Tailings are pumped as a slurry from the mill to the location of the impoundment and distributed using cycloning or spigotting methods (Lottermoser, 2010). These dispersal methods ensure a graded distribution of particles where coarser, more porous material is deposited near the outlet point at the perimeter of the structure and fines are deposited well away from the outlet toward the centre of the TSF. This ensures the structural integrity is preserved and reduces the likelihood of seepage and piping issues across the structure (Lottermoser, 2010). Furthermore, the finer particles settle away from the higher shear stresses at the confining walls.

The impoundment is raised sequentially, where initial retaining embankments are erected followed by incremental deposition as demand grows (Lottermoser, 2007). The construction of tailings dams adheres to the standards applied in the construction of conventional water storage dams (Lottermoser, 2010). The impoundment is raised in one of three ways; upstream, vertically (centre line) or downstream. Upstream raising is the most common method which is applied in more than half of the tailings dams globally (Davies & Martin, 2000). The preference for this method is likely due to the financial advantage. Upstream raising requires the least amount of construction materials, with the lowest overall cost (Soares et al., 2000). However, upstream raising comes at a greater cost in terms of stability, as this method incites a high susceptibility to erosion and failure resulting in the highest failure rate of 1 out of every 20 dams failing (Davies & Martin, 2000). This high susceptibility to erosion and failure is due to the difference in the approach used to deposit of new material on the impoundment structure. In downstream and centreline raising, new material is placed outside the impoundment or on top of the existing embankment respectively. In upstream raising, the material is placed within the impoundment making it more susceptible to piping and static liquefaction (Davies & Martin, 2000; Kossoff et al., 2014).

An alternative to tailings storage, is disposal. Riverine disposal, submarine disposal, wetland retention, backfilling and dry stack are other approaches in the disposal and storage of tailings (Lottermoser, 2010). These are generally classified into two broad categories which are direct and indirect disposal (Adiansyah et al., 2015). Direct disposal involves discharging into various water bodies which is subcategorised into riverine tailings disposal (RTD) and submarine tailings disposal (STD) for disposal in rivers as well as oceans and lakes respectively (International Maritime Organization, 2012).

Tailings impoundments are often preferred due to their limited impact on the ecology of the region in comparison to RTD and STD. In addition, the pond water in tailings dams, inhibits the generation of surface dusts and acid mine drainage by deterring oxidation (Kossoff et al., 2014). Tailings impoundments bestow the largest impression of any mining activity on the landscape due to their sheer magnitude in volume and size in area (Kossoff et al., 2014) Tailings also leave a large environmental footprint temporally due to the required lengthy management and rehabilitation periods (Department of Industry Tourism and Resources, 2007). Their failure results in the devastation of the surrounding region, with some impacts enduring for years after the event. Therefore, in order to prevent such catastrophic events from occurring, the root causes of TSF failure need to be understood.

2.2.2 Root causes

The objective of tailings impoundments is the containment of the waste materials for an indefinite period. However, due to a variety of factors, failure occurs resulting in the seepage, spillage, and erosion of tailings with considerable consequences for the surrounding region. **Table 2-1** below summarises the most commonly recognised causes of TSF failure.

Table 2-1: A brief summary of the commonly recognised causes of TSF failure from consulted literature which vary from dynamic loading resulting in liquefaction, structural and foundational failure, overtopping as a result of meteorological events, subterranean flows, maintenance issues, a combination of multiple causes and undetermined or unreported failure.

Cause of failure:	**Description:**	**Reference:**
Liquefaction	Resulting from dynamic loads such as seismic events, vibrating equipment or mine blasting, the applied stresses cause the solid material to behave like a liquid and eventually overflow or static loads which can induce liquefaction over time.	(Davies & Martin, 2000) (Lottermoser, 2010) (Silva Rotta et al., 2020)
Structural	Structural elements such as rapidly raising the dam wall height, the mode of dam raising, slope instability, mine subsidence, insufficient or incorrect compaction and the choice in construction materials may result in failure through a number of mechanisms such as increased pore pressures.	(Lottermoser, 2010) (Rico et al., 2008)
Foundational	If the bearing capacity of the foundation cannot withstand the loads imposed by the impoundment, failure along a failure plane occurs.	(Hustrulid et al., 2001)
Overtopping	Elevated or unusual meteorological events such as flood inflow, high rainfall and rapid snow melting raise the phreatic surface of the dam past a critical level resulting in excessive water levels. This in turn elicits overtopping and collapse.	(Lottermoser, 2010) (Rico et al., 2008)
Seepage	Excessive subterranean flows such as seepage and piping result in erosion which in time, forms liquid channels or pipe throughout the structure, which undermine the stability of the structure resulting in failure.	(Van Niekerk & Viljoen, 2005)
Maintenance	Improper water management, failure to heed warning signs, erosion, inadequate monitoring protocol and improperly maintained operational logs are examples of how substandard maintenance programmes may result in avoidable failure.	(Davies & Martin, 2000)
Combination	Almost all historical occurrences of failure have been a result of multiple causes occurring simultaneously.	(Rico et al., 2008)

In addition to the widely accepted causes of failure described above, there are some observed trends in literature concerning related contributing factors. Additionally, according to available data, the likelihood of failure is greatly increased in active dams in comparison to abandoned and inactive maintained dams with a reported 83%, 15% and 2% of failure incidences respectively (Rico et al., 2008). It is likely that the diagenesis that occurs in tailings following deposition contributes to the structural stability of tailings impoundments over time. Cementation and oxidation for instance, may have a role to play in improving the liquefaction resistance over time which explains the reduced failure occurrence in abandoned and inactive dams (Troncoso, 1990). Climate change has also been attributed as a contributing factor to failure due to the extreme meteorological changes brought about by a continual increase in the average temperature of the globes climate system. Increased snow melting is one key example of global warming induced change, as this contributes to excessive water levels and results in overtopping failure in impoundments (Rico et al., 2008).

Furthermore, historical evidence suggests that periods of rising commodity prices correlate with high failure rates in tailings impoundments 2 to 3 years following the market peaks (Davies & Martin, 2000). This highlights the importance of factors such as maintenance, which are often overlooked as serious contributors to catastrophic failure. Based on this observed trend, it can be deduced that during periods of peak demand where prices are optimum, safety and legislative restrictions and protocols which form part of a maintenance framework, are relaxed, and perhaps overlooked (Davies & Martin, 2000). This direct interaction between negligence and evident failure, brings to light the consequences of TSF failure.

2.2.3 Consequences

The key issues mentioned above in TSF failure have a direct impact on sustainability in terms of the effects on the environment, society, and the economy. This is evident from the impact of TSF failure on the landscape, public health, and safety as well as the resulting financial implications from the reparations and the hindrance of mining activities. The impacts of tailings dam failures on the surrounding region can vary between immediate, where repercussions occur within hours to months of the incidents and medium to long term, where repercussions are experienced within years to centuries (Kossoff et al., 2014). There is also an overlap between the environmental, social, and economic impacts that affect the regions downstream.

The immediate impacts of TSF largely result from the inundation of the downstream region in the toxic material. The toxic material enters surrounding waterbodies resulting in the mass death of aquatic life. A combination of burial, impact, contamination, and extreme changes in the water quality following the spillage of tailings such as over sedimentation and significant drops in pH results in the death of tonnes of aquatic species (Grimalt et al., 1999). Terrestrially, the primary consequences following the flow of tailings, are drowning, suffocation and poisoning of humans as well as land roaming animals such as cattle for kilometres downstream (Chandler & Tosatti, 1995; Davies & Martin, 2000; Foulds et al., 2014). The inundation of the surrounding region results in significant damage and loss of property including farmland, businesses, and homes, which also directly impacts the economy of the area (Grimalt et al., 1999). Operation of the facility typically ceases for a period following failure up until an attempt is made to mitigate or manage the impact of the failure which has a direct impact on the financial burden and profitability of the mine (Mining Journal Research Services, 1996). This is one of two key economic consequences of TSF failure; business interruption and environmental remediation (Kossoff et al., 2014). However, in some cases, production resumes as soon as two weeks following the event, further compromising the ecology in the area (U.S. Environmental Protection Agency, 2010).

In terms of the longer term, the impacts tend to be insulated by the affected environment due to natural processes such as sediment and aqueous dilution, and riverbed and flood plain uptake (Kossoff et al., 2014). Nevertheless, evidence of contamination persists in various areas such as the water bodies as well as the soils and sediments. Contamination hubs endure for years following the failures, particularly regions in close proximity to the site of the breech (Olías et al., 2012). The erosion and subsequent excavation of the channel bed, bank, floodplain sediment and riparian vegetation during the failure event and during reparation measures, results in a highly unstable river channel. Furthermore, the contaminated or un-remediated soils and sediments in the flood plains adversely impact the animal husbandry and crop production previously supported by the affected region further highlighting the economic impacts of TSF failure (Kossoff et al., 2014).

With the evident severe and frequently irreversible consequences of TSF failure, careful and well-designed maintenance and management of these facilities is of paramount importance (Adiansyah et al., 2015). In order to limit or completely avoid the repercussions of TSF failure described above, maintenance should be prioritised in a mining facility.

2.2.4 Monitoring

A monitoring program ensures the consequences described in section 2.2.3 are avoided through careful tracking of the tailings impoundments' behaviour as well as regular maintenance during operation and post operation (Lottermoser, 2010). Three key aspects of a TSF are addressed in the monitoring programme: the performance, the stability as well as environmental facets of the dam (Lottermoser, 2010). These assuage the potential risks associated with tailings during the operational and closure phase in the TSF, as summarised in **Table 2-2** below.

Table 2-2: A summary of the potential risks associated with tailings storage and the typical associated mitigation measures in the monitoring programme at two phases of the impoundments design life; the operational phase and at closure. The key components of the maintenance strategy to mitigate the liabilities are summarised according to the phase of the impoundment (Adiansyah et al., 2015; Department of Industry Tourism and Resources, 2007; Laurence, 2003; Lottermoser, 2010).

Potential liabilities:	Example:	Monitoring programme:
	Operational	
Malfunction of impoundment infrastructure	Leaking of drains, slurry pipeline	Water balance monitoring, infrastructure inspection and maintenance
Geotechnical failure	Bearing capacity or slope stability failure	Phreatic surface and pore pressure monitoring, height, and slope inclination monitoring
Seepage	Seepage through embankment wall, into groundwater, into sediments and soils	Water pressure and water level monitoring along groundwater flow paths and in the impoundment, computational modelling of groundwater flow, chemical analysis of surrounding groundwaters, seepage waters and surface water
Contamination	Infiltration of leakages, seepage and overflows into ground and surface water as well as surrounding arable land, interaction of tailings and process fluids with wildlife and livestock	Chemical analysis of surrounding groundwaters, seepage waters and surface waters, computational modelling of potential contamination impacts
	Closure	
Erosion	Wind pollution, gas pollution, failure of covers, undermining and overtopping the embankment wall	Cover inspection and maintenance, chemical analysis of downstream sediments and dust particles, infrastructure inspection and maintenance
Structural failure	Spillway failure, overtopping as a result of increased rainfall run-off	Meteorological observations, infrastructure inspection and maintenance

The dams’ performance is evaluated in terms of the rate of raising, consolidation and settlement, the water balance as well as concentrations of process chemicals. This safeguards against risks such as leakages, geotechnical failure, tailings overflow, seepage and infiltration which are identified and mitigated timeously. The impoundment stability is appraised in terms of the phreatic surface level, slope stability and pre-pressures. This ensures signs of seepage and erosion through the embankment walls, overtopping and dust pollution resulting from run-off and erosion and spillway failure are identified and managed prior to failure (Kossoff et al., 2014; Lottermoser, 2010).

The environmental interactions are gauged in terms of the monitoring of meteorological data, radioactivity and the chemical concentrations and mineralogy of the tailings, ground, surface and seepage waters, downstream stream sediments and dust (Kossoff et al., 2014; Lottermoser, 2010). This limits the interaction of the materials contained in the TSF with the surrounding ecology and terrain. Examples of this include wet and dry covers such as shallow water covers or gravel covers to limit wind and water erosion, as well as the ingress of water and oxygen into the waste.

2.3 Soil improvement

2.3.1 Overview

The concept of ground or soil improvement dates back thousands of years, to the first human civilizations that existed (Nicholson, 2015). In ancient Mesopotamia, wood and straw were used as inclusions to improve the engineering properties of the mud used for construction. This is the essence of soil improvement, whereby an existing and inadequate soil material is altered in order to perform a specific function (Nicholson, 2015). This useful technique has grown substantially over time, ushered by the growing demand for suitable land for development with a rapidly increasing population. As the population continues to grow, the available and suitable land continues to decrease (Briaud, 2014). Therefore, typically inept soils are altered to fit the needs for human activities, which are directly limited by the availability of competent soils (DeJong et al., 2010). Furthermore, soil improvement also provides a desirable alternative to foundation design. Typically where shallow foundations could have been used, soil improvement is selected as an alternative at almost half of the total cost (Briaud, 2014).

2.3.2 Objective

Where a potential site is limited in terms of the soil quality, a few avenues exist for consideration. The project can either be terminated, where no alternative to the existing site is available, there are no binding ties to the site and the financial implications associated with the site outweigh the benefit. Excavation is the second approach, where the poor soil material is removed and replaced with soils possessing improved characteristics for the intended use. The reconstitution of the design where additional elements are incorporated to compensate for the poor soil conditions is the third approach (Briaud, 2014; DeJong et al., 2010). Additional elements include structural elements such as piles can be incorporated in scenarios where more appropriate soils required for the project are located beneath poor soil strata.

Where these avenues are not desirable or fitting for the proposed project, soil improvement is chosen. Soil improvement aims to alter, advance and control (to a reasonable extent) the existing geotechnical characteristics of a soil material (Nicholson, 2015). The pertinent soil characteristics from a geotechnical perspective are the soils shear strength, compressibility, porosity, shrink or swell potential and stiffness. These indicate the sustainable loads prior to failure, the settlement and volume change behaviour, the rate of fluid transport through voids, the behaviour when losing or gaining with moisture as well as the stability of the soil material which are crucial elements in the design of foundations (Nicholson, 2015). These characteristics inform the design to ensure failure does not occur within the design life.

By altering these said characteristics, soil improvement limits potential undesirable effects the behaviour of the soil may have on the project. This typically includes the increase in shear and compressive strength and the manipulation of porosity. In this way, the compressibility and likelihood of liquefaction are reduced to prevent settlement and the flow of materials. Furthermore, the stability and bearing capacity are improved and the restriction or liberation of groundwater to limit groundwater flow or encourage drainage respectively, is possible. Evidently, soil improvement allows for the manipulation of the soil conditions on the site to suit a specific set of requirements and constraints stipulated in a project, providing a useful tool to geotechnical engineers.

2.3.3 Classification

Soil improvement can be broadly categorised into two methods: the use of foreign soil improvement material and the improvement of the soil without foreign inclusions (Salifu et al., 2016; Sharma & Ramkrishnan, 2016). Within these categories, exists five subcategories which expand on the two broad approaches. The use of foreign soil improvement material can be subdivided into three further categories of soil improvement: with reinforcement, with grouting and admixtures and lastly, with inclusions (Briaud, 2014). Similarly, soil improvement without foreign inclusions can be segregated into improvement in: coarse soils without admixture and in fine-soils without admixture (Briaud, 2014). This categorisation of the various soil improvements is illustrated in **Figure 2-2** below.

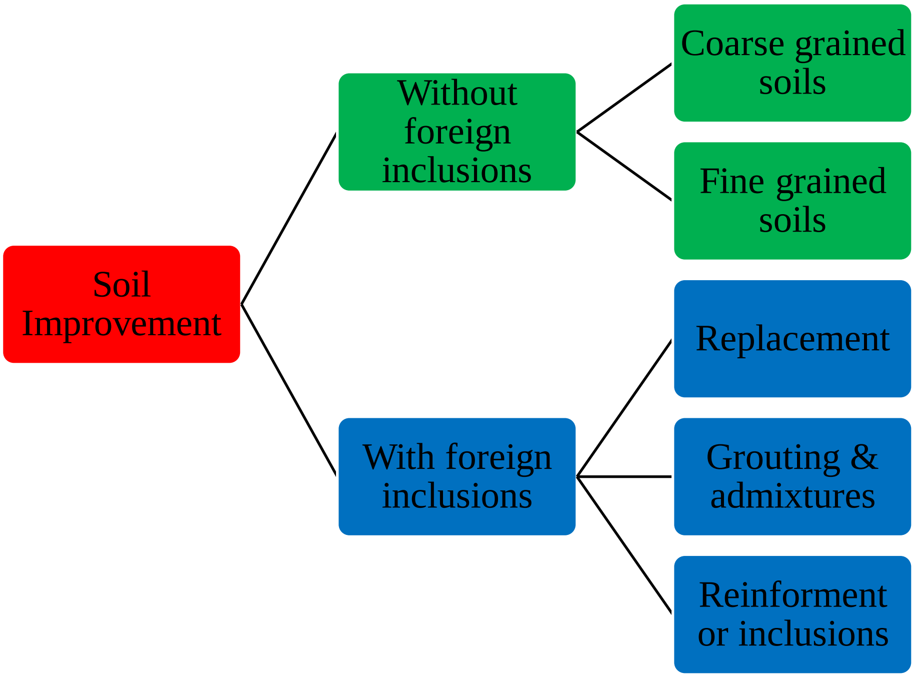

Figure 2-2: A diagrammatic representation of the categorisation of the various soil improvement techniques used in engineering. The broader categorisation of soil improvement into approaches with foreign inclusions and without foreign inclusions can be further subdivided according to the particle size of the soil as well as the type of foreign inclusion respectively (Briaud, 2014).

Within each subcategory, various methods and tools exist, to carry out that specific class of soil improvements, each with their own benefits and shortcomings. There are a multitude of soil improvement projects completed worldwide, each with their own specific requirements and limitations (DeJong et al., 2010). With a long and lucrative history of soil improvement, where current projects globally exceed total costs of US$6 billion annually and the development of numerous methods over the past 50 years, there are a number of methods which are implemented as common practice in the industry (Briaud, 2014; DeJong et al., 2010).

2.3.4 Conventional methods

The conventional and commonly used approaches applied in soil improvement are summarised in **Table 2-3** below.

Table 2-3: A summary of the conventional approaches to soil improvement and their mechanisms based on five central categories. These categories are namely soil improvement without admixtures in coarse and fine soils respectively, with reinforcement, with grouting admixtures and lastly with admixtures and inclusions (adapted from Briaud, 2014).

Category:	**Method:**	**Principle:**
Without admixtures in coarse soils	• Compaction • Dynamic compaction • Vibrocompaction	• Densification of soil as a result of an applied external force such as vibrations, dropping heavy weights and vibrations
Without admixtures in fine soils	• Displacement-replacement • Fill preloading	• Replacement of weak material with geotechnically superior material • Preconsolidation of compressible soils using fill
With reinforcement	• Ground anchors and soil nails • Geosynthetics • Mechanically stabilised earth (MSE)	• The use of steel concrete and geosynthetic products or a combination of these and pre-made rigid products in the soil material • The use of biological products such as vegetation to stabilise slopes
With grouting admixtures	• Particulate grouting • Chemical grouting • Jet grouting • Compaction grouting • Compensation grouting • Mixing	• Grouting voids, cavities and fissures in soil and rock material through the injection of particulate grouts such as cement or chemical grouts • High speed erosive grouting for the formation of columns or panels • Injection of grouts for densification and settlement reduction in soils • Mixing the soils with grouting materials
With admixtures and inclusions	• Aggregate columns • Lime treatment	• Driving aggregates to form stone columns, stone columns with geosynthetic encasement within the soil mass • The use of calcium oxide

The conventional approaches as described utilising foreign physical structures include but are not limited to geotextiles, wire meshes, cable nets, nails, and sheets. These approaches are often effective and durable hence their popularity in industry, however they are often costly and disruptive to infrastructure as well as plant growth (Salifu et al., 2016). The in-situ improvement methods utilising chemical or organic stabilising agents present issues in terms of ineptitude in wet conditions, as well as issues in being "ecologically unfriendly" (Salifu et al., 2016). Furthermore, these approaches are under public scrutiny for the potential environmental hazards they pose where a large majority of chemical grouts have been found to be toxic (Karol, 2003). These methods have also been associated with water poisoning, resulting in a global initiative for the removal of synthetic grouting materials (Karol, 2003). These methods are often energy intensive, requiring the production of vast quantities of synthetic materials such as cement as well as well as similarly energy intensive installation (Naeimi & Haddad, 2020).

With these evident shortcomings in the conventionally used techniques, there is a growing demand for more sustainable soil improvement techniques (DeJong et al., 2010). Bio-mediated soil improvement techniques for instance, have grown in their popularity as they seemingly absolve the issues posed by traditional methods (Umar et al., 2016). With a significant reduction in energy required as well as carbon emissions, these approaches mimic naturally occurring processes to improve the geotechnical properties of soil to a comparable scale as conventional methods, without the environmental concerns such as toxicity (L. Cheng et al., 2013a; DeJong et al., 2006, 2010). There are many available alternatives in industry however, a large focus has been placed on bio-mediated approaches and research for the promising results (Umar et al., 2016).

2.3.5 Novel approaches

The available but less frequently used approaches for soil improvement are summarised in **Table 2-4** below.

Table 2-4: A summary of the alternative and less commonly implemented soil improvement approaches and their mechanisms (adapted from DeJong et al., 2010; Briaud, 2014).

Category:	Method:	Principle:
Without admixtures in coarse soils	• Explosive compaction • Rapid impact compaction • Electric pulse compaction	Densification of soil as a result of an applied external force such as shock waves and vibrations from blasting, rapid blasting, or electric pulses
Without admixtures in fine soils	• Displacement replacement • Fill preloading • Prefabricated vertical drains and preloading • Vacuum preloading • Electro-osmosis • Thermal methods • Hydro-blasting compaction	• Replacement of weak material with geotechnically superior material • Pre-consolidation of compressible soils using fill, vacuum pressure, vacuum pressure and drainage and electrolysis • Inducing temperature changes to alter the soil such as ground freezing • Wetting soil and utilising explosives to exert a compacting force
With admixtures and inclusions	• Dynamic replacement • Microbial treatment • Natural products	• Driving and compacting sand to form sand columns or sand columns with geosynthetic encasement within the soil mass • Harnessing natural microbial processes such as biomineralization, biofilm formation and biogas production • The use of rigid natural products such as bamboo or timber

Limitations associated with the novel approaches include a limited suitability to various soil materials. For instance, dynamic replacement is best suited to cohesive or fine grained soils (Briaud, 2014; Premier Guarantee, 2018). Furthermore, the overall complexity of the methods in terms of specialized equipment and complex operation adds the burden of specialist labour as well as costly equipment in a soil improvement project (Ace, 2018; Sun et al., 2013). Perhaps, the most pressing limitation overall, the lack of long-term behaviour of these approaches which increases the risk of the project due to the unknown (Greenwood et al., 2018). Simpler and more adaptable bio-mediated approaches are once again highlighted in this respect as a prominent alternative. One such approach, is microbially induced calcite precipitation; a microbial treatment which harnesses the metabolic processes of bacteria to alter the structure of a material.

2.4 Microbially induced calcite precipitation

2.4.1 Overview

Microbially induced calcite precipitation (MICP) is a microbially mediated process resulting in the precipitation of calcium carbonate following the hydrolysis of urea in a calcium rich environment (Haouzi & Courcelles, 2018). In other words, facultative bacteria with a highly active urease enzyme such as *Sporosarcina pasteurii* (formerly *Bacillus pasteurii*) consume urea during metabolic processes (DeJong et al., 2010). During this process, urea is decomposed into ammonia (NH_3) and carbon dioxide (CO_2) catalysed by the urease enzyme. The (NH_3) and (CO_2) diffuse through the cell wall of the bacteria, and into the surrounding calcium rich solution (DeJong et al., 2010). In the presence of water (H_2O) in solution, the (NH_3) and (CO_2) ionize into ammonium (NH_4^+) and bicarbonate ions (HCO_3^-) (Umar et al., 2016). Equation 1 to Equation 3 below, describe the hydrolysis of urea as well as the resulting ionization of ammonia and carbon dioxide.

$$\mathrm{CO(NH_2)_2 + H_2O \xrightarrow{urease} 2NH_3 + CO_2} \quad \text{Equation 1}$$

$$\mathrm{2NH_3 + H_2O \rightarrow 2{NH_4}^+ + 2OH^-} \quad \text{Equation 2}$$

$$\mathrm{CO_2 + H_2O \rightarrow {HCO_3}^- + H^+} \quad \text{Equation 3}$$

During the ionization of NH_3, hydroxyl ions (OH^-) are formed which increase the local pH and collectively alkalise the bacterial environment to a pH of approximately 9 (DeJong et al., 2006; Van Passen, 2009). Thus, the OH^- and HCO_3^- ions react, forming carbonate ions (CO_3^{2-}) (Burne & Chen, 2000; Castanier et al., 1999). Calcium ions (Ca^{2+}) from the surrounding calcium rich solution react with the CO_3^{2-} ions forming calcium carbonate ($CaCO_3$) which precipitates as a crystal out of solution (Haouzi & Courcelles, 2018). Equation 4 and Equation 5 below, describe the precipitation of calcium carbonate. The overall urea hydrolysis and calcium carbonate precipitation is shown in Equation 6.

$$\mathrm{{HCO_3}^- + H^+ + 2OH^- \leftrightarrow {CO_3}^{2-} + 2H_2O} \quad \text{Equation 4}$$

$$\mathrm{Ca^{2+} + {CO_3}^{2-} \rightarrow CaCO_3 \downarrow} \quad \text{Equation 5}$$

$$\mathrm{CO(NH_2)_2 + 2H_2O + Ca^{2+} \xrightarrow{urease} 2{NH_4}^+ + CaCO_3 \downarrow} \quad \text{Equation 6}$$

Due to the negative charge of bacterial cells, they are attracted to the soil particle as well as the calcium ions (Ca^{2+}) in solution (Hall-Stoodley et al., 2004). In this way, the calcium carbonate crystals form bridges between the individual soil particles, binding them together (Soon et al., 2013). This cementation is therefore responsible for the improved geotechnical properties of a soil treated using MICP (Harkes et al., 2010).

There are alternative biologically mediated processes to MICP responsible for inducing calcite precipitation (DeJong et al., 2010). These various alternative calcite precipitation mechanisms besides urea hydrolysis include aerobic oxidation, denitrification and sulphate reduction (Sharma & Ramkrishnan, 2016). The urea mechanism is ideal in terms of waste resource recovery because the urea compound is found in the urine of mammals (Lambert & Randall, 2019; Okyay et al., 2016). Due to the ureolysis mechanism possessing the lowest Gibbs Free energy (kJ/mol), this reaction has a higher propensity to occur in comparison to aerobic oxidation, denitrification, and sulphate reduction (Morel & Hering, 1993). If urea is present in the system, ureolysis will likely prevail which is attributed to the pH change that occurs, essentially altering the environmental conditions and prohibiting the alternative processes (Pikuta et al., 2007). However, if the pH exceeds 11, urea hydrolysis will not occur (Randall et al., 2016). Controlling the pH is a useful function in preventing the premature hydrolysis of urea in applications such as the storage of fresh urine for use as cementation media in MICP (Henze & Randall, 2018). An additional benefit to maintaining high pH values is the prevalence of carbonate ions. One might question the suitability of these basic conditions for the bacterial community however, research has shown the resilience of *sporosarcina pasteurii* at pH values of approximately 11.2 (Henze & Randall, 2018).

2.4.2 Factors affecting the MICP process

Based on the chemical reactions described in Equation 1 to Equation 6, it is evident that the MICP process is affected by numerous components both innately and extraneously (Sheng et al., 2020). These factors are predominantly the bacterial species, bacterial concentration, temperature, pH, the ionic strength of the urea solution, the chemistry of the cementation solution, the grouting strategy as well as the soil properties (Lambert & Randall, 2019; Tang et al., 2020). These factors play a key role in the bacterial growth, metabolism and precipitation which are key determinants in the success of MICP (DeJong et al., 2010).

The bacterial strains primarily influence the crystallisation of the calcium carbonate precipitate. Various species of bacteria which mediate MICP, secrete disparate crystal types, sizes and morphology (Sheng et al., 2020). Larger crystals are favourable in coarse grained soils, whilst smaller crystals are favourable in fines in terms of improved cementation between particles (Al Qabany et al., 2012; Al Thawadi, 2008; Zeng et al., 2019). This is apparent with regard to the pores between particles where cementation occurs, where larger pores require larger crystal networks and contrarily, smaller pores require smaller networks. In terms of the soil properties, geometric compatibility between the microorganisms and the soil is key to establishing a homogenous bacterial community throughout the soil (Haouzi & Courcelles, 2018). As microorganism size varies between 0.5 and 3 μm, the lower limit for biomineralization is set at silt, however with the use of open inoculation, this can be extended to clays (DeJong et al., 2006; Mitchell & Santamarina, 2005). In addition to a varying crystal formation, bacterial strains vary in the activity of their urease enzyme. Research indicates that a high urease activity corresponds to a higher rate of calcium carbonate precipitation (Al-Salloum et al., 2017; Okwadha & Li, 2010; Stocks-Fischer et al., 1999; Whiffin, 2004).

Another way to attain greater carbonate precipitation is to increase the bacterial concentration in the soil. Higher urease activity corresponds to an increase in the microbial community (Okwadha & Li, 2010). Thus, the concentration of the bacteria in the inoculation solution has been found to correspond to the rate of calcium precipitation (Okwadha & Li, 2010). This in turn results in improved geotechnical characteristics such as an increased compressive strength, shear strength and reduced volumetric strains (Chou et al., 2011). In addition to the concentration of bacteria, higher strengths are observed at increasing concentrations of the cementation solution or the calcium carbonate source (Umar et al., 2016). However, cementation concentrations reach a peak, typically past 1 M, where microbial activity decreases with further increases in concentration past this point (Umar et al., 2016). This is a result of the increase in ionic strength induced by increasing the cementation solution concentrations which in turn inhibits the function of the microbial environment (De Muynck et al., 2010; Lambert & Randall, 2019; Whiffin, 2004).

Ureolysis is a temperature dependent process which has been found to increase with escalating temperatures (Ferris et al., 2004; Nemati & Voordouw, 2003). Typical temperatures between 20°C – 37°C provide optimum conditions for urease activity where increasing temperature to an upper limit of 70°C is proportional to higher rates of urease activity (Okwadha & Li, 2010; Whiffin, 2004).

The pH is a key player in the efficacy of MICP due to its considerable impact on urease activity which catalyses the hydrolysis of urea and in turn, sparks calcium precipitation (Stocks-Fischer et al., 1999). Calcite precipitation typically begins at a pH of 8.3, reaching a peak in ureolytic activity at a pH of approximately 9 (Stocks-Fischer et al., 1999). Higher pH values have been found to increase the rate of calcium ion depletion, resulting from a higher concentration of carbonate ions in basic conditions (Henze & Randall, 2018). Therefore, maintaining pH at a maximum value of 11.2, enables a faster rate of calcite precipitation whilst supporting the survival of the bacterial community (Henze & Randall, 2018).

The grouting or treatment dispensation strategy plays a crucial role in the distribution of the treatment media throughout the soil (Sheng et al., 2020). Therefore, grouting technology (grouting method, grouting speed, and grouting pressure) is critical to the in-situ implementation of the MICP cementation method. The inhomogeneity of MICP cemented soil is one of the most challenging factors that restricts the up-scaled use of MICP in field applications (L. Cheng et al., 2013a; Shahrokhi-Shahraki et al., 2015). Using different grouting methods, it is possible to alter the homogeneity and distribution of calcium carbonate precipitation after MICP treatment, and consequently the strength and porosity of MICP treated soil. However, this method is likely to cause the clogging of precipitated calcium carbonate crystals around the injection point and their non-uniform distributions over time (Sheng et al., 2020). Three prevalent treatment methods are recurring in the literature consulted: injection of reactant solutions in saturated soils (Al Qabany et al., 2012; Harkes et al., 2010; Whiffin et al., 2007), surface spraying and lastly percolation (Cheng et al., 2013; Chu et al., 2012; Stabnikov et al., 2011). Immersion and pre-mixing are less customary; however, these methods have been successfully used in MICP research (Wen et al., 2019; Yasuhara et al., 2012; Zhao et al., 2014).

Injection or grouting is regulated through the grouting speed and pressure, which is key to in-situ implementation of this technique. The grouting speed and pressure directly impact the distribution of the treatment media throughout the soil and thus the homogeneity and efficacy of the treatment (Sheng et al., 2020). This is also true for surface spraying and percolation techniques, where the distribution of the treatment media has a direct impact on the strength and porosity of the soils. The issues facing the three prevalent methods include clogging in proximity to the distribution points and the creation of preferential flow paths and inhomogeneity of the cementation as a result (Cheng et al. 2013; Sheng et al., 2020). Sequentially, effective and homogenous treatment over greater depths, areas and volumes of soil is a great challenge, particularly in large-scale and in-situ applications (Cheng et al., 2013; Shahrokhi-Shahraki et al., 2015). Spraying and surface percolation techniques in particular, presents a singular challenge in terms of achieving substantial treatment depths. Spraying has largely been limited to the treatment of the crustal layer in the range of centimetres similarly to surface percolation reaching larger surface depths, up to 1m in magnitude (Cheng & Cord-Ruwisch, 2012; Zomorodian et al., 2019). Nevertheless, results indicate that the spray technique remains effective in-situ for the improvement of the crustal layers shear strength (Zomorodian et al., 2019). Furthermore, spraying methods have been found to be more suitable to cohesive soils such as clays, due to their low porosity where injection methods are less applicable (Liu et al., 2020a).

Other issues faced primarily in large-scale and in-situ applications include creating and maintaining favourable environmental conditions and retention periods to allow for the continuation of the precipitation reaction as well as the recycling of excess treatment media (Shahrokhi-Shahraki et al., 2015). Immersion and submerged treatment techniques sustain similar deficiencies in terms of the recycling of treatment media. These methods achieve a significantly higher homogeneity and overall efficacy in terms of treatment due to the free penetration of the liquid media into the soils (Li et al., 2011; Zhao et al., 2014). However, vast quantities of treatment media are required for the full immersion of samples. This is a major limitation for large scale application as even greater quantities would be required, casting the feasibility of large-scale application of this technique into doubt. Various MICP research compares the percolation, spraying and immersion methods in terms of calcium uptake. Results indicate that in comparison to the spraying and immersion methods, surface percolation achieves the lowest calcium intake (Cheng & Cord-Ruwisch, 2012; Chu et al., 2014; Van Passen, 2010).

Another key issue to consider regarding the MICP process, is the precipitation of toxic compounds with increasing pH values. Ammonium and nitrate are by-products of urea hydrolysis and are hazardous, particularly at higher concentrations, to human health, vegetation, as well as indigenous microbial communities (Haouzi & Courcelles, 2018). Furthermore, ammonium can potentially be converted into nitric acid by the bacteria or released as a gas that is deposited into the atmosphere (Haouzi & Courcelles, 2018; Rajasekar et al., 2017). Consequences of this include the eutrophication and acidification of ecosystems which considerably affects the native organisms (Haouzi & Courcelles, 2018).

2.4.3 Soil type

The soil type influences the efficacy of the biologically mediated treatment approaches based on the compatibility of the soil size with the microbial community (DeJong et al., 2010). This compatibility is determined by the ability of the microorganisms to move freely between the soil particles which is determined by the size of the pore space between each particle (Mitchell & Santamarina, 2005). Soils with a higher percentage of fines pose a challenge to this migration of microbes, particularly during treatment when there is a continued reduction in pore spaces as treatment proceeds and microbial communities and calcifications coat the soil particles (DeJong et al., 2010). The soil type additionally inhibits the application of particular treatment administration methods, for similar reasons. The in-situ injection method for instance, has a lower limit in clays as a result of the difficulty in microbial migration (DeJong et al., 2010). **Figure 2-3** below illustrates the limits of various treatment methods as well as treatment administration techniques according to the soil types.

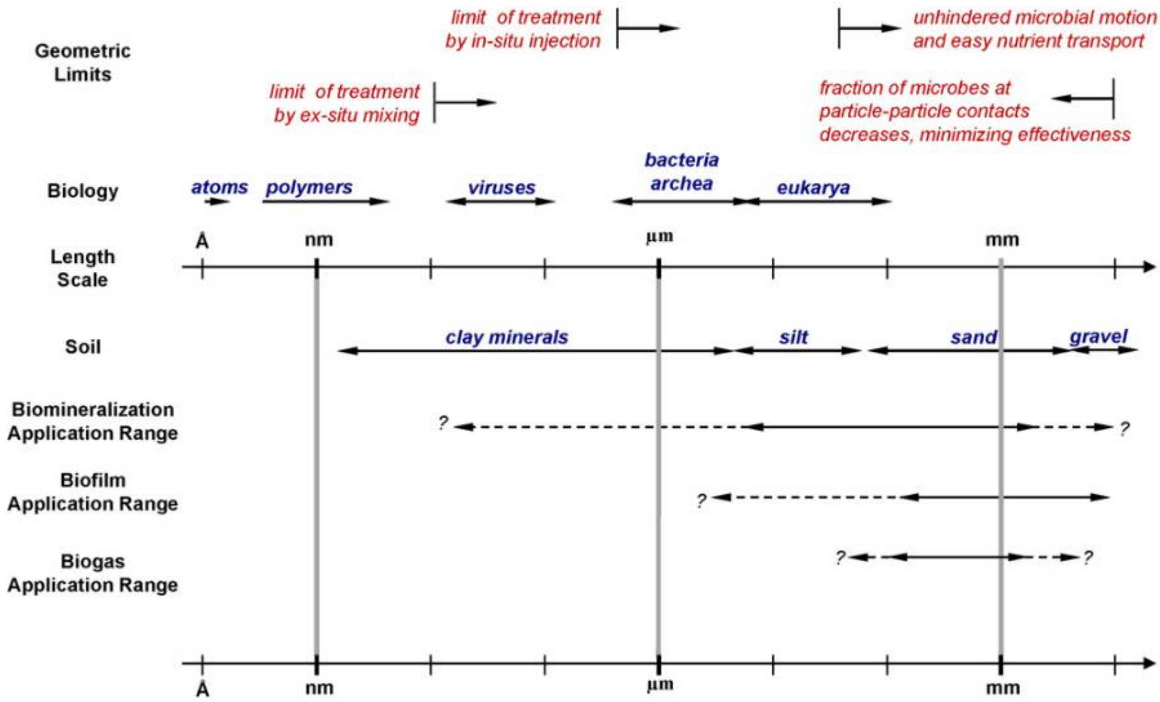

Figure 2-3: The geometric limits of biologically mediated calcite precipitation treatment administration methods or applications according to soil type and the relevant bio-mediated soil improvement system. The geometric limits observed include the biology of the microbial community utilised, and the soil type or particle size which then determine the application range of the three treatment approaches or uses, namely: biomineralization, biofilm production and biogas production (extended from Mitchell and Santamarina, 2005 by DeJong et al., 2010).

2.4.4 Treatment dispensation

The methods of treatment dispensation in MICP vary according to the application of the treatment. Surface percolation and spraying methods are frequently used for surface treatment applications to mitigate issues such as wind erosion (Jiang et al., 2019; Maleki et al., 2016). On the other hand, injection methods are more commonly used in applications requiring larger treatment depths (Haouzi & Courcelles, 2018). The common treatment dispensation techniques encountered in MICP literature alongside their corresponding treatment depths and common field applications are summarised in **Table 2-5** below. Saturated flow and immersion have largely been limited to research.

Table 2-5: A summary of the common treatment dispensation methods encountered in literature with the corresponding maximum treatment depths and field applications.

Method:	Maximum treatment depth:	Field application:	Reference:
Spraying	<40 cm	Erosion resistance, dust control, re-vegetation, construction, soil improvement	(Gomez et al., 2015) (Zomorodian et al., 2019) (Stabnikov et al., 2016)
Surface percolation	<1 m (fine grains) >2 m (coarse grains)	Erosion resistance, dust control, construction, soil improvement	(Liang Cheng & Cord-Ruwisch, 2014) (L. Cheng et al., 2013a) (Stabnikov et al., 2016)
Injection	>5 m	Construction, soil improvement	(Whiffin et al., 2007) (Liang Cheng et al., 2019) (Naeimi & Haddad, 2020)
Saturated flow	1 m	N. A	(Jiang & Soga, 2017) (Liang Cheng & Cord-Ruwisch, 2012)
Immersion	Varies	N. A	(Wen et al., 2019) (Zhao et al., 2014)

2.4.5 Inoculation

Two methods for the inoculation of the soil samples are available, namely isolated inoculation and open inoculation, each with its own benefits (Henze & Randall, 2018). Isolated inoculation occurs when the dry soil is placed in the reactor and de-ionized (DI) water is pumped through the reactor followed by five times the pore volume of the selected bacterial culture. The DI water removes dust from the reactor and allows for the identification of any system leakage. After the bacterial culture is recycled through the reactor, the system is allowed time (for instance, four hours) for acclimatization before cementation treatment commences. The method is evidently advantageous in terms of maintaining a hygienic environment as well as allowing for the regulation of the flow rate before treatment dispensation occurs (Henze, 2017). In addition to this, the likelihood of contamination by foreign bacteria in the system is reduced. However, this method has been shown to display the effects of preferential flow following inoculation. This results in predominant inoculation of the preferential flow path which impacts the cementation of the sample. Cementation occurs primary along the preferential flow path, limiting the cementation occurring homogenously throughout the entire sample (Whiffin et al., 2007). Evidently, only a portion of the sample would undergo changes in the identified geotechnical parameters, which is a major limitation in field application.

Open inoculation occurs when the dry soil is mixed with the selected bacterial culture in a beaker, saturating the soil. The reactor is filled with the saturated soil ensuring the liquid level matches the soil level, draining any bacterial culture in excess of this. Closing the reactor, the system is allowed time (for instance, four hours) for acclimatization before cementation treatment commences. This method is advantageous in terms of ensuring homogenous inoculation of the soil, preventing the development of preferential flow paths. However, the likelihood of contamination by foreign bacteria as well as the adjustment of the flow rate during the cementation treatment are limitations to the method (Henze & Randall, 2018).

In research conducted by Henze (2017), isolated inoculation proved to be ineffective in solidifying the entire sample of soil in the reactor, where only a small piece of solidified soil was discovered, surrounded by loose soil in each reactor following treatment. In the open inoculation however, the method was found to be effective in solidifying the entire sample of soil in each reactor, all of which could be handled as one discrete column. These samples, unlike those retrieved from the isolated inoculation, were able to be tested for compressive strength which ranged between a minimum of 290 kPa and a maximum of 870 kPa. These compressive strengths can be compared to those achieved by 40% lime bricks (Henze, 2017). Open inoculation was also used to solidify aggregates or soils using the urea from human urine in research by Lambert and Randall (2019). The resulting bricks produced achieved a compressive strength of 2700 kPa, which is comparable to conventionally produced bricks (Lambert & Randall, 2019). In research concerning the valorisation of copper mine tailings, open inoculation was once again used for each bio-column (de Oliveira et al., 2021). The greatest compressive strength following treatment was observed in the beach sand column, reaching 1850 kPa (de Oliveira et al., 2021).

2.4.6 Saturation

The degree of saturation (S) in a soil is defined as the ratio between the total volume of water or fluid (V_w) and the total volume of voids (V_v). The total volume of voids is a sum of the volume of air (V_a) and the volume of water of fluid contained in the soil sample. The typical ranges of the degree of saturation are dry (S = 0%), humid (S = 1 – 25%), damp (S = 26 – 50%), moist (S = 51 – 75%), wet (S = 76 – 99%) and saturated (S = 100%) (Chebet, 2017).

In previous MICP research conducted at UCT, the soil samples were completely saturated or submerged in treatment media for inoculation as well as cementation (de Oliveira et al., 2021; Henze & Randall, 2018; Mukhari, 2018). However, research has indicated that the degree of saturation can be reduced whilst the soil is able to retain a higher soil strength at the same $CaCO_3$ content. The degree of saturation higher than 80% was observed to have a marginal impact on the strength, crystal formation as well as the rigidity of the samples (Cheng et al., 2013)

2.4.7 Heavy metals

Another factor to consider, which has a significant impact on the bacterial community and consequently the MICP process, is the presence of heavy metals in the biosphere of the microbes. Heavy metals are generally classified as elements with a density higher than 5.0 g/m^3, encompassing 45 elements which include Fe, Mn, Pb, Cu, Zn, Cd and Hg. Soil typically contains higher Fe and Mn concentrations therefore the pollution effects of these elements are generally neglected (Chu, 2018). The availability of heavy metals has been found to limit and in certain cases inhibit the total bioactivity of the bacteria and thus the remedial potential (Mugwar & Harbottle, 2016; Ruggiero et al., 2005). This typically depends on the concentration of the heavy metals that the bacteria are able to withstand before growth is inhibited. This is referred to as the metal's minimum inhibitory concentration (MIC), which is the lowest metal concentration that inhibits microbial growth (Mejias Carpio et al., 2018). Heavy metals have also been found to have an impact on the composition of the microbial community or genetic variation alongside the activity (Xie et al., 2016). There exists a very close relationship between soil enzymes and soil microbes and thus heavy metals additionally impact the enzyme activity significantly. This is independent to the impact caused as a result of the death of microorganisms but rather as a direct consequence of the destruction of the active groups of the enzyme caused by the presence of heavy metals (Chu, 2018).

Microorganisms and heavy metals have a strong affinity, where the absorption, migration or transformation of these elements is made easy by the biological macromolecules including the organism's enzyme activity centre. These macromolecules continue to absorb the heavy metals reaching a limit where they become inactive, resulting in the disease and death of the bacteria (Begum et al., 2009; Giller et al., 1998; Nwuche & Ugoji, 2008; Sadler & Trudinger, 1967). Here, the composition is altered where specific microorganisms are able to adapt to the specific heavy metal irrespective of the concentrations and other strains without this ability die. Hence, this results in the degradation of microbial biodiversity in the soil (Chu, 2018; Pérez-De-Mora et al., 2006). Therefore, the presence of specific heavy metals as well the concentration of the said elements needs to be considered when evaluating the toxicity of heavy metals to the bacteria. Dependent on the specific heavy metal present, it may be particularly detrimental to the bacteria present irrespective of the concentrations. This is also dependent on the type of bacteria. For instance, aerobic-heterotrophic bacteria were found to be more sensitive to metal groups including Ni and Cu and metals such as Cu, Cd, Hg, Mn, Cr and Zn proved to be more toxic. This is similar to symbiotic nitrogen fixers where a greater toxicity was observed in Cd, Pb, Hg followed by Cu, Cr, Mn, Ni and Zn (Ahmad et al., 2005). Most living systems contain heavy metals such as copper, nickel, and zinc at sufficiently low concentrations to provide the essential nutrients without causing toxicity (Diels et al., 2006). Beyond this however, the elements can be to the detriment of the organisms. For instance, research by Mugwar and Harbottle (2016) has shown that the presence of relatively low concentrations of copper (~13–32 mg L^{-1} or 0.2–1 mM) have still adversely inhibited the growth of the *Sporosarcina pasteurii* bacteria. However, de Olivera et al. (2021) found that only at much higher copper concentrations, where the MIC fell between 128–256 mg L^{-1}, there was a considerable decline in the cell density of the *Sporosarcina pasteurii* bacteria. Thus, irrespective of concentrations, heavy metals have been found to adversely impact the bacterial populations exposed to them. As aforementioned, some bacterial communities evolve metal tolerant populations depending on the differences in sensitivity of various organisms to specific metals and their concentrations. In these cases, the proliferation metal-tolerant bacterial is heightened whilst simultaneously, the quantity and biodiversity of the microbial populations decline following exposure to heavy metals (Kozdrój & Van Elsas, 2001; Xie et al., 2016; Yao et al., 2017).

In other cases, the response of the bacteria to heavy metals was contrary to the above where no significant impact was observed. Mwandira et al. (2017) found that the impact lead had on the microbial growth of *Pararhodobacter sp.* was inconsequential which agreed with related research. However, it is important to note that this varies according to the bacterial species used as different microorganisms have varying inhibitory concentrations according to the element (Govarthanan et al., 2013; Naik & Dubey, 2013). Evidently the impact heavy metals have on the bacteria in MICP research is varied and is dependent on a number of factors which include but are not limited to the bacterial strain, the specific heavy metal as well as its concentration. These factors could either lead to a decline in microbial growth, the proliferation of more tolerant microbes or no significant change in the bacterial community.

2.4.8 Applications

The various applications of MICP include soil amelioration, rehabilitation of building materials, sequestration of pollutants (Gat et al., 2017). These applications have been implemented in various environments which include coastal, desert and urban environments, although the applications are limited in terms of clayey deposits and soils (Gomez et al., 2015; Salifu et al., 2016; Sheng et al., 2020). A growth in the research of MICP and its application has brought to light the versatility and adaptability of the technique, with emphasis on its sustainability in terms of soil improvement. In addition to laboratory scale research, field-scale applications such as the research conducted by Gomez and colleagues as well as Whiffin and colleagues, demonstrates the large-scale application and feasibility of MICP as a soil improvement technique (Gomez et al., 2015; Whiffin et al., 2007).

Various potential applications, some of which have been successfully implemented in literature are discussed by DeJong et al. (2010) and other authors:

- Soil stabilisation results in an increase in bearing capacity, a reduction in settlement and the prevention of liquefaction due to the cementation of the soil particles. Projects involving tunnelling and the excavation or construction of slopes can be carried out with minimal disruption from loose soil and collapses. Existing slopes may be stabilised as a prevention measure for slope failure (Salifu et al., 2016).
- Maintenance includes a variety of structures such as earth dams as well as foundations. This encompasses erosion or scour prevention such as the prevention of erosive piping, wind erosion as well as erosion resulting from the flow of water (e.g., rainfall). This also

includes the remediation of fissures or surface protection through bio-deposition or plugging-cementation in porous media such as rocks and concrete (De Muynck et al., 2010; Decho, 1999).

- Contamination prevention limits the interaction of harmful toxins or compounds with water bodies, ground water as well as soils. This is primarily achieved through the creation of impermeable and reactive barriers in addition to emergency immobilization of contaminants. Impermeable barriers inhibit the subsurface transport of contaminants which protects groundwater and aquifers. Reactive barriers proactively treat groundwater as it flows, ensuring contaminants are removed from the groundwater system. Emergency immobilization performs the same function, with a rapid response.
- Sustainability is a key advantage of MICP with various benefits resulting from the natural process such as carbon dioxide sequestration. Natural gas storage, aquifer storage and aquifer recovery are further examples of the uses and potential applications of MICP for environmental rehabilitation.
- Bioremediation is a cost effective, and environmentally friendly alternative to conventional treatments such as chemical precipitation, ion exchange and adsorption to mitigate heavy metal contamination (Cheung & Gu, 2007; Kapoor, 1995; Li et al., 2013; Matheickal et al., 1997). MICP is a popular biomineralization approach in bioremediation for the sequestration, immobilisation, leaching and solid-phase capture of inorganic contaminants including heavy metals in contaminated water or soil (Decho, 1999; Ivanov & Stabnikov, 2016; Kang et al., 2014; Li et al., 2013; Salifu et al., 2016).
- Currently, the MICP process is being used in the production of bio solids commercially as well as in research. BIO Mason, a US based company, is one such example where bio-bricks are grown and sold for use. Related research by Lambert & Randall (2019) looks at manufacturing bio-bricks using microbial induced calcium carbonate precipitation and human urine of comparable strength to conventionally produced bricks. Also using human urine, Henze & Randall (2018) interrogated the formation of sand columns at elevated pH values (> 11) using *Sporosarcina pasteurii*. This is similar to research by de Oliveira et al., (2021), where bio-columns were produced from copper mine tailings and beach sand, once again using *Sporosarcina pasteurii*.
- MICP has been widely used in bio-cementation of soil particles and specifically for the mitigation of wind erosion or dust suppression (Bang et al., 2011; de Oliveira et al., 2021; Meyer et al., 2011; Son et al., 2012). Here the crustal sand layer is cemented following MICP treatment to tackle issues such as dust storms, reduced visibility, air and surface water pollution as well as desertification (Zomorodian et al., 2019).

2.5 Geotechnical parameters

2.5.1 Overview

Stability experimentation will investigate the extent to which the general research objective, investigating the feasibility of implementing MICP in soil stabilisation, is satisfied. The geotechnical testing will investigate two primary components of soil stability: shear strength and porosity.

2.5.2 Shear strength testing

The shear strength (τ) of a soil is defined as its ability to resist sliding or shear stresses. Shear failure occurs when the soil fails to resist deformation caused by the continuous displacement of its individual particles which then slide past each other (Nolutshungu, 2017). The soils' ability to resist this deformation is derived from the effective cohesion (c) and the frictional resistance ($\sigma'_n \times \tan \phi'$) between the soil particles, comprised of the product of the effective normal stress (σ'_n) and the tangent of the effective friction angle (ϕ'). Therefore, the shear strength as shown in Equation 7, is used as a measure of the soil's ability to sustain loads.

$$\tau = c' + \sigma'_n \times \tan \phi' \qquad \text{Equation 7}$$

The standard triaxial test and set-up as shown in **Figure 2-4** below, is used to determine the shear strength of soils in both the drained and undrained condition.

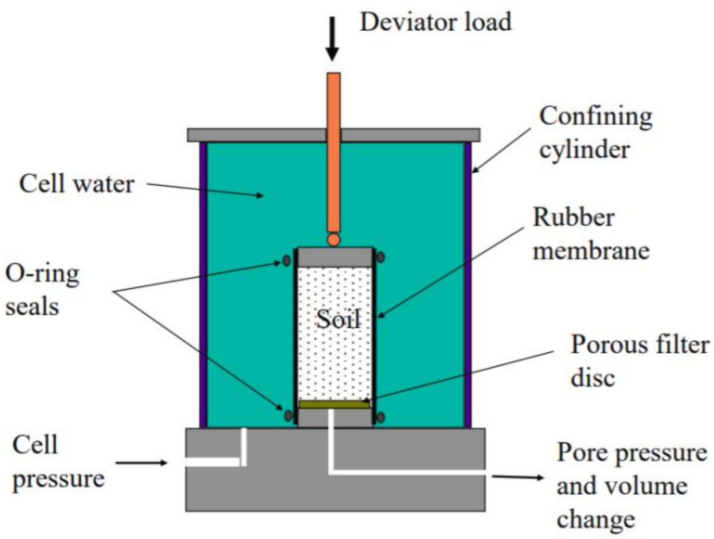

Figure 2-4: The standard triaxial test apparatus used to determine the shear strength parameters of a confined soil sample. The deviator load as well as the confining cell pressure resulting from the pressurised cell water apply stresses to the soil contained within the rubber membrane. O-ring seals and the rubber membrane isolate the soil sample within the confining cylinder ensuring no cell water infiltration into the soil sample occurs. The pore pressure and volume changes are monitored. (Nolutshungu, 2017).

The typical equipment used to analyse soil samples for triaxial testing is illustrated in **Figure 2-4**. The test determines the shear strength parameters of the specimens. These parameters can then be used in slope failure modelling. The specimen is subjected to three stresses: the effective vertical axial stress or major principal stress, the effective confining pressure or minor principal stress and the pore pressure. The four-stage test procedure involves the system and specimen preparation, saturation, consolidation, and shearing. The failure envelope, Mohr circle and stress paths are plotted and the angle of shearing resistance (ϕ), cohesion (c) and shear strength (τ) for the specimen is recorded.

The cohesion of a soil is fairly simple to determine from the Mohr Circle plot once the failure envelope defined by Equation 7 is plotted. This is simply the y-intercept of Equation 7, with the shear stress axis. The friction angle, however, can be defined at three distinct points in the soil's failure profile. Before shearing phase in the triaxial test commences, the soil is only subject to the radial stress or cell pressure in the load cell (Nolutshungu, 2017). Once shearing commences, the deviator load begins to increase whilst the confining pressure is maintained, and thus the deviatoric stress and strain increases until failure is reached. The stress-strain behaviour of the soil before failure is reached when considering the peak friction angle, is primarily dependent on the relative density and the consolidation of granular and cohesive soils (Basu, 2020b). In dense granular soils, there is less available void space for the rearrangement of soil particles that occurs during shearing and thus particles instead climb over each other resulting in an increase in volume or dilatancy (Basu, 2020b). Additional energy is thus required to overcome the additional resistance resulting from the dilatancy, which is exhibited by a peak in the stress versus strain profile before the sample reaches critical state. Likewise, over consolidated cohesive soils exhibit a peak in the stress strain profile before critical state is reached. At this point, the peak failure of the soil is defined by the peak friction angle at failure. Past the peak, the critical state is eventually reached, where the soils continue to deform at "constant volume, shear stress, normal effective stress and velocity" as the triaxial test progresses (Poulos, 1981; Roscoe et al., 1958). At the critical state, the resistance of the soil is purely frictional, and this second failure point can be defined by the critical state friction angle. In loose granular soils and normally consolidated cohesive soils on the other hand, the critical state is reached without exhibiting dilative behaviour. The soils approach the critical state without reaching a peak and demonstrate contractive behaviour. With continued shearing at even greater strains, the residual state as defined by the residual friction angle is reached, where cohesive soil particles have eventually become aligned in defined slip planes (Basu, 2020a; Davison & Springman, 2000; Kulhawy & Mayne, 1990). This residual friction angle is typically slightly lower than the critical state angle by several degrees whereas in cohesionless soils, the two angles are effectively equal (Davison & Springman, 2000; Kulhawy & Mayne, 1990).

Emphasis is placed on the friction angle as the most important parameter in evaluating the loading response of soils (Sadrekarimi & Olson, 2011). Therefore, it is crucial to explore the variables affecting the friction angle as well as the range of values obtained by other authors during testing. The values of this parameter from various sources are summarised in **Table 2-6**. Sources investigating predominantly gold tailings, low plasticity clays and poorly graded sands were consulted to evaluate the values of the friction angles obtained in these soils.

Table 2-6: A summary of the friction angle values obtained by various authors in the consulted literature for tailings, clays, and sands. Sources with primarily gold tailings, low plasticity clays and poorly graded sands using triaxial testing were included for comparison with the materials used in this research.

Soil description:	Friction angle (°):	Cohesion (kPa):	Test method:	Test conditions:	Source:
Gold tailings	36.6	10.7	Direct shear	CD	(Islam, 2021)
Coal tailings	22.2	38.9			
Red mud tailings	34.4	26.3			
Copper tailings	38 – 40	0 – 32	Triaxial	CU	(Hu et al., 2017)
Iron tailings	32 – 41	7.4 – 8.8			
Gold tailings	38.9 – 44		Triaxial	CD	(Rassam & Williams, 1999)
Copper tailings	34				(Mittal & Morgenstern, 1975)
Copper tailings	33 – 37				(Volpe, 1979)
Gold tailings	28 – 40.5				(Blight & Steffen, 1979)
Swedish tailings	37 – 42		Triaxial	CD	(Pousette, 2007)
	40 – 43			CU	
Copper tailings	39.1 – 41.1		Triaxial	CD	(Bhanbhro, 2014)
Gold and copper tailings	37 – 43	0	Triaxial	CD	(Rodriguez & Edeskär, 2013)
	40	0		CU	
Gold tailings	34	0	Triaxial	CU	(Chang, 2011)
Quick Draining Cohesionless Materials	26 – 50	0			(Byrne & Berry, 2008)

Soil description:	Friction angle (°):	Cohesion (kPa):	Test method:	Test conditions:	Source:
Low plasticity clay	28	12			(NAVFAC, 1971)
Poorly graded sand	29 – 41				(Geotechdata.info, 2009)
Low plasticity clay	18 – 35				
Low plasticity clay	14.85 – 40.38		Ring shear	CD	(Xu et al., 2018)
NC fine grained soil	4 – 30.5		Direct shear		(Arvanitidis et al., 2019)
OC fine grained soil	4.2 – 35.4				
Loose granular soil	31 – 51.8				
Dense granular soil	29 – 52				
Poorly graded sand	29 – 49.2				(Ching et al., 2017)
Uniform coarse sand	35 – 44				(Kulhawy & Mayne, 1990)
OC and NC sands	30 – 50				
NC Clay	15 – 45				
Poorly graded sand	38.7 – 46.5		Direct shear		(Al-Mhaidib, 2005)
Beach sand	41 – 49		Direct shear		(Stark et al., 2014)
Cape Flats sand	31.22 – 32.17	19.90 – 20.53	Triaxial	CU	(Nolutshungu & Kalumba, 2018)
Cape Flats sand	29 – 30	19.0 – 21.5	Triaxial	CD	(Wanyama et al., 2016)

The values in **Table 2-6** agree with the conventionally accepted values for tailings, sands, and clays. Typically, clays exhibit lower friction angles in comparison to the more angular cohesionless soils such as sands and tailings (Byrne & Berry, 2008; Geotechdata.info, 2009). A wide variation can be seen in values obtained by the different authors for the same soils. For instance, Rassam & Williams, (1999), report the gold tailings friction angle as ranging between 38.9–44°. Blight & Steffen, (1979), alternatively found the friction angle to be 28–40.5° for the gold tailings in their research. Nonetheless, it is evident that there is also significant overlap in the results obtained for the different materials between authors. A number of variables in the tests conducted contribute to this variation in the friction angles obtained. One such variable is the relative density, where the friction angle of a specific soil may vary depending on whether the particles are loosely or densely packed (Basu, 2020b).

Although emphasis is placed on the friction angle as the most important parameter in evaluating the loading response of soils, due to this said variation with factors such as stress level, soil fabric and particle damage, this has been contested (Sadrekarimi & Olson, 2011). This variation is observed in granular soils, where the shear resistance is mobilised through interparticle sliding friction and geometrical interference (Lee & Seed, 1967; Rowe, 1962; Terzaghi et al., 1996). These two parameters, the interparticle sliding friction and the geometrical interference are defined by the interparticle friction angle and the geometrical interference friction angle respectively which vary with particle surface roughness and effective confining pressures (Lee & Seed, 1967; Rowe, 1962; Sadrekarimi & Olson, 2011). As with most granular soils, the peak friction angle increases with increasing angularity, surface roughness and relative density of the soil grains (Arvanitidis et al., 2019; Stark et al., 2014). Regarding cohesive soils, the friction angle has been shown to decrease with an increasing clay content or clay sized particles such as fine grained mine tailings (Akayuli et al., 2013; Haider et al., 2011; Islam, 2021; Najjar et al., 2015; Otoko, 2014). Further variations include the impact of the moisture content. For instance, small increases in the water content of a soil resulted in large reductions in shear strength (Yates et al., 2018). Similarly, Li (2018) found that cohesion and the friction angles decrease with water content and increase with dry density. Sadrekarimi & Olson (2011) found that the yield friction angle, although independent of consolidation stress, is inversely related to the void ratio during consolidation. Research by Al-Mhaidib (2005) indicated that the internal friction angle of the sand being studied, increased with an increasing shearing rate. Similarly, Xu et al., (2018) found that the shear rate in the range of 0.1°/min to 10°/min had a slight impact on the peak strength in both low liquid limit silt clay and high liquid limit clays.

Ideally, the measured friction angle should be a function of the intrinsic characteristics of the soil and not external variables such as the testing apparatus or the specimen dimensions. That being said, it is evident that a number of factors inherent to the soils, can result in significant variations in the friction angles. However, in this research, external factors which could result in the high friction angle readings of this research, rather than variables such as the plasticity index or OCR which are innate to the soil should be considered. The reason being, these external variables such as the boundary conditions and the sample geometry, are potentially the more likely cause of the readings in the soil parameters beyond what is typically the norm in literature as encountered in this research. An example of the boundary condition can be found in soft organic soils where the interaction of the high compressibility and the high friction angles with the end restraint condition, exacerbates the non-uniform stresses and strains developed during standard laboratory testing. Muraro & Jommi, (2019) go on to articulate that laboratory test data is not reflective of the true material behaviour of the soils tested, but merely the response of the samples to the test conducted. This is due to the irregularities that develop during the test in the stresses and strains. Therefore, results reflect the reading of that specific parameter rather than its actual value.

Regarding sample geometry, the impact on the friction angle readings can be observed in the variation of the specimen size. The sample size of a triaxial specimen has been found to change the mechanical properties of a soil (Cerato & Luteneger, 2006; Hu et al., 2011; Skuodis et al., 2019). This change is dependent on the soil type where drained triaxial tests were carried out on two specimen sizes of sand and clay and no impact was observed in the sand specimens (Skuodis et al., 2019). In the clays however, it was found that the shear strength results were significantly affected by the selected specimen size. A difference of up to 7° in the effective friction angle in loose sands has been encountered in various literature, however dense sands were found to have a negligible difference (Skuodis et al., 2019). An even greater difference of 12,12% was obtained between samples of varying diameters in another study (Monteiro et al., 2018). In related research, the impact of the sample size was found to be more pertinent at greater confining pressures, where the peak friction angle was found to decrease with increasing sample size (Tan, Lee, and Sivadass, 2008). Furthermore, a smaller specimen size may not be truly representative of the fabric and structure of the soil selected (Chew & Bharati, 2011). It is for this reason that larger sample sizes are recommended for testing to more accurately observe the field shear and deformation behaviour (Skuodis et al., 2019). In the undrained shear strength testing of marine clay, Chew & Bharati (2011) found that the large diameter triaxial samples achieved results ranging between 20 to 70% lower than the conventional triaxial sample sizes. Similarly, Skuodis et al., (2019) found that the peak friction angle decreased from 26.87° to 24.50° in samples with diameters of 50 and 100cm respectively. In the sand samples however, the sample size showed no significant impact on the shear strength behaviour, similarly to related studies on coarse grained soils (Hu et al., 2011; Skuodis & Norkus, 2014).

In addition to the diameter of the specimen, or sample size, the sample slenderness i.e., the H/D ratio, has also been found to impact the readings of the friction angle value in a soil specimen. Traditionally, the international standard for triaxial testing is a sample slenderness of H/D = 2 based on early triaxial research (Peri et al., 2019). However, in later research, the ratio of H/D = 1 has been found to exhibit more stable and homogenous behaviour, with reduced sample disturbance (Ibsen, 1994; Jacobsen, 1970; Nielsen et al., 2013; Omar & Sadrekarimi, 2014; Sabaliauskas & Ibsen, 2018). For instance, in experimental work with sands samples where H/D = 2 – 2.3, significant nonuniformities were found (Kirkpatrick & Belshaw, 1968; Shockley & Ahlvin, 1960). Some authors found that the slenderness ratio has a negligible influence on the shear strength of the tested material (Olson & Campbell, 1964). However, another parameter closely linked to the slenderness of the soil sample needs to be considered. This is the aforementioned boundary condition. Typically, rough end caps in the form of filter stones with the same diameter as the sample are used instead of the smooth, lubricated and enlarged alternative. Similarly to the slenderness, this alternative end restraint results in more homogenous and less disruptive testing (Peri et al., 2019). However, the H/D ratio and the end restrains are linked, where the failure conditions are a function of both these variables, and the isolated analysis of each is complex. This is shown in research by Bishop & Green, (1965) where rough end caps were observed to increase the shear strength parameters of sand. However, this effect was found to be most significant when the slenderness ratio was equal to one rather than 2. This agrees with a majority of authors where higher strength properties are found when H/D=1. However, this is not the case with the friction angles where the variation between samples with a slenderness ratio of 1 and 2 respectively has been found by the majority to be insignificant. With regard to the rough end restraints however, there is general consensus amongst authors that rough restraints lead to greater readings of strength properties, including the friction angle (Peri et al., 2019). Nonetheless, there is a variation in behaviour with different soil types. In granular soils, rough ends have been found to induce higher friction angles that are on average, 3.6˚ greater than the norm (Omar & Sadrekarimi, 2014). Similarly to Raju et al., (1972) and Drescher & Vardoulakis, (1982) where rough ends resulted in higher friction angles. This is an overestimation of the parameter and is thus deemed unsafe for design (Drescher & Vardoulakis, 1982). On the other hand, some authors found that in cohesive soils, no pertinent difference in the shear strength parameters is observed when changing to enlarged, smooth ends and only minor reductions are induced (Casagrande & Poulos, 1964; Olson & Campbell, 1964). Contrary to both the decreasing or unchanged friction angle, one authors found an increase of 5˚following a change to smooth ends (Sachan, 2011). Evidently, there is more variation and less consensus amongst authors concerning the impact of the end restraint on the friction angle of cohesive soils.

Another external factor which may impact the shear strength parameters obtained for a soil, is the test quantity. The test quantity is considered a governing factor in the accuracy of soil strength parameters (Skuodis & Norkus, 2014). The recommended quantity of the said tests varies according to author where quantities vary between 2 – 4 tests, a minimum of 3 tests or at least 9 tests (Bond & Harris, 2008; Skuodis & Norkus, 2014). The impact of the test quantity is evident in the variation between the friction angles where the test quantity of 3 and 36 was compared for the same soil. At 3 and 36 tests the friction angle of the Klaipėda sand was approximately 16˚ and 27˚ respectively (Skuodis & Norkus, 2014). Alongside test quantity, is the testing method. In triaxial tests for instance, the friction angles for the same soil specimen have been found to be substantially higher than their direct shear counterparts (Bhanbhro, 2014). Therefore, this could also be a contributing factor to high angles of internal friction obtained during research. Evidently, numerous authors encountered uncharacteristically high friction angles in both granular and cohesive soils due to a number of external factors. Any number of these factors could result in atypical friction angles. In addition, any combination of these factors, rather than an isolated cause, could be instrumental in atypical results.

2.5.3 Porosity

Porosity (Φ) is defined as the ratio of the void volume to the total volume of a soil (Chebet, 2017). The porosity can be determined numerically following the determination of the specific gravity of the soil. The specific gravity is determined following the Density Bottle method, outlined in the D854-14 ASTM International standard (ASTM International, 2014). Specific gravity (G_s) is a dimensionless ratio between the mass of a specific volume of solids to the mass of the same volume of water at 4°C. Using Equation 8 below, the specific gravity of the soil can then be determined. M_1, M_2, M_3, and M_4 is the mass of the density bottle, mass of the bottle and dry soil, mass of the bottle, soil and water and the mass of the bottle full of water only respectively in grams.

$$G_s = \frac{G_{s\,(l)} \times (M_2 - M_1)}{(M_4 - M_1) - (M_3 - M_2)} \qquad \text{Equation 8}$$

Using Equation 9 below with the determined specific gravity, the volume of solids (V_s) is determined.

$$V_s = \frac{M_s}{G_s \times \rho_w} \qquad \text{Equation 9}$$

The volume of voids (V_v) and void ratio (e) can then be used to determine the initial porosity (Φ_i) of the soil samples before treatment numerically using Equation 10, 11 and 12 respectively, below.

$$V_v = V - V_s \qquad \text{Equation 10}$$

$$e = \frac{V_v}{V_s} \qquad \text{Equation 11}$$

$$\Phi_i = \frac{e}{1+e} \qquad \text{Equation 12}$$

Once the samples have undergone treatment, the porosity of the specimens is once again determined using the Density Bottle method.

2.5.4 Impact of MICP on geotechnical parameters

The impact that calcite precipitation has on the shear strength of various soils, particularly fine-grained soils, has not been as frequently encountered in the consulted literature as the impact the treatment has on the compressive strength. However, the Mohr-Coulomb shear strength parameters, comprised of the angle of internal friction and cohesion, are the most widely used design parameters to describe the shear strength of soils (Cheng et al., 2013). From a geotechnical perspective, the shear strength is a more useful and appropriate measure of strength for various engineering applications such as the design of foundations and retaining structures as well as slope stability analysis. Soils also generally tend to fail in shear thus it is the more pertinent measure of strength for soil materials (Nolutshungu, 2017).

The porosity of the soils following treatment is expected to decrease, resulting from the deposition of calcium carbonate crystals in the voids between the soil particles. This is because the deposition of calcium carbonate occurs in the inter-granular spaces of the soil particles (Dhami et al., 2016). As the crystals continue to grow during treatment, the pore sizes of the soil skeleton are reduced and clogged due to the bio-cementation occurring (Cardoso et al., 2018; de Oliveira et al., 2021). This gradual reduction in the pore throat size in the soil fabric reduces the void ratio as well as the hydraulic conductivity of the soil (DeJong et al., 2006; Montoya et al., 2013; Pakbaz et al., 2018; Soon et al., 2014). In other words, this reduction in pore size has a consequent effect on the movement of fluids through the soil specimen. This limits the essential treatment media from homogenously penetrating the soil and restricts the growth potential for additional crystals. This would in turn reduce the availability of oxygen to bacteria in regions where the porosity has been reduced and restrict bacterial activity (de Oliveira et al., 2021; Frederickson et al., 1991). This dichotomy furthermore limits the access of the bacteria for successful nucleation and during the deposition of calcium carbonate, the pores become progressively smaller (Navdeep Kaur Dhami et al., 2016). The soil type contributes to this where the efficacy of MICP treatment is also dependent on the ability of the microorganisms to move freely between the soil particles which is determined by the size of the pore space between each particle (Frederickson et al., 1991). Soils with a higher percentage of fines and thus smaller pore spaces, present a greater challenge to this migration of microbes. This difficulty is exacerbated once again, by the reduction in pore spaces as the calcite precipitation continues.

The pore sizes of the soils have also been shown to have an impact on the rate at which urea is consumed during the MICP process. In research by Dhami et al. (2016), a larger pore size was initially slow in the rate of urea consumption whilst smaller pore sizes were found to consume urea at a faster rate in the initial treatment days. Thereafter, as the treatment days progressed, this was reversed where the urea content in the smaller grained soils increased, and the coarser grained soils continued to decline at a growing rate. This declining microbial activity is attributed to the precipitated crystals enveloping active bacteria as well as a blockage of pore spaces, i.e. a reduction in porosity which impedes the flow of cementation media to the microbes Dhami et al., 2016; van Paassen et al., 2009). Therefore, it can be said that the interaction between the reduction pore sizes as a consequence of MICP and the soil grain sizes influences the rate of deposition at various treatment stages.

Similarly to the porosity, the impact MICP has on the shear strength of treated soil is according to the consulted literature, a positive one (DeJong et al., 2006; Soon et al., 2014). In a study exploring the impact biological stabilisation has on the shear strength of a swelling fine-grained soil comprised of silty sand, kaolinite, and bentonite, the soils cohesion as well as angle of internal friction were shown to increase following treatment (Saffari et al., 2017). This can be seen largely across the board in related research (Al Qabany & Soga, 2013; Chou et al., 2011; DeJong et al., 2006; van Paassen et al., 2010; Whiffin et al., 2007). Looking farther into this phenomenon, the influence of the bacterial concentration as well the composition of the culture media used was explored. It was found that the growth in the cohesion and the friction angle were both a function of the of the bacterial concentration, and with growing concentrations, more significant increases in the soils shear strength was observed (Saffari et al., 2017). Although the use of culture media in the treatment of the soils similarly improves the shear strength, this has significantly lower impact than the use of both the culture media and the bacteria. This validates the conjuncture that the increase in shear strength in soils brought about during MICP treatment is predominantly achieved by the bio-geochemical processes of the microorganisms (Saffari et al., 2017). Once again, the author highlights the limited research and application of MICP in fine grained soils and their mechanical behaviour, emphasising furthermore the gap concerning expansive fine grained soils (Saffari et al., 2017).

An intuitive question following a review of MICP is how exactly the process impacts the shear strength and the inner workings of this process in the treated soils micro-fabric. According to (Saffari et al., 2017), there are two means which directly and indirectly impact the shear strength of the soils structure namely:

1. Changes in the grain boundary, shape, and roughness
2. Changes in the particle bonding

The accumulation of the calcite crystals on the surfaces of the soil particles results in the above variations which in turn affect the way the soil grains roll and slide against each other and thus the sliding friction. The crystals also bond the soil grains to each other which affects the soils cohesion (Cardoso et al., 2018; Pakbaz et al., 2018). This is reflected in the resulting changes in the friction angle and cohesion or apparent cohesion of the treated soils (Saffari et al., 2017). Following MICP treatment, a greater shear strength was observed in the fine-grained swelling soil investigated where both the angle of internal friction as well as the cohesion increased (Saffari et al., 2017). This is corroborated by a number of authors where laboratory tests have shown significant increases in soil strength following MICP treatment (Al Qabany & Soga, 2013; Chou et al., 2011; DeJong et al., 2006; Pakbaz et al., 2018; van Paassen et al., 2010; Whiffin et al., 2007). These substantial increases include a 44 – 86% increase in shear strength of a sandy soil following 15 days of MICP treatment achieved by Pakbaz et al., (2018). This increase was a result of both the cohesion and the angle of internal friction. Furthermore, this increase has been observed in both the peak as well as the residual friction angle (Montoya & De Jong, 2015; Omar et al., 2016). Although both the angle of internal friction as well as the cohesion increase, the consulted literature indicated that the latter shear strength parameter undergoes a significantly greater increase following calcite precipitation. A greater increase in the cohesion was observed exhibiting the greater impact MICP has on the soils cohesion in contrast to the friction angle (Pakbaz et al., 2018; Saffari et al., 2017). In the study conducted by Pakbaz et al. (2018) for instance, the increase in cohesion grew from a negligible value to approximately 25kPa. On the other hand, the increase in the friction angle was limited to approximately 6 degrees. Nonetheless, an increase in the shear strength is to be expected following MICP treatment in soils. However, conflicting results concerning the cohesion have been reported where limited cementation was achieved and consequently a less significant increase in cohesion (Canakci et al., 2015; Chou et al., 2011). In the case of Chou et al. (2011), this was largely attributed to the experimental sample preparation method used which deviated from the norm specified by authors such as (DeJong et al., 2006).

It can therefore be surmised that the impact MICP has on the geotechnical parameters of a soil is a positive one, with the intention of soil improvement. The calcite produced by the bacteria cement the soil particles together and clog the void spaces of the soil skeleton. This effectively increases the shear strength in terms of the friction angle as well as cohesion and decreases the void ratio (DeJong et al., 2006; Muhammed et al., 2018; Soon et al., 2014). In general, the consulted literature has shown that with an increase in the calcium carbonate content, the shear strength of the treated soil grows as a result of the increase in cohesion and the friction angle (Consoli et al., 2009; Montoya & De Jong, 2015; Omar et al., 2016).

3. Methodology

3.1 Overview

The proposed methodology was comprised of three distinct phases: Phase 1: Preparation, Phase 2: Treatment and Phase 3: Testing and analysis. Phase 1 entailed the preparation of the components required for the proposed treatment. This included culturing the microbial community, preparing the cementation media, preparing the nutrient broth as well as characterising the soil to allow for a comparative analysis in Phase 3. Phase 2 followed with the treatment of the soils using the solutions prepared in Phase 1. The treatment media was administered via injection, where various doses and inoculation periods were investigated. Phase 3 concluded the experimentation with the testing and analysis of the treated specimens produced in phase 2. A control specimen was tested and analysed alongside the treated specimens to determine the efficacy of the method in stabilising the soils.

3.2 Phase 1 – Preparation

3.2.1 Geotechnical characterisation

Three soil types were treated for this study. A clay, sand and gold tailings sample were characterised before treatment to determine the geotechnical characteristics that define the soils behaviour. The clay used was a red brown Durbanville clay sample from the Western Cape Region. The sand used was a beige, clean quartz sand called Cape Flats Sand predominant in the Western Cape region of South Africa (Wanyama et al., 2016). The gold tailings used was a sage green sample from a gold mine in the Gauteng region. The sample obtained was in close proximity to the embankment wall at the TSF and thus coarser particles were anticipated. Before commencing the various characterisation tests and procedures, the soil samples were oven dried for 24 hours and a mechanised crusher was used to disaggregate the clay sample according to the D421-85(1998) ASTM International standard (ASTM International, 1998).

Particle size distribution

A component of soil characterisation is determining the particle size distribution. Three particle size distribution test methods, dry sieving, wet sieving and hydrometer sedimentation analysis were carried out in accordance with the D7928-17 ASTM International standard (ASTM International, 2017c). Representative samples were obtained by riffling or quartering, giving minimum mass of 2.5 kg. Approximately 1000 g of the soil sample was used for each sieve analysis experiment.

Using the grading curve generated by the particle size distribution test data, the soils were characterised using the USCS method following the D2487-17 ASTM International standard (ASTM International, 2017a). The typical geotechnical characteristics of each soil type were thus identified for use in determining the respective moisture contents.

Wet sieve analysis

Experiment: Wet sieve analysis

Outline: This method is used in preparation of the coarse-grained material for the particle size distribution. The wet sieving is followed by dry sieving of the remaining coarse material and the hydrometer analysis of the fines.

Dry sieve analysis

Experiment: Dry sieve analysis

Outline: This method is used to determine the particle size distribution of cohesionless, coarse grained soils i.e., particles greater than 75 µm. Where greater than 90% of the initial sample mass is contained in the 75 µm sieve, the wet sieving method is to be used for the determination of the particle size distribution.

Hydrometer sedimentation

Experiment: Hydrometer sedimentation

Outline: This method is used to determine the particle size distribution of fines i.e., particles less than 75 µm. Where less than 10% of the initial sample mass passes the 75 µm sieve, the hydrometer method is not required. The results from hydrometer sedimentation are combined with the wet and dry sieving results to form a continuous particle distribution curve.

<u>Classification of fine-grained soils</u>

Fine-grained soils with particles smaller than 425 µm were classified following the determination of the Atterberg limits. The Atterberg limit tests provide an indication of the effect water content has on the consistency of the fine-grained material. Based on the soil's behaviour, the liquid limit and the plastic limit are determined, and the plasticity index can be found on a plasticity chart (Chebet, 2017). Based on this chart, the fine-grained soil is classified as a clay or silt and organic soil with a low or high plasticity based on the determined plasticity index and liquid limit, relative to the A-line and the 50% liquid limit line.

The liquid and plastic limits define the soils relationship between moisture content and the strength and stiffness. The three Atterberg limit test methods, liquid limit, plastic limit and linear shrinkage were carried out in accordance with the D4318-17 ASTM International standard (ASTM International, 2017b).

Atterberg limits

Experiment: Casagrande's method – Liquid limit test

Outline: This method is used to determine moisture content at which the soil begins to behave as a liquid and flows when subjected to a small disturbing force.

This is defined as the liquid limit and is determined using the Casagrande apparatus.

Experiment: Plastic limit test

Outline: This method is used to determine the moisture content at which the soil begins to plastically deform. This is defined as the plastic limit and is determined by hand rolling threads of the sample of approximately 3 mm diameters. Observing the threads, the moisture content at which crumbling begins is determined to be the plastic limit.

Experiment: Linear shrinkage test

Outline: This method is used to determine the moisture content at which the soil undergoes no further changes in volume or shrinkage upon the soils drying. This is defined as the linear shrinkage and is determined by oven drying a sample in a shrinkage trough for 24 hours and determining the length of the shrinkage which occurs as a fraction of the original specimen's length.

Compaction

Compaction was carried out in order to determine two key geotechnical characteristics of the soil, namely the optimum moisture content as well as the maximum dry unit weight. The soil compaction was carried out in accordance with the D698-12(2021) ASTM International standard (ASTM International, 2012).

Compaction

Experiment: Compaction – Standard Proctor test

Outline: The Standard Proctor test is carried out to compact the soil material at various moisture contents and dry densities. The moisture curve for the specimen can then be completed and the optimum moisture content (OMC) and the maximum dry density (MDD) identified from the peak of the curve. A minimum of five moisture contents, corresponding to five data points on the moisture curve are determined when conducting compaction on the soil specimens.

Specific gravity

The specific gravity was determined in order to compute the porosity of the untreated soils. The untreated porosity of the soil allowed for the volumes of treatment media to be determined which ensured complete saturation. This allowed for an improved treatment efficacy in terms of greater homogeneity. The specific gravity of the untreated soil samples was determined using the Density Bottle Method as outlined in the D854-14 ASTM International standard (ASTM International, 2014). The porosity of the treated specimens was calculated using the specific gravity determined. The change in porosity was analysed to determine the success of the MICP treatment process.

Specific gravity

Experiment: Specific gravity – Density Bottle Method

Outline: The Density Bottle Method determines the specific gravity of the solid soil particles by quantifying the mass of a volume of water at 4°C equivalent to the mass of the soil particles. The ratio between these masses is defined as the specific gravity of that soil material. This ratio can be used to determine the porosity of the soil material numerically.

Porosity

Calculation: Porosity – Numeric method

Outline: The pre-treatment and post-treatment porosity of the soil materials can be determined based on the specific gravity determined by the Density Bottle Method. The porosity describes the volumetric ratio between the voids and the total volume of the specimen. This allows the treatment volumes to be determined in 10% excess of the voids to ensure complete saturation.

Summary

The treatment volume used depended on the void ratio of the specific soil and thus required different volumes to reach OMC or saturation. Therefore, the required volumes of treatment media could be prepared according to the geotechnical characterisation of the soils.

3.2.2 Ammonia-yeast stock solution

The selected culture media, the ammonia-yeast stock solution (ATCC®1376), contained ammonium sulphate ($(NH_4)_2SO_4$), yeast extract and tris buffer (tris(hydroxymethyl)aminomethane) at concentrations of 10 g/L, 20 g/L and 15.75 g/L respectively. Each ingredient of the stock solution was prepared individually in a 1 L shake flask with deionised (DI) water and autoclaved to ensure the sterilization of each ingredient occurred separately. This was done in order to avoid the milliards reaction, which results in inactive bacteria (Lambert & Randall, 2019). Thereafter, the three solutions were mixed aseptically at room temperature, producing the stock solution or nutrient broth. The stock solution was used to prepare the agar plates for the propagation of the bacteria, as well as in the cementation media to support bacterial metabolism during cementation.

In each 1 L shake flask, 10 g of $(NH_4)_2SO_4$ and 20 g of yeast extract were mixed respectively with 400 mL of DI water. In a (500 mL) beaker, 15.75 g of tris buffer was added to 150 mL of DI. A 32% HCl solution was added to the beaker to adjust the tris buffer solution to a lower pH of 9 to achieve optimum growth conditions for the bacteria. In the remaining shake flask, 200 mL of DI water was added, followed by the pH adjusted tris buffer solution. The three shake flasks were autoclaved and finally the three solutions were mixed aseptically at room temperature forming the ammonia yeast stock solution or nutrient broth.

3.2.3 Agar plates

To prepare the agar plates, the nutrient broth solution was replicated with one variation. To the 1 L shake flask containing $(NH_4)_2SO_4$, 20 g of bacteriological agar was added, followed by the 400 mL of DI water. The rest of the procedure was followed without change, resulting in the agar plating solution. The agar plating solution was immediately poured into petri dishes, following the autoclaving and mixing. Once the solution had solidified, the poured agar plates were stored for later use in sealed plastic bags at 4°C and were ready for streaking.

3.2.4 Concentrated bacterial culture

The bacteria, *Sporosarcina pasteurii*, was cultivated from a glycerol stock culture by Daniel De Olivera (UCT Water Research Group) following the methodology implemented by various MICP researchers (Lambert & Randall, 2019). *Sporosarcina pasteurii* was cultured under controlled laboratory conditions to achieve a standard cell concentration for all experiments. The handling of the bacteria, in the concentrated culture or otherwise, as well as the mixing and handling of the stock and agar solution or agar plates was conducted under a fume cupboard. This was done to ensure an aseptic environment was maintained.

Sporosarcina pasteurii was retrieved from cryostock and whilst maintaining aseptic conditions, a heated loop wire was used to collect it. A sample of the bacteria infesting the loop wire was then streaked onto the prepared ATCC®1376 agar plate. Incubating the plate at 30°C for 48 hours, the bacteria was propagated in order to harvest individual colonies for the preparation of the starter culture.

Following the 48-hour incubation, the streaked agar plate was examined to confirm the presence of individual bacterial colonies. In the presence of colonies, a heated loop wire was used to collect the bacterial colonies, which were then deposited into 10 mL of stock solution in a sterile McCartney bottle. This starter culture was then incubated at 30°C on the SSL1 orbital shaker (Stuart, Staffordshire, United Kingdom) at an incline at 120 rpm, maintaining aerobic conditions. Following 16 hours, the inoculum was harvested at the late exponential phase, prior to entering the stationary phase in development.

In order to produce the required volume of concentrated bacterial culture to inoculate the soils, an inoculation train was implemented. This was achieved by sub-culturing (six) 10 mL starter cultures into 90 mL of stock solution in each of the six sterile 500 mL Erlenmeyer flasks, which were all then incubated for 16 hours as described above. The six 100 mL inoculums were mixed under the fume cupboard, producing a final 600 mL which was incubated for 16 hours as described above.

3.2.5 Assessing ureolytic activity

Christensen's Urea Agar (CUA) is an indicator agar plate used to confirm the availability of viable *Sporosarcina pasteurii* cells in the concentrated bacterial culture. The media discerns between dead and viable *Sporosarcina pasteurii* cells in addition to distinguishing foreign species contaminating the culture stock. Urea degradation commences at a neutral pH and begins to increase as the reaction continues. Phenol red indicator is added to the indicator agar plates, which undergo a colour change to red or pink, following the degradation of urea (Christensen, 1946).

To prepare the indicator agar, 250 mL of DI water was heated in a conical flask. In the heated DI water, 6g of CUA was dissolved forming a solution. The CUA media was adjusted to a pH range between 6.6–7 and autoclaved for two hours to inhibit foreign bacterial contamination. Urea at a 20 g/L concentration was filtered through a 0.22 µm pore syringe filter and added to the CUA media under aseptic conditions at 50 to 55ºC. The CUA plating solution was immediately poured into petri dishes, following the autoclaving and mixing. Once the solution had solidified, the poured agar plates were ready for streaking to confirm the availability of viable *Sporosarcina pasteurii* communities prior to the commencement of experiments.

3.2.6 Cementation media

The synthetic urine was comprised of 0.3 M of urea, 0.3 M of calcium chloride and nutrient broth at a concentration of 3 g/L. The urea concentration was selected in order to mimic the concentrations found in human urine (Randall and Naidoo, 2018). An equimolar concentration of calcium chloride was selected based on previous research conducted by the UCT Water Research Group, where results indicated that an increased concentration of calcium ions gives rise to a greater MICP efficiency (de Oliveira & Fahn, 2019; Lambert & Randall, 2019; Mukhari, 2018). The treatment media comprised of the concentrated nutrient culture and the cementation media was used in the second phase of the methodology to induce MICP and improve the geotechnical characteristics of the selected soils.

3.3 Phase 2 - Treatment

3.3.1 Experimental set-up

The experimental reactors used to conduct the experiments are shown in **Figure 3-1** below. Nine stainless steel reactors in total were constructed by Charles Nicholas and Swayiza Masimthembe from the Civil Engineering Workshop for the purposes of this research.

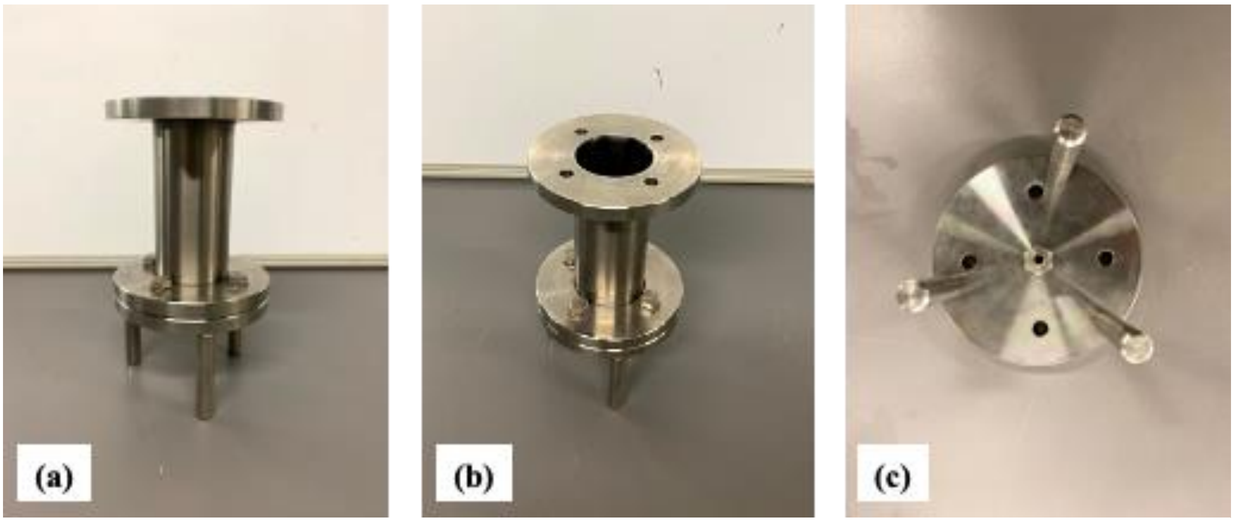

Figure 3-1: Schematic of the experimental reactors used to house the soil samples and conduct the experiments showing the **(a)** front or side view **(b)** top view **(c)** bottom view. The inoculated soil was compacted within the reactor, ready for treatment dispensation via the injection. Filter paper at the base of the reactor ensured no particulates escaped with the liquids, preventing blockages in the drainage channels.

Each soil type was allocated three reactors to ensure triplicate results were produced and the average for every soil was reported for each experiment. This was done to ensure the statistical error in each experiment could be indicated by the standard deviation of each average. This allowed for the repeatability of the experiments. The column reactor with a diameter and height of 50 mm and 112 mm respectively, was designed for compatibility with the standard triaxial testing equipment. This brough the sample slenderness or the H/D ratio to approximately 2 in each specimen contained in the cylinders. The stainless-steel column was supported by three legs and equipped with a drainage outlet at the centroid of the base plate. The top of the reactor remained open to mimic the conditions of large-scale application. The sand cylinder had the greatest drainage with drainage channels leading to a single, large orifice as shown in **Figure 3-2**.

The clay cylinder had the lowest drainage, with three, small and angled orifices. The gold tailings fell between the sand and the clay with drainage channels leading to three small, angled orifices. These drainage conditions were intended to emulate the in-situ drainage conditions of each soil. The porosity of each soil also greatly affected the volume of flow through each sample. Therefore, these conditions were expected to be reflected in the dosage fluctuations per treatment and per day.

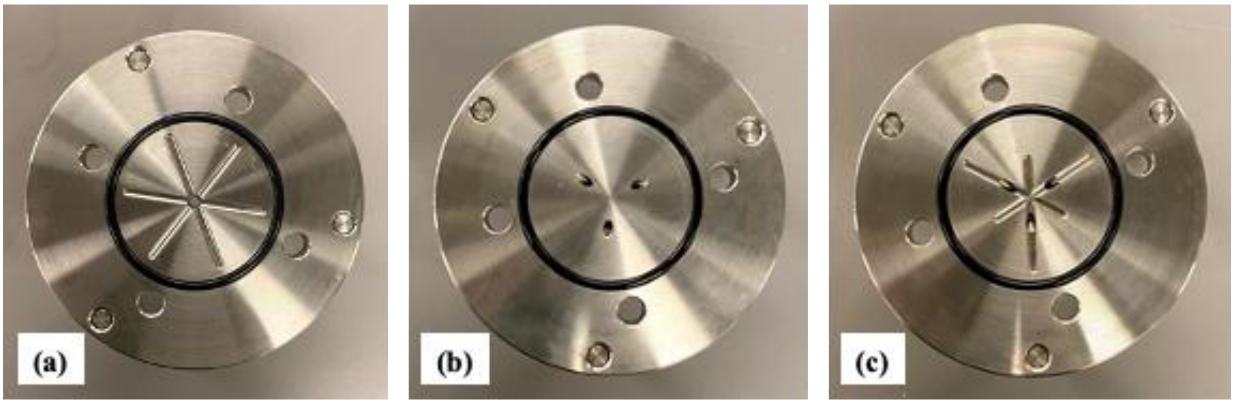

Figure 3-2: The drainage orifices of the **(a)** sand, **(b)** clay and **(c)** gold tailings. The treatment was injected into the compacted soil columns within the experimental reactors. The reactors remained open for the duration of the treatment to mimic in-situ conditions. Samples were taken from the drainage outlet, where excess liquid media was drained.

The filter paper was placed to line the base reactor, ensuring that no fines clogged the drainage outlet. The injection was used to steadily dispense the cementation media by hand to the inoculated soil at three distinct points in the soil mass. These points were approximately the midpoint of each third of the soil mass contained in the reactor. This was done to approximate the homogenous distribution of the cementation media, whilst considering that this is the key limitation to the injection method. A timer was used to ensure consistency of the flow rate whilst administering the injection. Excess fluid was drained from the reactor using the discharge outlet. Samples were collected from the influent contained in the injection, as well as the effluent expelled by the discharge port.

3.3.2 Inoculation

Based on the results of Henze's study and the aims of this research, open inoculation was selected as the method of choice for this research (Henze, 2017). Improving the shear strength of the soil in addition to reducing the porosity, which were key research aims, is possible through the cementation achieved with open inoculation. Furthermore, the research aimed to determine the feasibility of the methodology for use in sand, clay, and gold tailings, where large scale application is considered. It was evident that isolated inoculation could not be applied in soil stabilization, as large expanses of land or slopes cannot be isolated and treated in a reactor.

Open inoculation (see section 2.4.5) was chosen to populate the soil samples with bacteria to mediate the calcium precipitation process. Firstly, oven dried gold tailings, dune sand and clay respectively were weighed out and placed in glass beakers. The concentrated bacterial culture (see section 3.2.4) was poured into each of the soil samples. The saturated soils were observed to ensure the liquid level matched the soil level in the beaker and any excess fluid beyond this was removed. The inoculated soils then rested for four hours to allow for bacterial acclimatization. The soils were thus prepared for cementation treatment to commence.

3.4 Phase 3 - Testing and analysis

3.4.1 Analytical methods and sampling

The observation procedure entailed collecting effluent and influent samples to monitor the systems variation in pH, urea, dissolved calcium, and ammonium concentrations. The Thermoscientific Gallery (TSG) (ThermoFisher Scientific, Massachusetts, United States) was used to analyse the samples and determine these concentrations. The calcium and ammonium range within the calibration of the TSG are 10 – 200 mg/L and 1-5 mg/L respectively, and the upper limit for nitrogen is 5 mg/L (Lambert & Randall, 2019). This was initially measured colorimetrically and thereafter, the TSG automated the process. These ranges were then used to determine the appropriate dilution for the samples to generate readings within the detectable range. The TSG does not differentiate between ammonium ions and ammonia. It measures the total ammoniacal nitrogen (TAN) comprised of ammonium ions and ammonia in the sample. The prevalence of either ammonium ions or ammonia is dependent on the pH of the solution where a pH above 9, results in predominantly ammonia in solution. A dilute solution, such as the prepared treatment media described in section 3.2.4, will be closer to a neutral pH of 7 and thus predominantly ammonium ions would be prevalent in the solution. To ensure no further reactions occurred following sampling, the calcium and ammonium specimens were refrigerated and then analysed within 30 minutes. The urea specimen was analysed following an hour. A pH probe (HI1131B, Hanna Instruments, Rhode Island, United States) was used to measure the pH of the samples. The ammonia samples were acidified prior to analysis, adjusting the pH to ensure the conversion of nitrogen to ammonium (rather than gaseous ammonia) as well as ensuring a suitable pH for the Jack Bean urease enzyme. The sampling procedure followed for each element can be found in Appendix A.

Following the preparation of the samples, the TFG was set-up and prepared for the analysis of samples. The refrigerated samples were decanted into sample cups and placed in the TFG for analysis. The precipitation was quantified by the difference in influent and effluent calcium concentrations as shown by Equation 13 to 15 below. Calcium and ammonium results were reported following analysis. Calcium usage as shown in Equation 13 was monitored throughout the experiments to observe the systems response to treatments and trends in calcium precipitation. Similarly, the urea degradation was also quantified by the difference in influent and effluent ammonium concentrations.

$$\Delta\text{Calcium} = \text{Ca}_{\text{Influent}} - \text{Ca}_{\text{Effluent}} \qquad \text{Equation 13}$$

$$\Delta\text{CaCO}_3 = \frac{\Delta\text{Calcium}}{\text{M}_{\text{Ca}}} \times \text{M}_{\text{CaCO}_3} \times \text{V}_{\text{Reactor}} \times \Phi_{\text{Sand}} \qquad \text{Equation 14}$$

$$\text{CaCO}_3\ \text{precipitated} = \frac{100}{\text{V}_{\text{Reactor}} \times \Phi_{\text{Sand}}} \times \frac{\Delta\text{CaCO}_3}{\rho_{\text{aCO}_3}} \qquad \text{Equation 15}$$

The TSG measures the concentration of ammonia, and not urea. Therefore, the changes in the ammonium concentrations were used to determine the hydrolysis of urea. This quantified the urea utilised in biomineralization, which determined the rate of precipitation (de Oliveira et al., 2021; Lambert & Randall, 2019; Mukhari, 2018). A three-point calibration of the pH range between 4.01, 7.01 and 12.592, using Hanna pH buffers and saturated calcium hydroxide solution, was performed prior to data collection. The third calibration point was the pH of saturated calcium hydroxide solution at 21°C.

Using the procedures described above, samples of 1 mL were taken daily from the influent and the effluent to monitor the systems response during treatments for the duration of each experiment.

3.4.2 Trial experiment

The trial experiment was conducted in order to verify that the proposed methodology was technically sound. The trial result gave an indication whether MICP had any meaningful impact on the geotechnical characteristics of each soil type. In addition, the trial aimed to determine the response of the various soil types to the treatment process. MICP research has primarily focused on applications in desert or beach sand rather than tailings or clay (Liu et al., 2020b). Variables from related research conducted by Henze (2018), which successfully met his research aims, were assumed in the experiment in order to analyse the initial data and improve or alter the methodology where necessary (Henze & Randall, 2018).

The specimens were inoculated for four hours before treatment commenced. A retention time of three hours was selected between treatments, equating to three treatments daily. Following Henze's study, 42 treatment cycles were selected, equivalent to 14 treatment days (Henze & Randall, 2018). This varied from the referenced study, as treatment dispensation was manual, therefore treatment did not continue overnight. The standard concentrations and volumes discussed in section 3.2 were used throughout the trial experiment. Samples were to be taken twice, daily between treatments following the procedures outlined in section 3.4.1.

3.4.3 Microbially induced calcite precipitation experiment

This experiment was the primary focus of the research and was conducted in order to determine the effect MICP had on the shear strength and porosity of the three soil types. The experimental procedure followed was the same as the trial experiment, excepting the number of treatment cycles. An inoculation time of 4 hours was selected (Henze & Randall, 2018). Five treatments were administered daily over a period of nine days equating to 45 treatment cycles. The standard concentrations and volumes discussed in section 3.2 were used throughout the trial experiment. Samples were to be taken twice daily, between treatments following the procedures outlined in section 3.4.1.

3.4.4 Triaxial testing

The standard triaxial test detailed in section 2.5.2 was used to determine the shear strength of each column in the consolidated undrained condition. The D2850-15, D4767-11(2020) and the D7181 (2020) ASTM International standards were used to dictate the testing procedures carried out on the specimens. The primary equipment used to analyse the sample was the Geocomp Load Trac III / Flow Trac III system for triaxial testing. For the purpose of this research, the

Consolidated Undrained (CU) condition was monitored. Eighteen unsaturated specimens in total were subjected to the triaxial tests. The specimens were transferred into the triaxial cell following the procedure used for undisturbed samples.

The axial strain (%) and deviatoric stress (kPa) results generated by the triaxial software following the failure of the specimens were processed in order to plot the Mohr's circles. As described in section 2.5.2, the specimen is subjected to three stresses: the effective vertical axial stress or major principal stress, the effective confining pressure or minor principal stress and the pore pressure. The major principal stress was determined from the raw data which is the sum of the deviatoric stress and the minor principal stress. The midpoint between the principal stresses was then calculated to determine the radius and the centre of Mohr's circle. Once the principal stresses, the radii and the centres were determined, Mohr's circle was plotted for each soil at three confining pressures of 25, 50 and 100kPa. The Mohr-Coulomb failure envelope could then be plotted, lying tangential to the point of failure of the three circles and intercepting the shear stress axis. The standard straight-line equation as shown by Equation 16, indicating the gradient (m) and the y-intercept (c) of the x and y values, can then be re-written as Equation 17 on the shear stress (τ) versus normal stress (σ_n) axes. Equation 7 describes the relationship between the shear stress, normal stress, angle of internal friction or shearing resistance (ϕ) and the cohesion (c) of the soil.

$$y = c + mx \qquad \text{Equation 16}$$

$$\tau = c + \tan\phi \times \sigma_n \qquad \text{Equation 17}$$

Thereafter, the shear strength parameters could be determined where the y-intercept and the inverse tangent of the gradient of Equation 16 is equal to the cohesion and the angle of internal friction of the soil shown in Equation 7. This process was repeated for each of the soils respectively to determine the required shear strength parameters for the untreated and MICP treated sand, clay, and gold tailings.

Following the completion of the triaxial tests, the failed soil samples were removed from the testing equipment and allowed to air dry. The samples of the unadulterated and MICP treated soils were then taken for further analysis after the conclusion of the triaxial tests.

3.4.5 Porosity

The method detailed in section 3.2.1 was used to determine the porosity of each specimen before and after treatment.

3.4.6 Scanning electron microscopy and energy dispersive x-ray spectroscopy

The scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS) was carried out by the Electron Microscope Unit (EMU) at the University of Cape Town. The SEM analysis allowed for microscopic images of the treated and untreated soil particles to be obtained to examine the structure of the soils with magnifications ranging between x 1000 to x 10000. This was also done to determine the extent to which calcite crystal precipitation occurred in the soils following treatment. The EDS analysis was used to determine the elemental composition of various points of interest from the microscopic images obtained of the soil specimens.

3.4.7 Inductively coupled plasma mass spectrometry

The inductively coupled plasma mass spectrometry (ICP-MS) is a destructive elemental analysis that was carried out by the Central Analytical Facilities in the ICP-MS & XRF unit at Stellenbosch University. Here the major and minor elements contained in the untreated and treated gold tailings were determined to concentrations in parts per million (ppm). This was carried out in order to determine if the gold tailings contained any heavy metals and if the MICP treatment process affected the composition of the soil.

3.4.8 Analysis

The data generated from the stabilization testing was analysed in order to determine the effect MICP had on the geotechnical characteristics of the selected soil. The key geotechnical parameters hypothesised to change following MICP were shear strength and porosity. The margins at which the experimental variables were altered was determined, allowing for a comparative analysis to satisfy the specified research objectives and questions posed. The analysis was comprised of the following categories: soil characterisation, treatment fluctuation, urea, ammonia and calcium fluctuations, crystal morphology, shear strength and heavy metals.

In soil characterisation, the soil samples were classified through particle size distribution tests, Atterberg Limit tests, specific gravity tests and finally the compaction test. The soils were tested before and after treatment to quantify the changes in the material characteristics, specifically the porosity, following MICP treatment. The treatment fluctuation analysis involved observations of the treatment volumes entering each cylinder on a daily basis and monitoring how this changed with time. The urea, ammonia and calcium fluctuations were monitored to determine the chemical changes occurring in each system which are indicative of how the reactions in the MICP process are progressing. Here the influent and effluent concentrations of urea, ammonia and calcium were monitored to determine the extent to which each system was consuming urea, releasing ammonia as a by-product, and successfully precipitating calcium. The crystal morphology was determined next to analyse the crystal structure of the calcite precipitated between the soil particles. This once again provided a visual to determine whether or not cementation of the soil particles occurred in the specimens. The shear strength analysis comprised of processing the data obtained from the consolidated undrained triaxial tests conducted on the soils. Here the deviatoric stress versus the strain for the samples were plotted to identify the failure point of the soils. This was used to plot the Mohr's circles and corresponding failure envelopes of the soils to determine the shear strength parameters. Once again, the shear strength parameters before and after treatment were compared to quantify the extent to which MICP treatment successfully improved the shear strength parameters of the soils. Lastly, ICP-MS analysis was used to determine the elements in the gold tailings in order to determine if the specimen contained any heavy metals, which play a part in the success and efficacy of MICP treatment. This was also done in order to determine how these elements were altered by the treatment process.

4. Results and discussion

4.1 Soil characterisation

The results of the soil characterisation following the procedures described in section 3.2.1 were used to identify the geotechnical characteristics of each untreated soil investigated in this study. The sieve analysis and hydrometer analysis for coarse grained and fine-grained soils respectively was used to determine the particle size distribution for the soils investigated. For the clay, the Atterberg limits tests were carried out to determine the liquid limit, plastic limit, and the linear shrinkage of the soil. The specific gravity was found using the small pycnometer method, which was then used to calculate the porosity. The compaction test was carried out in order to establish the maximum dry density and the optimum moisture content of each soil. Once this characterisation was complete and the soils were classified, one key parameter, the specific gravity, was investigated after treatment. This was in order to determine the change in porosity of the soils following calcite precipitation.

4.1.1 Grading curves

The results of the sieve analysis as well as the hydrometer analysis are plotted in **Figure 4-1** below to illustrate the particle size distribution of the sand, clay, and tailings. The shape parameters and grading coefficients for the sand and tailings used to classify the soils are summarised in **Table 4-1** below. The sand, gold tailings and clay have a percentage of fines passing the 0.075mm sieve of 0.20%, 0.49% and 52.50% respectively. The tailings used were obtained closer to the embankment wall where coarser materials are typically encountered in a TSF.

The sand is uniformly graded where a majority of the soil particles fall within the same grain size boundary and are roughly the same size. The tailings are uniformly gap graded, which falls under a poorly graded description (Chebet, 2017). Therefore, similarly to the sand, a majority of the soil particles lie within the sand boundary. However, a central size, in this case approximately 0.3 to 0.6mm, is missing from the soil. The clay appears to be well graded with fines. More than 50% of the particles are smaller than 0.075mm therefore the fines form the largest fraction of the soil (Chebet, 2017). This soil classification plays a significant role in the success of bio-mediated soil improvement techniques (Umar et al., 2016; Zhao et al., 2014). The freedom of movement between soil particles is paramount for microorganisms to migrate between particles and coat them in order to commence calcite precipitation (DeJong et al., 2006). A greater particle size means greater voids in the soil grain structure where air or water is free to move (DeJong et al., 2006; Zhao et al., 2014). Therefore, in addition to the means of treatment administration, the particle size determines the geometric limits of MICP (Umar et al., 2016). Soils with a higher percentage of fines, such as the clay used in this study, pose a challenge to the movement and coating of soil particles due to the significantly smaller void spaces (DeJong et al., 2006). Another important factor that the particle size affects is the shear strength of the soil. Coarser particle grains result in a higher angle of internal friction, which is observed in gravels and sands

(Cheng et al., 2013). Fines typically have much lower angles of internal friction, where the soil strength is typically derived from cohesion (Wood, 1990).

Table 4-1*:* The shape parameters and grading coefficients for the sand and tailings used to classify the soils are summarised. The shape parameters (D_{10}, D_{30}, D_{60}) are used to obtain the grading parameters; the coefficient of curvature and the coefficient of uniformity (C_u, C_c).

Soil:	D_{10}	D_{30}	D_{60}	C_u	C_c
Sand	0.20	0.30	0.62	3.18	0.74
Clay	0.07	0.11	0.17	2.52	0.96
Tailings	0.17	0.28	0.91	5.22	0.49

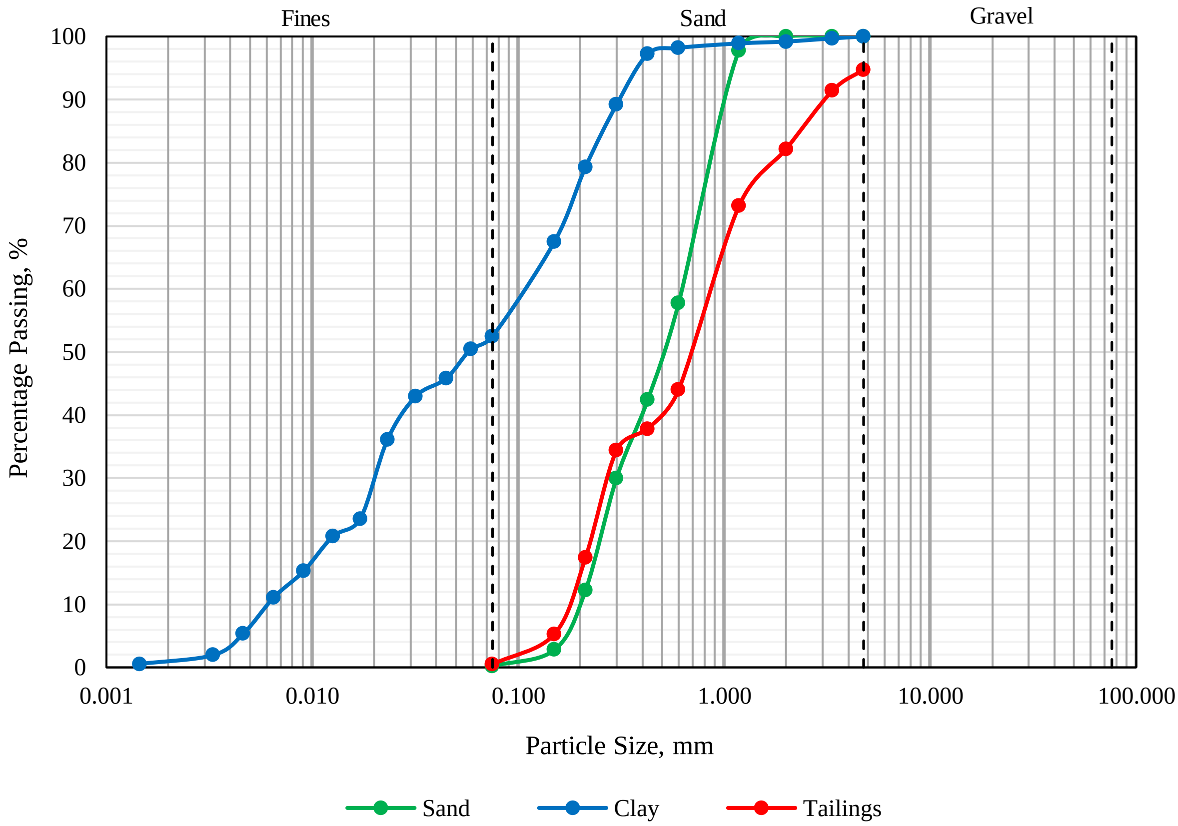

Figure 4-1*:* The particle size distribution of the sand, clay and tailings showing the particle size in (mm) plotted against the percentage (%) of the sample by weight that is finer than that sieve size. Gravel, sand, and fines grain size ranges are indicated as 76.2 to 4.75mm, 4.75 to 0.075mm and less than 0.075mm respectively. The curvature of the sand, clay and tailings distributions can be described as uniform, well graded with an excess of fines and gap graded (Hyde et al., 2021).

Evidently, **Figure 4-1** indicates that all three of the soils have a significant proportion of sand sized particles (between 0.075 and 4.75mm) which are larger, coarse-grained particles (Hyde et al., 2021). Therefore, higher angles of internal frictions can be anticipated, even in the clay soil.

Thus, the response of the various soils to the MICP treatment needs to be evaluated, taking into account the varying particle sizes and the particle shapes.

4.1.2 Atterberg limits

The liquid limit (LL), plastic limit (PL) and linear shrinkage (LS) of the clay was determined in order to classify the fines fraction (<0.075mm) of the soil according to its water absorption behaviour and limits. The results of the Atterberg Limit tests are summarised in **Table 4-2** below.

Table 4-2: The results of the liquid limit, plastic limit, and linear shrinkage tests of the clay soil. The plasticity index was calculated as the difference between the liquid limit and the plastic limit. The clay was found to exhibit low plasticity.

Parameter:	Value (%):
Liquid Limit	30.3
Plastic Limit	20.5
Plasticity Index	9.8
Linear Shrinkage	2.2

4.1.3 Specific gravity and porosity

The specific gravity of each of the soils was determined in order to calculate the porosity. Determining the change in porosity following calcite precipitation was one of the key objectives of this research. A decrease in porosity following MICP treatment is an indicator of a reduction in pore space between the soil particles. The premise for this lies in the definition of the volumetric relationship of porosity, which is the ratio of the volume of voids to the total volume of a particular soil (Chebet, 2017). Once calcium carbonate crystals are formed between particles of soil, the volume of solids will grow, effectively reducing the volume of voids between particles (DeJong et al., 2010). The specific gravity or particle density is expected to increase following treatment as the density of the solids is expected to increase after calcite precipitation. The results of the particle density tests using the small pycnometer, are summarised in **Figure 4-2** and **Figure 4-3** (Hyde et al., 2021).

The results suggest that sand potentially achieved the greatest success in terms of the deposition of calcium carbonate between the soil particles and the subsequent increase in particle density. The crystal formation was significant enough to alter the density and porosity of the soils. The aforementioned impact the particle size of a soil has on the extent of calcite precipitation as well as the migration of the microbial community between particles needs to be considered once again. With regard to clay, the particle size likely had a role to play in the deposition of crystals as there is a substantial fraction of fines (54%) which tend to fill the voids between larger soil particles (Cardoso et al., 2018). Therefore, the microorganisms were potentially challenged in finding sufficient nucleation sites in the significantly smaller particles and voids to effectively mediate the MICP process (DeJong et al., 2006; Umar et al., 2016). In terms of tailings, the larger, coarser grained particles should be ideal, similarly to sand. Therefore, another variable is identified, which could likely inhibit the reduction in voids. This is the potential issue of heavy metal toxicity which is explored further in section 4.5

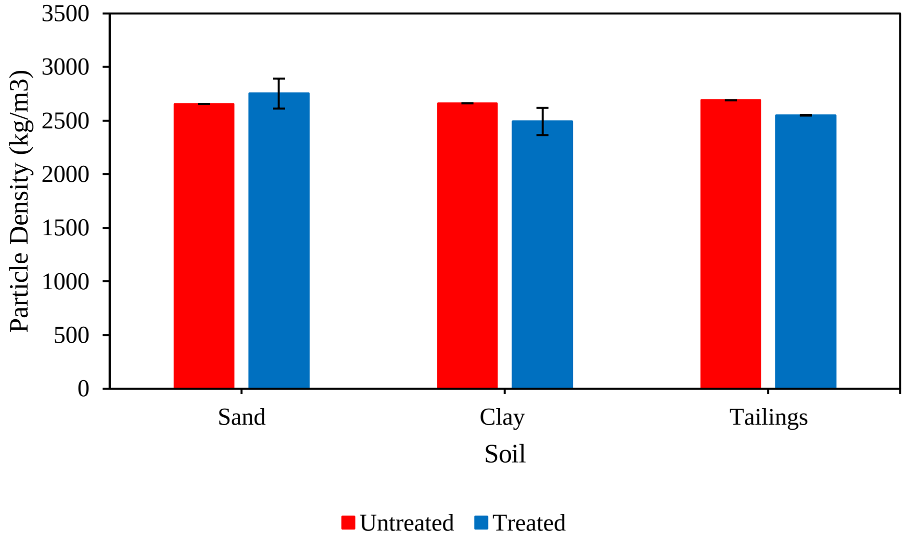

Figure 4-2: The variation in the average particle density in kg/m^3 of the untreated and MICP treated sand, clay, and tailings soils. Sand is observed as the only soil to achieve an increase in particle density following treatment. Tailings and clay both achieved decreases in particle density following treatment with clay reaching a greater decrease than tailings (Hyde et al., 2021).

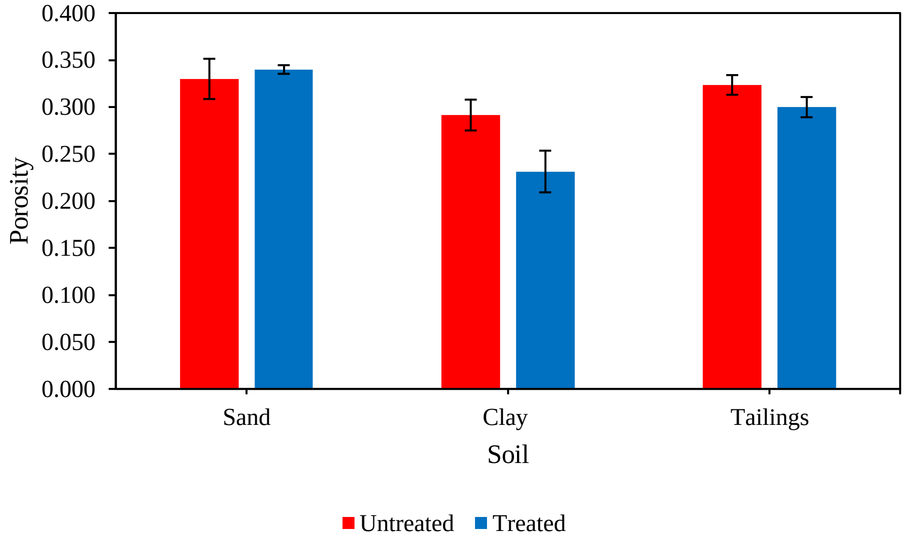

Figure 4-3: The variation in the porosity of the untreated and MICP treated sand, clay, and tailings soils. Sand is observed as the only soil to achieve an increase in porosity following treatment. Tailings and clay both achieved decreases in porosity following treatment with clay reaching a greater decrease than tailings (Hyde et al., 2021).

Limited success in terms of calcite precipitation should not necessarily result in an increase in porosity. Theoretically, the soil would remain unchanged by the treatment and maintain its natural porosity. The increase in porosity is potentially as a result of the design of the treatment system. The samples were not contained within any membrane or such enclosure when the cementation media was dispensed. They were simply compacted into the cylinder following inoculation, and the treatments commenced. Tailings and clay have been identified as having higher fractions of fines in comparison to sand in section 4.1.1 where tailings and clay have a percentage of fines of 52.50% and 0.49% respectively and sand has a percentage of 0.20%. During treatment, the effluent from the clay and tailings cylinders had greater discoloration in contrast to that of sand. The tailings and clay columns exhibited a greater run-off of soil particles following dosing, and retained the channels created during injection for longer periods. These observations are shown in **Figure 4-4** (Hyde et al., 2021).

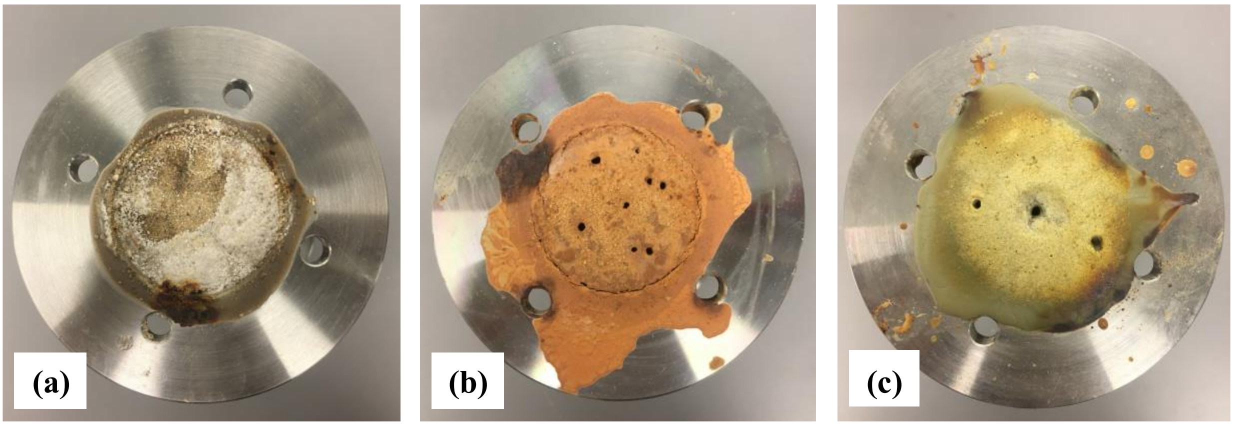

Figure 4-4: Observations made during the treatment dispensations concerning the erosion of soil particles as well as the retention of injection channels in the **(b)** clay and **(c)** gold tailings specimens. **(a)** Sand alternately, exhibited no erosion and did not retain the shape of the injection channels (Hyde et al., 2021).

A decrease in the average porosity of each soil following treatment as a direct consequence of the deposition of calcium carbonate crystals in the voids between the soil particles was expected as shown in **Figure 4-3**. In both clay and tailings, a decline in the porosity is observed. Sand is the only system where a rise in the porosity is observed. This is contrary to the observations made in the calcium concentrations to follow in section 4.3.3 below. In every system there was evidence of calcium being utilized therefore all the soils should have decreased in porosity to varying degrees. As shown in **Figure 4-4**, the sand cylinder had the least erosion and surface flow following the dispensation of the treatment media. The clay and the tailings on the other hand, underwent significant erosion during treatment. It was also observed that only very fine particles of the clay and tailings materials would rise to the surface as the treatment flowed through the specimens. Thus, the soils with a greater fines content underwent erosion which would result in an increase in porosity, rather than the decline observed. This is something to be considered with the implementation of the injection method. Furthermore, sand allowed for more closure of the temporary injection channels, which frequently closed or disappeared immediately after the removal of the needle, in comparison to the other soils. In terms of the gold tailings and the clay however, the injection channels were typically retained following treatment where the small orifices created were visible after treatment as shown in **Figure 4-4**. This is attributed to the lower fraction of fines in sand which are easier to remove and flush, and thus resulted in the erosion of the tailings and clay specimens which contain higher fractions of fines. This is therefore the likely cause of the observed reduction in particle density as opposed to the expected increase in particle density following calcite precipitation. Concerning the increase in porosity in the sand however, this is attributed to the drainage orifice in the sand cylinders. The sand cylinders had the largest drainage orifice as detailed in section 3.3.1. Some sand particles were likely washed-out during treatment once calcite precipitation had cemented most of the soil at the effluent as well as the filter paper together.

4.1.4 Compaction

The compaction tests were carried out in order to obtain the maximum dry density as well as the optimum moisture content for the given soils. Determining these two parameters allows for the optimum moisture content corresponding the maximum dry density to be identified. This moisture content was used to ensure that the soils were compacted to their densest state, similarly to compaction that is carried out in situ (Briaud, 2014). A summary of the OMC and MDD data obtained from the compaction tests is summarised for each soil in **Table 4-3** below.

Table 4-3: A summary of the maximum dry density in kg/m^3 and the corresponding optimum moisture content percentage (%) determined for each soil type during the compaction tests.

Soil:	**Maximum dry density (kg/m^3)**	**Optimum moisture content (%)**
Sand	1716	8.0
Clay	1822	14.2
Tailings	1781	13.9

Additionally, the OMC was used instead of the moisture content at 100% saturation based on a study carried out by Cheng et al., (2013) which indicated that a degree of saturation higher than 80% had minimal impact on the strength, crystal formation and rigidity of the specimens investigated. This research has shown that higher strength can be obtained at lower saturations (Cheng et al., 2017; Jiang et al., 2019; Lambert & Randall, 2019).

4.1.5 Soil classification

Following the completion of the characterisation tests, the sand, clay, and tailings were classified as the following soils in **Table 4-4** below:

Table 4-4: The final classification for the sand, clay and tailings soils investigated using the results from the sieve analysis, hydrometer analysis and the Atterberg limits. The key parameters required in the classification of the soils are indicated.

Soil type:	Key parameters:	Classification:	Symbol:
Sand	D_{10}, D_{30}, D_{60}, C_u, C_c	Poorly Graded Sand	SP
Clay	D_{10}, D_{30}, D_{60}, C_u, C_c, LL, PL, PI	Low Plasticity Clay (Sandy Lean Clay)	CL
Tailings	D_{10}, D_{30}, D_{60}, C_u, C_c	Poorly Graded Sand	SP

The soil classification gives a good indication of the behaviour of the soil which aids in understanding its response to the treatment. Upon examining the results from the triaxial testing of the untreated and treated soil specimens, significantly high shear strength parameters were reported for all the soils (see section 4.4). This was particularly evident in the untreated soils, therefore the particle shape and texture needed to be evaluated beyond the size of the particles. This was explored further in section 4.3.4 where the scanning electron microscopy images of the soils before and after treatment were analysed.

4.2 Treatment fluctuation

The fluctuation of the volume of cementation media administered to each cylinder was observed throughout the duration of the experiment. The fluctuations per day as well as the fluctuations per treatment (5 treatments were administered daily) were recorded and presented in **Figure 4-5** and **Figure 4-6** below (Hyde et al., 2021).

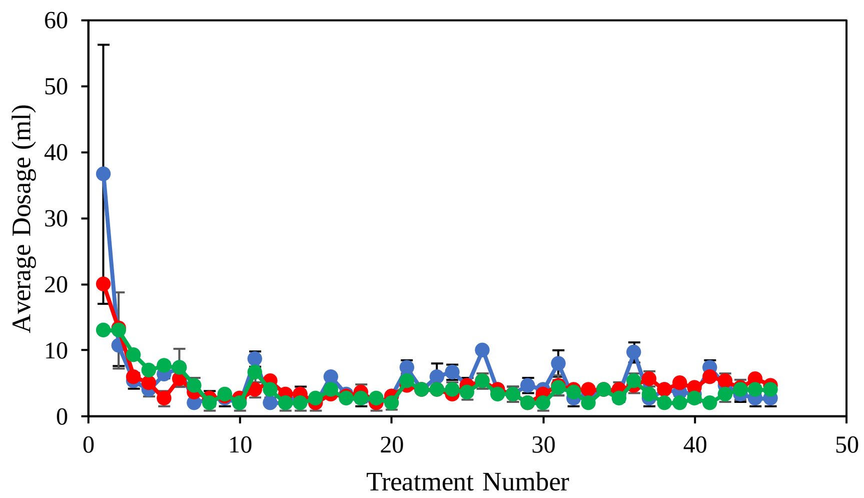

Figure 4-5: The average treatment fluctuations per treatment for sand, tailings, and clay. The variation in the average dosage volume in ml for the 45 treatments was observed for each soil. Sand was observed to accept the highest dosage of cementation media for each treatment on average, followed by tailings and lastly, clay (Hyde et al., 2021).

The similarity between the fluctuations per day and the fluctuations per treatment is evident: the average total dosage undergoes a sharp decline following the first treatment day and the first treatment number respectively. This is indicated by the significantly large standard deviations observed in the initial treatments of the first treatment day. This was apparent when administering the media as the ease of injection (resistance to the flow of fluid from the syringe) was lowest for treatment no.1 and treatment day 1. This correlated to the total volume administered which was highest for treatment no.1 and treatment day 1. This was due to the interaction of the soils' porosity and the drainage in each cylinder.

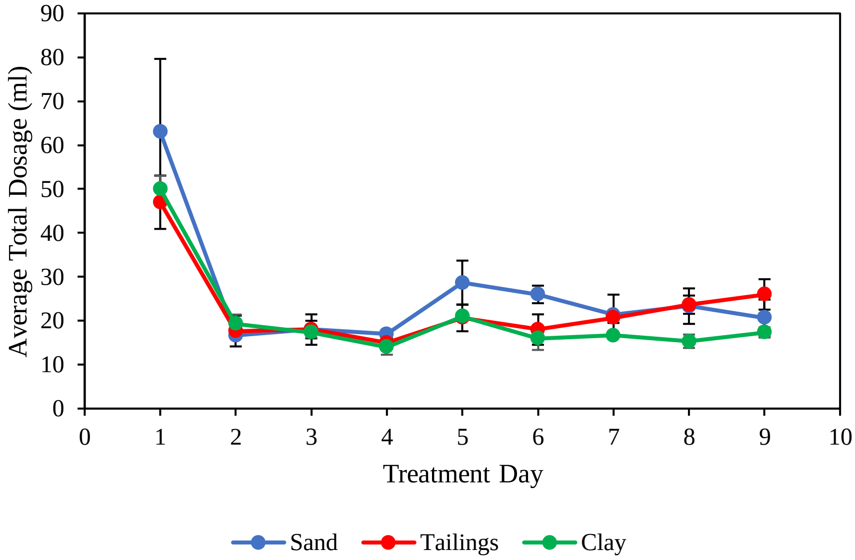

Figure 4-6: The average treatment fluctuations per day for sand, tailings, and clay. The variation in the average total dosage volume in ml for the 9 treatment days was observed for each soil. Each treatment day consisted of 5 hourly treatments. Sand was observed to accept the highest total dosage of cementation media for each treatment day on average, followed by tailings and lastly, clay (Hyde et al., 2021).

The sand cylinder had the highest drainage (see section 3.3.1) with a single, large orifice leading directly to the outlet port that allowed for the greatest flow of liquid through the sand. In addition, the particle size distribution of the soil had a significant impact on the permeability of the column. The larger, coarser particle sizes of sand allowed for greater movement of the treatment throughout the soil sample in comparison to the clay. Clay, by its very nature, electrostatically attracts water to surround each soil particle allowing for the absorption of water (Basu, 2020c). This clay-water chemical interaction makes it increasingly difficult for liquids to flow through the soil skeleton, as evidenced by the typically low permeabilities in clay materials (Basu, 2020c). The clay cylinder exhibited the lowest drainage, with three, small and angled orifices leading to the outlet port which inhibited drainage. With regards to the tailings, the predominance of coarse, larger particles also allowed for greater flow through the soil, similarly to sand. The additional drainage channels leading to the outlet port allowed for greater flow of liquid through the tailings cylinders. Therefore, these conditions were reflected in the dosage fluctuations per treatment and per day. Although the different columns and soils had varying drainage conditions, the commonality was in the volumes of effluent generated in the first treatment day. All nine of the cylinders, independent of the soil type, generated 0 mL of effluent after the fifth and final treatment of treatment day 1. Following day 1, the cylinders typically released effluent following the second or third treatment where clay characteristically generated the lowest volumes, followed by tailings with sand often generating the highest volumes. This once again, was indicative of the drainage conditions of each soil type and the design of each cylinder.

Sand was observed to have the highest average dosage throughout the treatment numbers and treatment days whilst clay was observed to have the lowest. Tailings fell between the two soils on average with a slight increase toward the final treatment number and the final treatment day respectively. The fluctuations in treatment aligned with the overall efficacy of the MICP treatment on each soil. Once again, tailings fell between the two other soils in treatment response.

Maintaining consistent dosage volumes proved to be one of the main difficulties faced in carrying out this research. Each cylinder typically behaved independently, across the different soils. It was observed that particular cylinders would reach saturation faster than others upon dispensation of the treatment. Some cylinders also exhibited greater drainage than their counterparts, with higher volumes of effluent collected after each treatment. This was not initially evident in terms of the treatment fluctuation data shown above, which achieved higher standard deviations in the first three treatment days and gradually decreased with increasing treatments as the volumes stabilised. However, the variability became more distinct once the fluctuations in the influent and effluent for each cylinder's urea, ammonia and calcium concentrations were plotted. Here, a notably higher standard deviation was observed as shown in **Figure 4-7** to **Figure 4-9** in section 4.3 below.

4.3 Urea, ammonium, and calcium fluctuation

The urea hydrolysis, ammonium production and calcium carbonate precipitation of each system for the sand, gold tailings and clay soils were monitored for the duration of the treatment days. This gave a chemical indication of the progression of the MICP process in each soil. Although a noteworthy variation was observed in terms of the urea, ammonium and calcium fluctuations for each cylinder's effluent, the general trends identified were particularly evident. Each cylinder followed an almost identical trajectory to its counterparts in terms of the rise and fall of the various concentrations. The variation was observed in terms of magnitude rather than behaviour. These fluctuations are discussed below.

4.3.1 Urea hydrolysis

The synthetic urine used as the cementation media, was comprised of 0.3 M urea and 0.3 M of calcium chloride in 3 g/L of nutrient broth. During the process of MICP, the bacteria with their highly active urease enzyme consume the urea during their metabolic processes (Dhami et al., 2013; Lee et al., 2018). The fluctuation in the average urea concentration over the treatment days is shown in **Figure 4-7** below (Hyde et al., 2021).

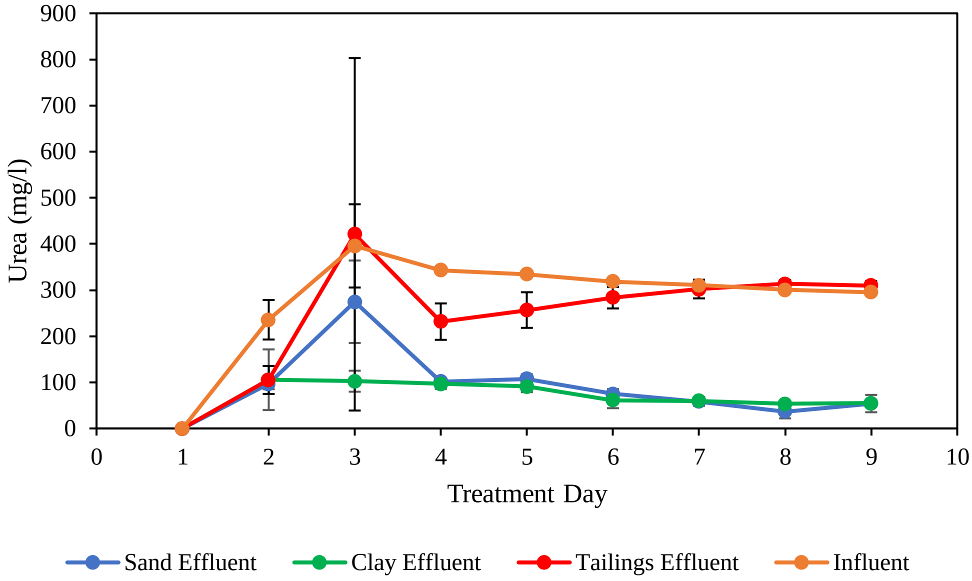

Figure 4-7: The average urea concentration in mg/L over 9 treatment days for the influent and effluent from the sand, clay and tailings cylinders following the dispensation of the cementation media. The average urea concentrations of sand and tailings are seen to peak during treatment day 3, whilst clay maintains a relatively flat curve throughout treatment (Hyde et al., 2021).

Clay exhibits a slight increase followed by a steady decline after treatment day three alongside sand for the remaining treatment. This indicates that initially, the hydrolysis of urea is relatively low and thus the effluent concentrations match those of the influent. Thereafter, the effluent concentration continually declines and approaches zero. This is similar to the effluent concentrations of urea in the sand system that are initially high, approaching the concentration of the influent. A likely source for this initially low hydrolysis of urea is the initial acclimatisation of the systems to the treatment media introduced. Once the bacteria had adjusted however, the urea hydrolysis increased, and this is evident by the decline in the effluent concentration after treatment day two and three in clay and sand respectively. In the case of sand and clay, the influent concentrations of urea are being successfully utilized by the bacteria and therefore, the effluent concentrations leaving the systems are approaching zero.

The gold tailings on the other hand, are seen to only slightly decline during treatment day 4 before a steady incline for the remaining treatment days. The trajectory followed by the tailings' effluent urea concentration closely follows that of the influent. This demonstrates that after the cementation media was fed to the tailings, the concentration of urea in the effluent remained relatively high. This is indicial of an issue with regards to the consumption of urea after treatment day 3 where the urea entering the tailings system is remaining largely unused and exiting the system through the effluent. In other words, the bacterial community within the tailings did not hydrolyse the majority of the urea in the feed for their metabolic processes. Sand and clay on the other hand, did utilise a significant proportion of the urea in the feed as evidenced by the steady decline in effluent concentrations from day 4 onwards.

The experiments were conducted in triplicates, and the average of the three cylinders was plotted to observe the fluctuations in average urea concentration between the influent and the various effluents (sand, clay, and gold tailings). For tailings in particular, an outlying concentration of urea was observed in cylinder 1 for the results of treatment day three. A reported 862 mg/L in comparison to the reported 195 and 206 mg/L for cylinders two and three respectively resulted in a large standard deviation. This explains the effluent concentration appearing to be higher than the influent concentration which would not be possible as this would be indicial of urea production in the gold tailings. The irregularity was likely a result of experimental error and so the average of cylinder two and three, and the exclusion of cylinder one will give a more accurate effluent urea concentration. This would bring the effluent concentration down below the influent concentration for urea on day 3 to 200 mg/L.

Furthermore, **Figure 4-7** illustrates the steady flattening of the average urea concentration curves for the sand and clay systems to significantly lower concentrations than the influent. This indicates that the rate of the reaction is slowing down as the effluent concentrations approach a constant value. Here the bacteria have likely acclimatised well to the systems and are steadily continuing to utilise the urea in the influent for continuing metabolic processes. The tailings system on the other hand, describes a different situation. The aforementioned tailings effluent urea concentration closely shadowing the influent urea concentration is diagnostic of the cessation of urea hydrolysis. The influent continues to feed the system urea rich synthetic urine, and gradually, the reaction slows to a stop where the concentration coming into the system is the same as the concentration leaving the system. Evidently, the urea in the influent is no longer being broken down and thus no urea is being hydrolysed whatsoever. Evidently, the bacteria in this system have not acclimatised to the system and are no longer performing their metabolic processes and are likely dying. In related research, this impediment was caused by the presence of heavy metals in the copper tailings that were used to create bio-solids using the MICP process (de Oliveira et al., 2021). Here, the presence of copper at low concentrations was still found to be toxic to the bacteria and prevented the completion of metabolic processes such as the hydrolysis of urea due to the dying microbes. Thus, the gold tailings in this research were tested for the presence of heavy metals and these findings were explored in section 4.5.

4.3.2 Ammonium production

One of the by-products of the hydrolysis of urea in the cementation media, as described above, is ammonia (NH_3). Once the cementation media was fed into the cylinders, the *Sporosarcina pasteurii* bacteria began to produce urease which broke down the urea ($CO(NH_2)_2$). The urea was then further decomposed into ammonia (NH_3) and carbon dioxide (CO_2), catalysed by the urease enzyme (Rajasekar et al., 2017; Yasuhara et al., 2012). The ammonia then ionizes into ammonium (NH_4^+). Therefore, the actual concentrations of NH_4^+ produced are revealing of the success of the decomposition of urea which sheds light on the success of the MICP process as a whole. This is shown in **Figure 4-8**, displaying the average TAN concentrations in mg/L over the treatment days for the influent as well as the effluent from the sand, clay, and tailings cylinders respectively (Hyde et al., 2021).

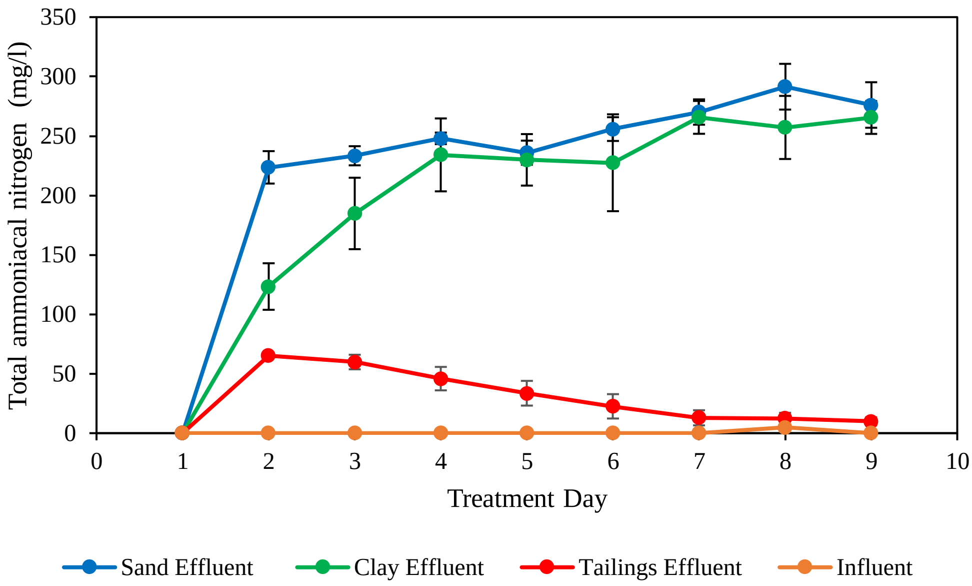

Figure 4-8: The average total ammoniacal nitrogen concentration in mg/L over 9 treatment days for the influent and effluent from the sand, clay and tailings cylinders following the dispensation of the cementation media. Sand and clay are seen to steadily increase, whilst tailings are seen to steadily decline in NH_3 concentration (Hyde et al., 2021).

According to **Figure 4-8**, the influent ammonium concentration begins at a starting concentration of 0 mg/L in the influent. This is before the hydrolysis of urea, which commences once the cementation media is fed into the cylinders. Once $CO(NH_2)_2$ is broken down, the effluent NH_3 concentrations are observed to sharply increase after one treatment day, and steadily increase for the remaining treatment days for sand as well as clay. This is indicative of the successful decomposition of urea in the influent resulting in growing concentrations of ammonium in the effluent (Haouzi & Courcelles, 2018). Tailings, however, exhibit a significantly lower peak in day two in contrast to day one, followed by a gradual decline in NH_3, which steadily approaches a concentration of 0 mg/L by day 9. Once again, these results highlight an issue with the tailings system, as shown by the declining ammonium concentration in the effluent and are symptomatic of an issue in the tailings columns with regard to the hydrolysis of urea as described in section 4.3.1 above. That is, an issue in urea hydrolysis would be evident in the production of ammonium which is a by-product of the decomposition of urea. Also to be noted, is the presence of ammonium in the influent during treatment day 8. This is potentially indicative of experimental error during the use of the TSG as the reaction described in Equation 1 requires contact with the facultative bacteria in order for urea to be broken down into ammonia and carbon dioxide (Cheng et al., 2017; Liu et al., 2020a). This is evidenced by the uniform 0 mg/l influent concentration throughout days 1 to 7 as well as day 9. However, the ammonium ions could have also been converted to ammonia gas depending on the pH, which would have quickly disappeared.

Proceeding with the ammonium production, the limitation in the tailings system could be a result of a number of factors including but not limited to the bacterial species, bacterial concentration, temperature, pH, the chemistry of the cementation solution as well as the soil itself (Sheng et al., 2020). However, on the basis of the apparent success of the first reaction (Equation 1) in the MICP process for the sand and clay columns, it can be deduced that the issue likely lies with the soil itself. This is derived considering that the other factors remained largely consistent throughout the experiment. The presence of particular heavy metals in soils at varying concentrations has been found to result in toxic environments for the exposed bacterial communities, eventually killing the microorganisms (de Oliveira et al., 2021; Mugwar & Harbottle, 2016; Ruggiero et al., 2005). Once again, this is likely the cause of the negative performance of the tailings system in terms of urea hydrolysis, as gold tailings are often highly contaminated with the heavy metals utilised in the processing of gold (du Plessis & Curtis, 2021; Mpanza et al., 2020). In order to confirm this, inductively coupled plasma mass spectrometry or ICP-MS analysis is required to determine the elements, particularly heavy metals, within the tailings. As with the urea hydrolysis and the ammonium production, the calcium carbonate precipitation results were examined to shed light on the success of the MICP process in treating the soils.

4.3.3 Calcium carbonate precipitation

The results of the average calcium concentrations confirmed observations made in the systems with regard to the performance of the sand, clay, and tailings systems in sections 4.3.1 and 4.3.2 above. As described by Equation 1, 2 and 3, once the urea enzyme catalyses the decomposition of urea into ammonia and carbon dioxide, the NH_3 and CO_2 are then ionized into ammonium and bicarbonate ions in the presence of water (Pakbaz et al., 2018; Sterianos. B, 1988). The resulting hydroxyl ions react with the bicarbonate ions forming carbonate ions. The calcium rich cementation media fed into each system then allows the final step to occur: the reaction of the carbonate ions with calcium ions, precipitating calcium carbonate crystals as described by Equation 4 and 5. Thus, the fluctuation of the calcium effluent concentrations in comparison to the influent concentrations in each system gives a good indication of the extent to which the reactions described are successful. Therefore, the average calcium concentration in mg/L over the treatment days for the influent as well as the effluent from the sand, clay and tailings cylinders respectively are shown in **Figure 4-9** below (Hyde et al., 2021).

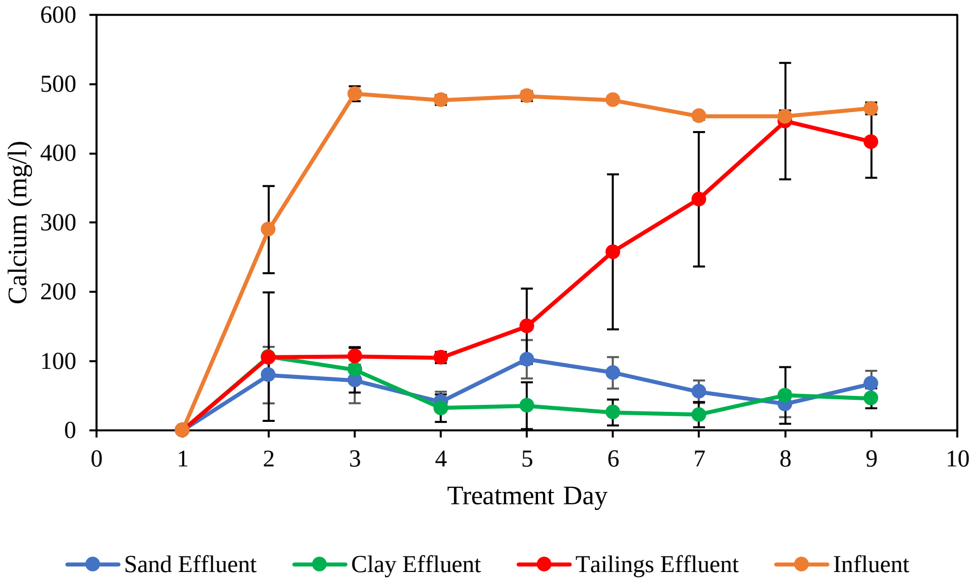

Figure 4-9: The average calcium concentration in mg/L over 9 treatment days for the influent and effluent from the sand, clay and tailings cylinders following the dispensation of the cementation media. Sand and clay are seen to maintain low flat curves, whilst tailings steadily climb to reach influent calcium concentrations (Hyde et al., 2021).

Once again, **Figure 4-9** is indicative of the successful completion of the required processes for the eventual precipitation of calcite into the soil structure. The influent maintained steady and high calcium concentrations for each systems utilisation. For sand and tailings, the resulting effluent concentrations are considerably lower maintaining concentrations below approximately 100 mg/L for the duration of the treatment days. This indicates that the calcium provided to the system by the influent is being utilised resulting in a decline in the effluent concentration. The tailings system appears promising from treatment day 1 to treatment day 4, where low effluent concentrations of calcium are maintained similarly to sand and clay. However, from treatment day 4 onwards, the average effluent calcium concentration rapidly rises, reaching the influent concentration on day 8. Evidently, the tailings system begins to increasingly react with fewer and fewer calcium ions until eventually, by day 8, the calcium ions fed into the system are leaving the system in the effluent. Once again, the tailings systems response is diagnostic of an inhibition to its optimal functioning. It appears that as similarly displayed in the urea hydrolysis and ammonium production components of the MICP process, something inhibits the efficacy of system in carrying out its function. In this case being, the precipitation of calcium carbonate crystals from available calcium ions in the influent solution. With regards to this component of the MICP process in particular, this phenomenon is not limited to this research. One of the main challenges associated with MICP, is the irregular precipitation of calcium carbonate crystals in the soil of choice (Cheng & Cord-Ruwisch, 2014; Whiffin et al., 2007). The heterogenous distribution of calcium carbonate crystals results in clogging and the creation of preferential flow paths which further exacerbates the non-uniform cementation (Rowshanbakht et al., 2016; Yasuhara et al., 2012). This is a potential cause in the performance of the tailings system, where a preferential flow path clogs the even permeation of the cementation media resulting in the reduced calcium usage efficiency.

In this particular instance, all three tailings' cylinders displayed no initial signs of preferential flow paths once the samples were removed after treatment day 9. However, once the samples were broken apart to inspect the interior of the specimens, some significant zones of cementation amongst the rest of the soil sample were observed as shown in **Figure 4-10**.

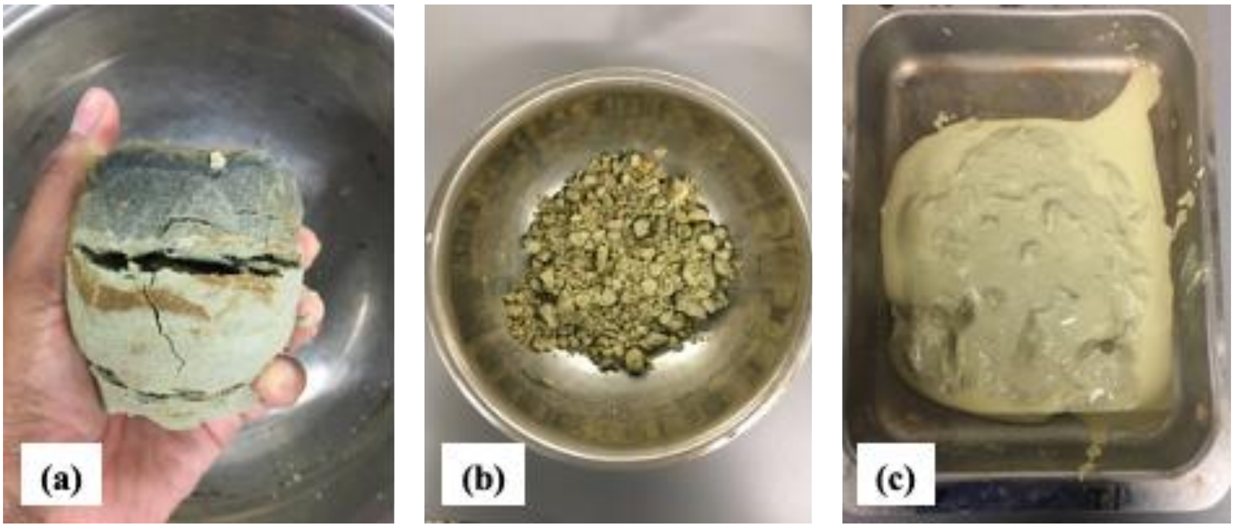

Figure 4-10: Images of the tailings soil samples after removal from the cylinders following the completion of the MICP treatment. **(a)** No preferential flow paths or zones of cementation were observed initially on the exterior of the treated sample. **(b)** However, upon inspection of the specimen's interior once the sample was broken apart, several small pellets of cemented gold tailings were discovered surrounded by uncemented soil. **(c)** Furthermore, following the specific gravity test which saturated the sample, the multiple inhomogeneous zones of cementation became more evident.

This was similar to the clay samples, where no significant zones of cementation were discovered upon first inspection after the final treatment day. In both the tailings and clay samples, no preferential flow paths were initially observed. Although, once the clay samples were broken up to observe the interior conditions, no zones of cementation were observed whatsoever unlike the tailings. The condition of the soils appeared to be essentially unaltered by the MICP process as shown in **Figure 4-11**. The sand columns on the other hand displayed visible signs of inhomogeneous cementation externally. Once the specimens were broken apart, the selective cementation was observed in the interior of the cylinders as shown in **Figure 4-12**

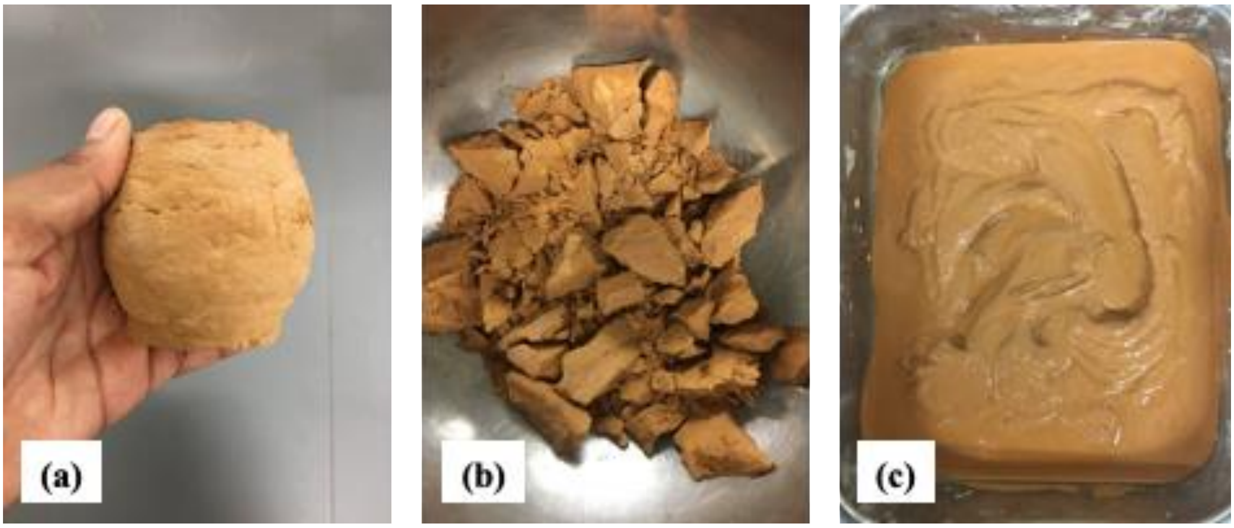

Figure 4-11: Images of the clay soil samples after removal from the cylinders following the completion of the MICP treatment. **(a)** No preferential flow paths were observed, and no inhomogeneous zones of cementation were found. **(b)** Upon inspection of the interior, no evidence of calcium carbonate precipitation occurring was found and the soil samples appeared to be unchanged. **(c)** Following the specific gravity test which saturated the sample, no pellets or zones of cementation were discovered, and the sample appeared to be smooth and unchanged.

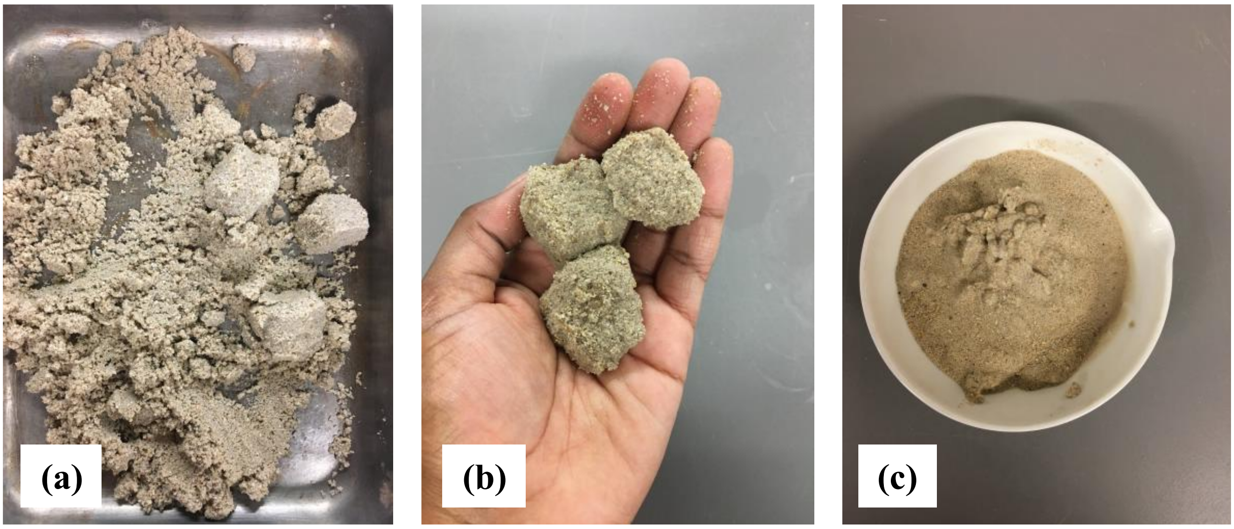

Figure 4-12: Images of the sand soil samples after removal from the cylinders following the completion of the MICP treatment. **(a)** Externally, significant zones of inhomogeneous cementation were found. Only the cemented portion of the samples remained intact following removal from the triaxial apparatus. **(b)** Significantly large pellets of cemented soil in comparison to the gold tailings and the clay were found in the interior of the sand specimen. **(c)** This was also evident following the specific gravity test which uncovered smaller pellets amongst the larger pellets initially observed before saturation.

Sand exhibited the highest level of cementation throughout the soil samples examined. Significant zones of cementation were found at the top as well as the bottom of the cylinders where the influent and the effluent entered and exited the system respectively. The cementation at the effluent is likely due to the slow drainage of the cylinders as shown in **Figure 4-5** and **Figure 4-6**. This allowed for prolonged exposure to the treatment media and thus more successful cementation at the bottom of the soil column. Each soil column displayed unique cementation throughout with one cylinder achieving smaller, more numerous portions of cemented soil and the remaining two achieving significantly larger, less frequent sections of cemented soil. Therefore, the preferential flow paths or inhomogeneous cementation are likely not the cause of the limited performance of the tailings system in terms of calcium usage. This confirms the observations made in section 4.1.3 surrounding the increase in porosity observed in the sand as well as the decrease in particle density in the clay and tailings. These variations from the expected behaviour are not indicative of unsuccessful cementation but are likely due to minor experimental issues such as the aforementioned erosion.

Although, unlike the tailings and the sand, no visible zones of cementation were observed in the clay soil, the SEM images in section 4.3.4 indicate microscopic evidence of calcite precipitation. Therefore, the calcium depleted in the effluent stream was successfully precipitated. In spite of selective cementation and the evidence of preferential flow paths, sand was able to maintain high calcium usage efficiencies and low calcium effluent concentrations. Likewise with the clay specimens, which exhibited no visible changes after precipitation and were still able to maintain similarly high usage efficiencies and effluent concentrations of calcium. This in turn highlights the more likely possibility of the toxicity of the tailings to the microbial community resulting from the concentrations of heavy metals. This will be explored further in section 4.5. The inhomogeneous precipitation throughout the tailings and clay can however explain the significant standard deviations in the calcium concentration and calcium usage efficiency data as each cylinder appeared to respond uniquely during the formation of the calcium carbonate crystals. Therefore, the crystal morphology of the calcium carbonate crystals was examined in order to probe further into the variation of MICP according to the soil type.

4.3.4 Crystal morphology

Using the SEM images shown in **Figure 4-13**, the treated soil particles were studied to determine the crystal morphology. In **Figure 4-13 (a)** Rhombohedral layers of calcite are prevalent between the particles of soil. The layer-like distribution varies significantly from individual crystals covering the surface of the soils in **(b)**. In **(b),** a substantial distribution of polyhedral and rhombohedral calcite crystals cover the surfaces of the soil particles. In **(c)**, the elongated orthorhombic aragonite crystals are scattered across the surface of the soil particles. A variety of sizes are evident in the rod-like crystals. In **(d)**, more elongated orthorhombic aragonite crystals are distributed across the soil specimen. However, the rod-like crystals are more thoroughly assimilated into the soil matrix and are not only scattered on the particle surfaces. In **(e)**, elongated orthorhombic aragonite crystals are sparsely distributed amongst the platy clay particles. In **(f)**, distinct clusters of elongated orthorhombic aragonite crystals are scarcely distributed on the surfaces of the platy clay particles.

Typically, the pH of the bacterial environment as well as the type of heavy metal present determine the morphological features of the crystals precipitated in MICP (Kang et al., 2014; Li et al., 2013). For a pH between 8 and 9, which is the target range for peak ureolytic activity, rhombohedral, sphere and needle shaped crystalline carbonate crystals are anticipated. (Li et al., 2013; Stocks-Fischer et al., 1999). Therefore, the calcium carbonate polymorphs observed in **Figure 4-13** are as expected in the sand, clay, and gold tailings.

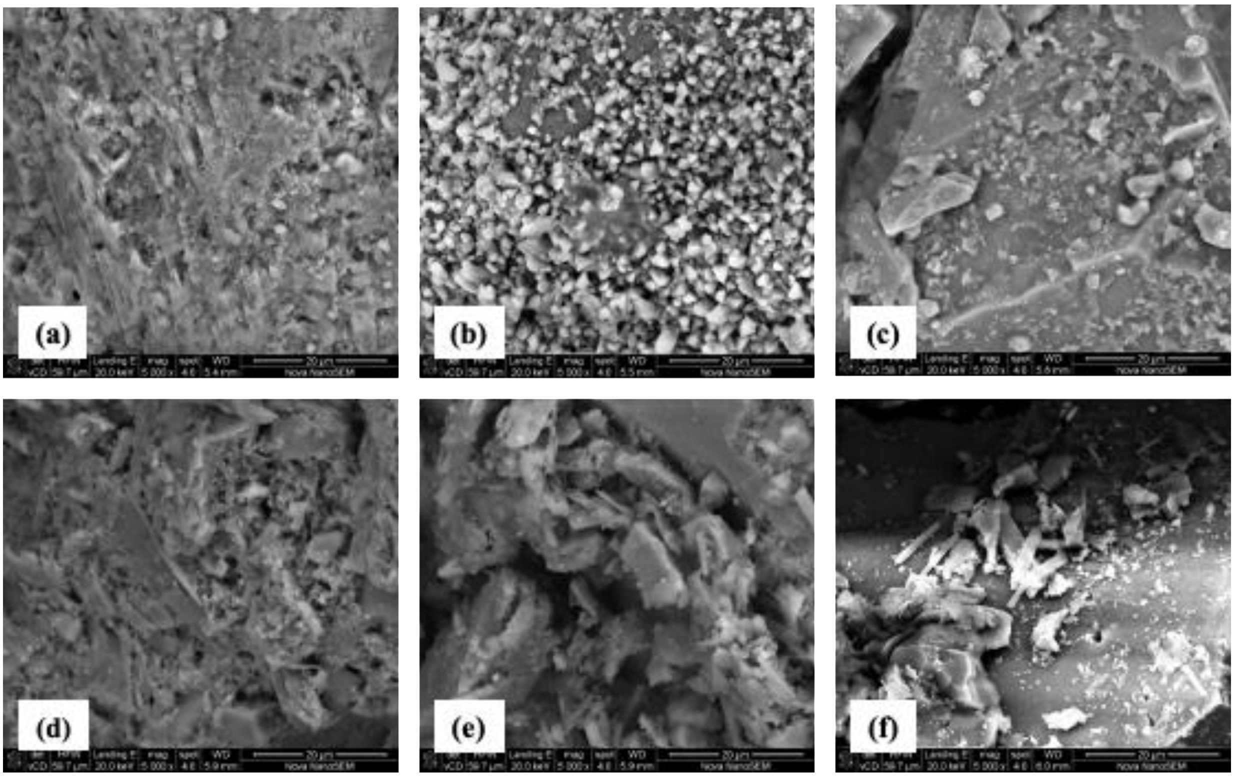

Figure 4-13: SEM Images of the calcium carbonate polymorphs in the MICP treated **(a)** rhombohedral calcite layers in sand at x 5000 magnification **(b)** polyhedral and rhombohedral calcite in sand at x 5000 magnification **(c)** scattered elongated orthorhombic aragonite in tailings at x 5000 magnification **(d)** assimilated elongated orthorhombic aragonite in tailings at x 5000 magnification **(e)** elongated orthorhombic aragonite in clay at x 5000 magnification and **(f)** clusters of elongated orthorhombic aragonite in clay at x 5000 magnification.

Concerning heavy metals specifically, rhombohedral, spherical and needle-like crystals are expected morphologies in the presence of Ni and Co, Cu and Cd and Pb and Zn compounds respectively (Li et al., 2013). With the exception of Cd, these elements were all present in trace and major concentrations in the treated and untreated gold tailings, therefore the elongated orthorhombic crystals do not follow this trend. However, numerous other heavy metals were found in the gold tailings, any number of which could influence the morphology of the crystal precipitates. Another factor to consider alongside the shape, is the distribution and prevalence of the calcite crystals in the soils.

The images of the crystal morphology in each of the treated soils above confirms the observations made in the urea, calcium, and ammonium fluctuations in section 4.3. Evidently, the distribution of calcite crystals follows the trends in calcium usage efficiency as well as urea breakdown efficiency in sand. Sand exhibited the greatest prevalence of calcium carbonate crystals, followed by the gold tailings and lastly, the clay. Clay exhibited largely surface level crystallisation whilst greater integration between soil particles and the crystals was visible in the sand and gold tailings. This varies from the trend observed initially where sand and clay exhibited the lowest effluent calcium concentrations and the highest calcium usage efficiencies in comparison to the gold tailings. Potentially, a longer treatment duration could remedy the limited crystal formation in the clay soils. It was necessary to follow the crystal morphology analysis with a scrutiny of the shear strength parameters of the soils in order to determine the extent to which the calcium carbonate crystals were able to alter and potentially improve the shear strength of the untreated specimens.

4.4 Shear strength parameters

The shear strength parameters for the untreated soils and the treated soils were determined by plotting the Mohr's circles and their corresponding Mohr-Coulomb failure envelope using the data obtained from the triaxial testing (see Appendix B). This was carried out in order to satisfy one of the key objectives; determining the effect MICP treatment has on the shear strength parameters of the soils, namely the angle of internal friction and the cohesion. The deviatoric stress in kPa versus strain percentage curves for the untreated and treated soils are shown in **Figure 4-14** and **Figure 4-15** respectively.

The sand and gold tailings samples first attained a peak before reaching critical state as shown in **Figure 4-14**, therefore the samples are identified as dilative or dense (Kalumba, 2021; Mendoza, 2017; Wood, 1990). This is indicative of additional frictional resistance resulting from the dense state of the soils. Additional external energy is required in order for the particles to overcome the frictional resistance caused by the soil grains as they "climb" over each other during the rearrangement occurring during shearing. The clay on the other hand, exhibited largely contractive behaviour, similarly to a loose sand and instead steadily approached critical state failure (Basu, 2020c; Kalumba, 2021; Mendoza, 2017). The deviatoric stress continues to rise as the critical state is approached. This is foreign to traditional contractive behaviour where typically no peak is observed. This is potentially indicative of slight dilatancy in the clay sample. As is common practice, the maximum deviatoric stress between the axial strains of 0 and 16% is used to compute the principal stresses in order to plot Mohr's circle for that particular sample. Therefore, the peak friction angle is reported as the angle of internal friction for the sand and tailings and the critical state friction angle is reported as the angle of internal friction for the clay. The peak friction angle is dependent on the relative density of the soil alongside the confining stress and the critical state friction angle is dependent on the frictional behaviour of the soil (Basu, 2020c; Wood, 1990). This could potentially result in uncharacteristically high angles of internal friction to an extent.

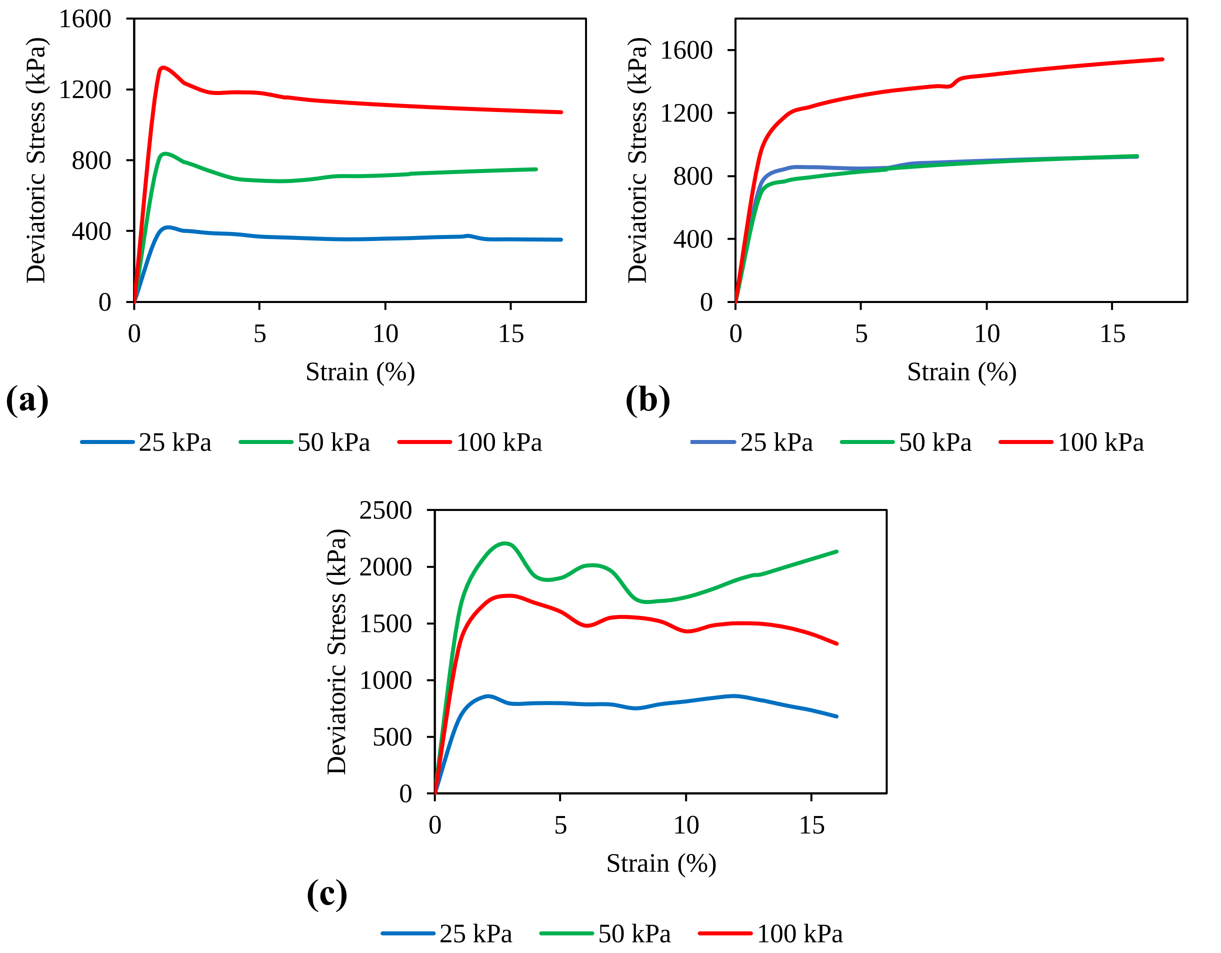

Figure 4-14: The deviatoric stress in kPa versus the percentage strain for the untreated **(a)** sand, **(b)** clay and **(c)** tailings samples are plotted to identify the failure point at peak and critical state of the specimens during consolidated undrained triaxial testing. The stress-strain relationship of the sand and tailings specimens during shearing affirmed the dense state of the soils, and that of clay was suggestive of a normally consolidated stress history in the sample.

Another likely cause for the high angles of internal friction, is work hardening plasticity. Work hardening plasticity is a phenomenon observed in materials such as soils which deform plastically during failure (Basu, 2020c; Nolutshungu, 2017). Once yielding occurs, the yield surface begins to change and the strength of the material (or soil in this case) begins to increase. Both the untreated clay and tailings exhibit strain hardening in their respective stress-strain curves, where the deviatoric stress steadily increases following yielding. This results in higher peak or critical state values, which in turn increases the major principal stress and the radius of the corresponding Mohr’s circle. Once the failure envelope is fitted to the larger circles, a steeper gradient is achieved. Strain hardening is not observed in the sand specimens, where the deviatoric stress begins to decline after yielding. However, high angles of internal friction are to be expected in sands, particularly beach or desert sands with a high relative density such as the soil used in this study.

In **Figure 4-14**, the curve for tailings at a confining pressure of 50kPa is significantly higher than that of the 100kPa confining pressure, which varies from the norm as the maximum deviatoric stress is anticipated at the highest confining pressure i.e., 100kPa. This appears to be an anomaly in the data, similarly to the clay at 25kPa as this is not observed again in stress versus strain curves for the MICP treated soils. It is also worth noting that the curve exhibits significant irregularity which is indicative of a potential issue with that particular specimen during testing.

In terms of the treated soils, strain hardening was once again observed in the clay and tailings materials and not in the sand specimens. Overall, the behaviour was largely comparable to the untreated soils with considerably similar stress versus strain curves as shown in **Figure 4-15**.

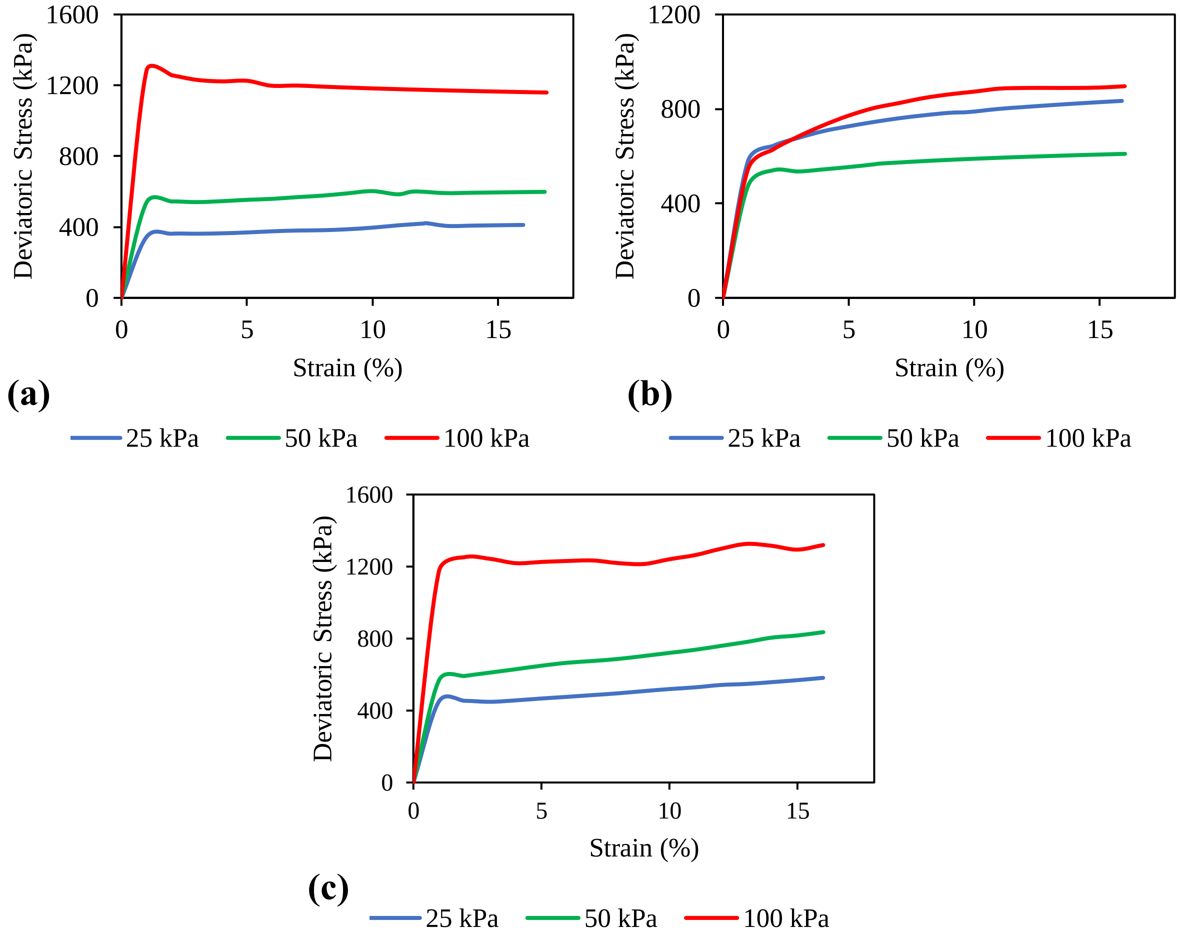

Figure 4-15: The deviatoric stress in kPa versus the percentage strain for the MICP treated **(a)** sand, **(b)** clay and **(c)** tailings samples are plotted to identify the failure point at peak and critical state of the specimens during consolidated undrained triaxial testing. The stress-strain relationship of the treated sand and tailings specimens during shearing affirmed the dense state of the soils and that of clay was suggestive of a normally consolidated sample stress history, similarly to the untreated specimens.

Here, the curve for tailings at a confining pressure of 50kPa is lower than that of the 100kPa confining pressure, which follows the anticipated benchmark as the maximum deviatoric stress is anticipated at the highest confining pressure i.e., 100kPa. The lower confining pressures, 50 and 25kPa respectively, display significantly lower peak stresses in the sand and tailings soils whilst clay results remain within a similar magnitude between 400 and 800kPa. Evidently, complex behaviour can be observed in terms of the stress-strain behaviour of the soils during triaxial testing. These curves do however give an indication of what to expect in terms of the effect MICP treatment has on the shear strength parameters of the soils investigated. In order to quantify this impact, Mohr’s circles with their corresponding Mohr-Coulomb failure envelopes are plotted in **Figure 4-16** and **Figure 4-17** to determine the angle of internal friction and cohesion for each the untreated and MICP treated soils.

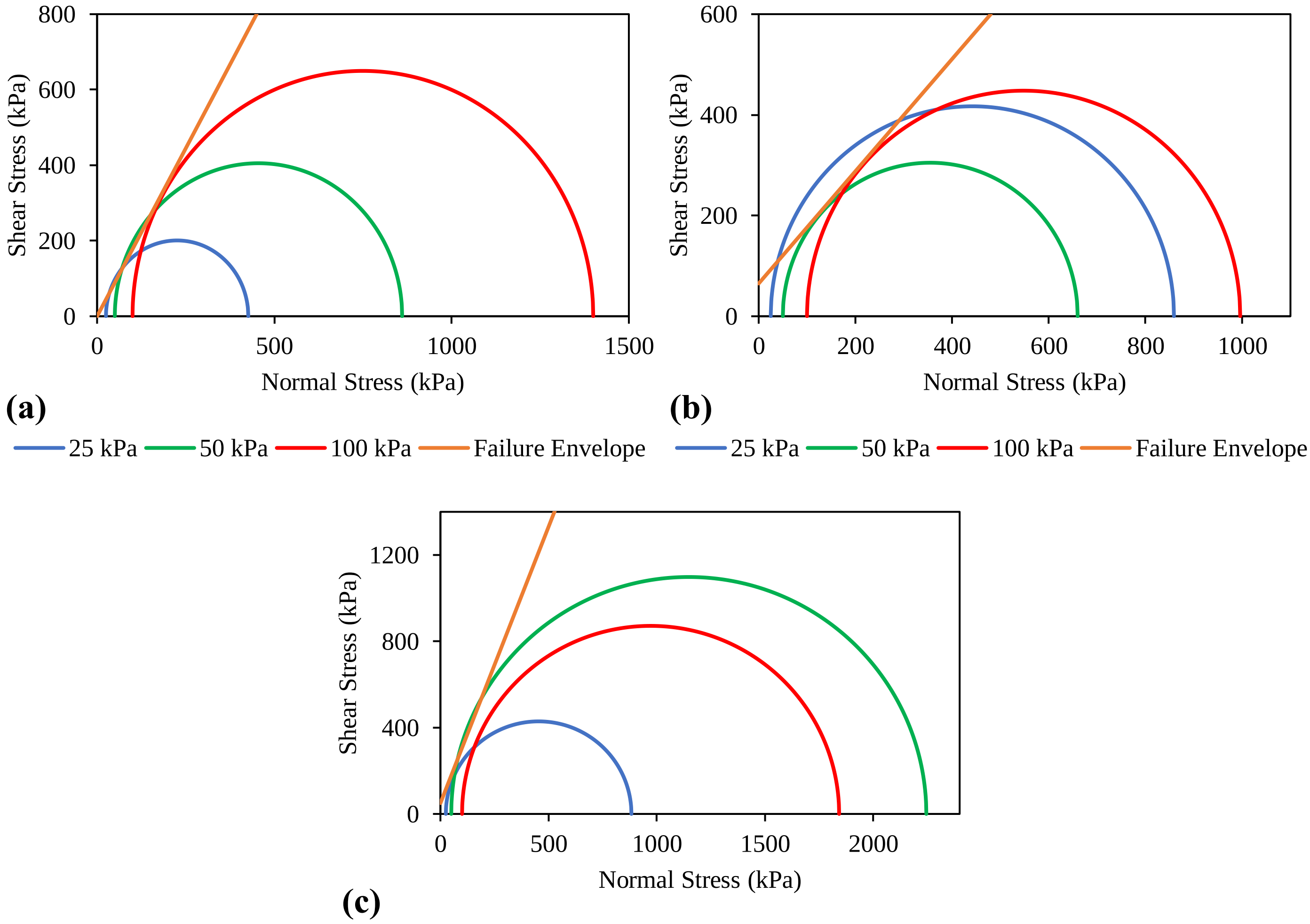

Figure 4-16: The Mohr’s circles for the shear failure of the untreated **(a)** sand, **(b)** clay and **(c)** gold tailings soils during consolidated undrained triaxial testing. The shear stress in kPa against the normal stress in kPa is plotted to determine the Mohr-Coulomb failure envelope for each of the soils at confining pressures of 25, 50 and 100kPa. All three of the soils exhibit uncharacteristically steep failure envelopes, which is indicative of high angles of internal friction.

The equations for the three failure envelopes used to determine the angle of internal friction as well as the cohesion for the untreated soils are summarised in **Table 4-5** below.

Table 4-5: A summary of the Mohr-Coulomb failure envelope equations determined after plotting the Mohr's circles of the untreated sand, clay, and gold tailings specimens. Three samples for each soil type were tested at confining pressures of 25, 50 and 100kPa respectively in order to plot the failure envelope tangent to the three resulting circles.

Untreated soil:	Mohr-Coulomb failure envelope equation:	Angle of internal friction:	Cohesion:
		ϕ°	c (kPa)
Sand	$\tau = \tan 60.6 \times \sigma_n$	60.6	0.0
Clay	$\tau = 60.0 + \tan 58.3 \times \sigma_n$	58.3	60.0
Gold tailings	$\tau = 50.0 + \tan 68.7 \times \sigma_n$	68.7	50.0

Steep failure envelopes are observed in all three soils, which is then used to determine the angle of internal friction. This failure envelope was fitted tangentially to the surfaces of the Mohr's surfaces. The y-intercepts which indicate the cohesion for each soil are as expected; lower cohesion is observed in the coarser grained sand, and a higher cohesion is seen for clay specimens. The tailings, however, achieves a slightly higher cohesion at 65kPa. Although the tailings had a higher fraction of fines, the material was largely cohesionless during handling and testing. This was particularly evident with regards to the inability to hold water. This is likely due to the outlying stress versus strain curve of the 50kPa confining pressure specimen, which resulted in a significantly larger Mohr's circle for the tailings. It can be said that the cohesion for the untreated tailings is likely significantly lower. The treated soils Mohr's circles and failure envelopes diverged from that of the untreated soils, as shown in **Figure 4-17** below.

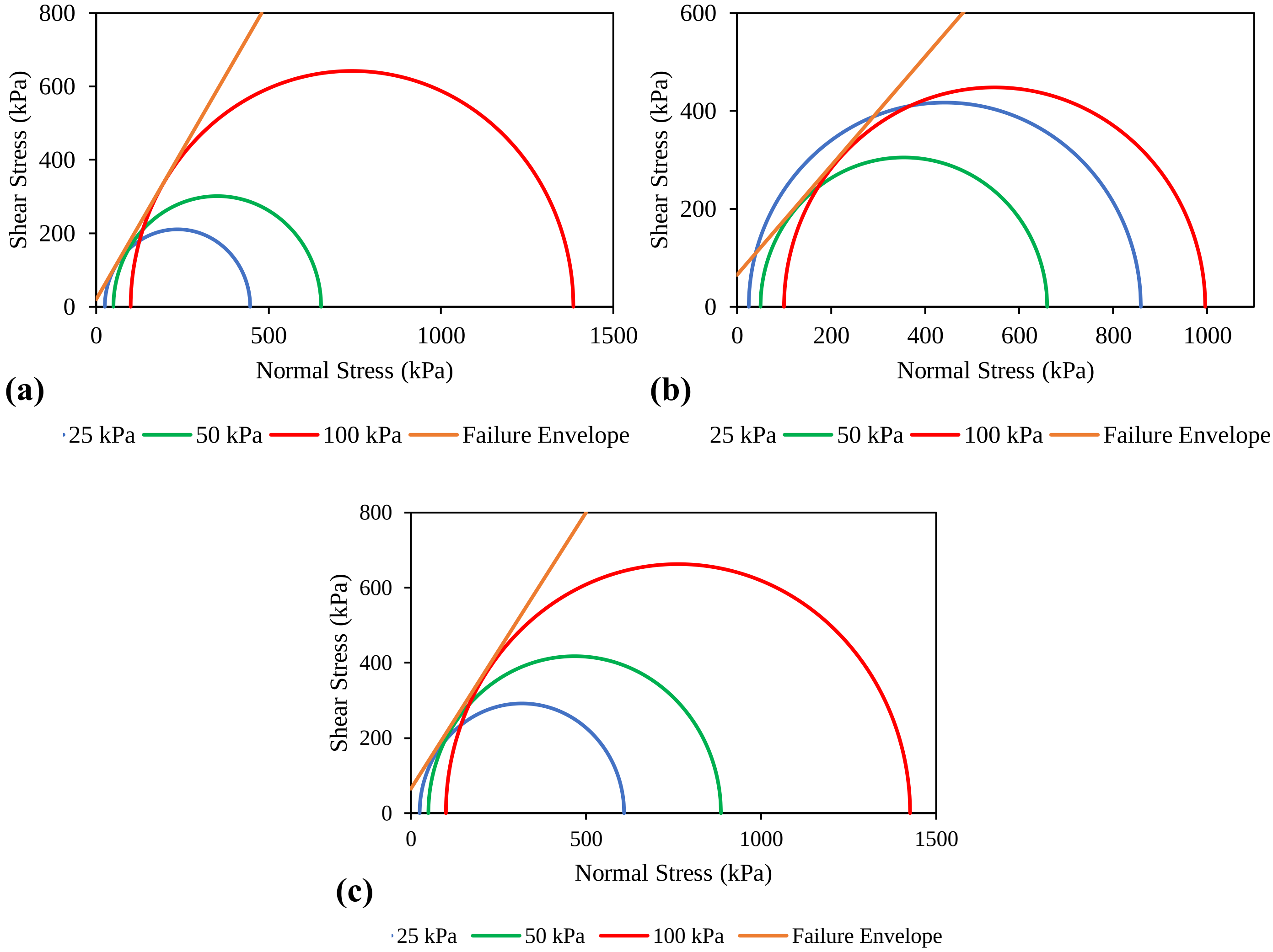

Figure 4-17: The Mohr's circles for the shear failure of the treated **(a)** sand, **(b)** clay and **(c)** gold tailings soils during consolidated undrained triaxial testing. The shear stress in kPa against the normal stress in kPa is plotted to determine the Mohr-Coulomb failure envelope for each of the soils at confining pressures of 25, 50 and 100kPa. All three of the soils exhibit flatter failure envelopes in comparison to their untreated counterparts, which is indicative of lower angles of internal friction.

The equations for the three failure envelopes used to determine the angle of internal friction as well as the cohesion for the untreated sand, clay and gold tailings are summarised in **Table 4-6** below.

Table 4-6: A summary of the Mohr-Coulomb failure envelope equations determined after plotting the Mohr's circles of the untreated sand, clay, and gold tailings specimens. Three samples for each soil type were tested at confining pressures of 25, 50 and 100kPa respectively in order to plot the failure envelope tangent to the three resulting circles

Treated soil:	Mohr-Coulomb failure envelope equation:	Angle of internal friction:	Cohesion:
		ϕ°	**c (kPa)**
Sand	$\tau = 20.0 + \tan 58.4 \times \sigma_n$	58.4	20.0
Clay	$\tau = 65.0 + \tan 48.1 \times \sigma_n$	48.1	65.0
Gold tailings	$\tau = 65.0 + \tan 55.8 \times \sigma_n$	55.8	65.0

In previous research conducted by Cheng et al., (2013) and Pakbaz et al., (2018), the angle of internal friction as well as the cohesion was found to increase following MICP treatment in some soils. Therefore, it was anticipated that these shear strength parameters for this study, would grow by a particular magnitude based on the efficacy of the treatment methodology. Remarkably, a decrease in the angle of internal friction was observed in the treated soils, which obtained flatter failure envelopes than the untreated specimens. Furthermore, an increase in the cohesion was observed for all the soils, which is an indication of an improvement in the shear strength of the materials. This aberration in the angles of internal friction is once again attributed to the complex behaviour of the soils during testing particularly with regard to the tailings. In addition, the relative density of the samples and potentially, the particle morphology of the soils, resulted in significantly higher angles for the untreated soils. Therefore, an improvement or increase in the angle of internal friction of the treated soils would be difficult to note. This is especially noteworthy for the tailings sample where not only particle morphology and relative density could have had an impact, but also the interaction of the heavy metals with the system.

Upon noting the uncharacteristically high angles of internal friction in both the untreated and treated soils, this necessitated further investigation in exploring more external variables which affect the friction angle of soils during testing. This review of literature is presented in section 2.5.2. These sources, investigating predominantly gold tailings, low plasticity clays and poorly graded sands, were consulted in order to compare the values of the friction angles to the gold tailings, low plasticity clay and poorly graded sand used in this research. **Table 2-6** shows a wide range of values obtained by the various studies, however the data generally shows that the clays typically have the lowest friction angles, followed by the tailings and sands which typically have friction angles in the same range. The same can be said for this research, where the clay friction angles were the lowest in both the untreated and treated soils followed by the sand and the gold tailings as shown in **Table 4-5** and **Table 4-6**. **Table 2-6** shows that the friction angles of tailings, sand and clays can reach values up to 44°, 50° and 45° degrees respectively. These values indicate that particularly during triaxial testing, higher shear strength parameters can be obtained than the norm for that particular material (Bhanbhro, 2014). However, the values obtained in this research for the untreated soils were 10.6°, 13.3° and 24.7° higher in the sand, clay, and gold tailings respectively than some of the maximum values encountered in the literature consulted in **Table 2-6**. This is therefore likely due to the calibration of the testing apparatus, which resulted in very high readings of the friction angles. The higher value of the tailings is to be expected as due to their high angularity, the material has been found to have a slightly higher friction angle than other natural granular materials (Mittal & Morgenstern, 1975; Vick, 1990). However, unless the soil is very densely packed, the friction angle typically remains below 45° (Byrne & Berry, 2008). Beyond this, other factors affecting the friction angle include, but are not limited to: water content, over consolidation ratio, dry density, shear rate, plasticity index, particle shape (Chan & Page, 1997; Sukumaran & Ashmawy, 2001), initial soil fabric (Been et al., 1991), interparticle friction (Matsuoka & Liu, 2003; Thornton, 2000), fines content (Murthy et al., 2007; Ni et al., 2004; Sladen et al., 1985) and particle damage (Bishop & Green, 1965; Lee & Seed, 1967; Li, 2018; Sadrekarimi & Olson, 2011; Scarpelli & Wood, 1982; Tarantino & Hyde, 2005; Vithana et al., 2012; Xu et al., 2018; Yates et al., 2018). These factors and their influence on the angle of internal friction are explored in section 2.5.2. Although the said factors could result in a significant increase in the angle of internal friction, the uncharacteristically high values presented in Table **4-6** are likely resulting from the experimental apparatus. The calibration of the load cell used to measure the deviator stress could cause such atypically high friction angles.

Fixating on the cohesion, an increase was observed throughout all the soils in this research as described above. The most significant increase in cohesion was seen in the sand, followed by the tailings and lastly the clay. This alludes to the fact that sand had the highest success with the MICP treatment concerning the objectives of this research, followed by tailings and lastly clay. Overall, the clay material obtained the lowest change in shear strength parameters. A summary of the shear strength parameters for the investigated soils is provided in **Figure 4-18** below to illustrate the changes following MICP treatment (Hyde et al., 2021). This is quantified as percentage changes in the angle of internal friction, cohesion and apparent cohesion of the untreated soils following MICP treatment as summarised in **Table 4-7** below.

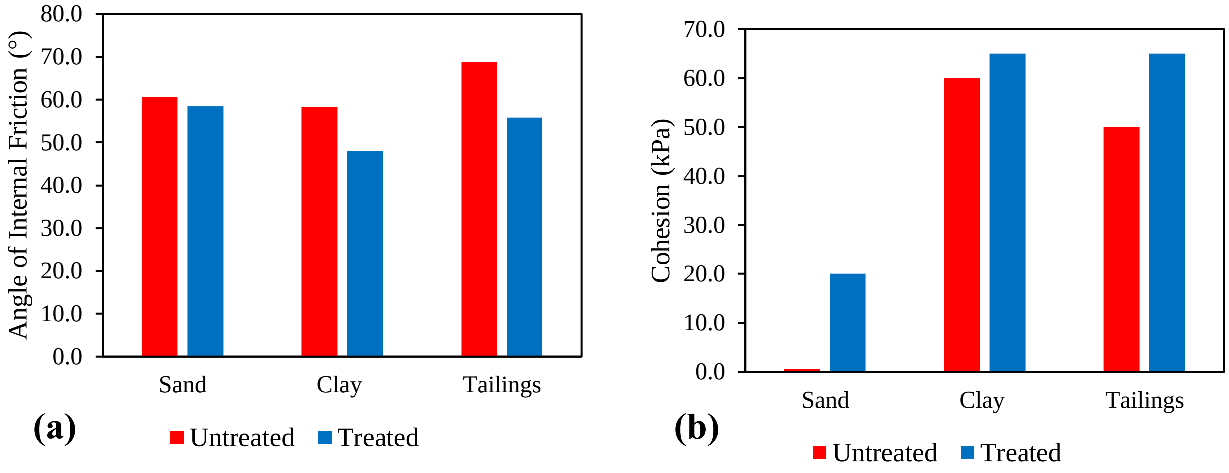

Figure 4-18: A summary of the shear strength parameters obtained from the consolidated undrained triaxial tests conducted on the untreated and MICP treated sand, clay, and gold tailings soils. The trend in terms of the angles of internal friction, is inconclusive, where all of the soils exhibit a decrease. Alternatively, an evident positive trend is observed concerning the cohesion of the soils, which all exhibit an increase (Hyde et al., 2021).

Table 4-7: A summary of the percentage changes in shear strength parameters obtained from the consolidated undrained triaxial tests conducted on the untreated and MICP treated sand, clay, and gold tailings soils. Overall, sand exhibited the lowest decrease and the greatest increase in the friction angle and cohesion respectively, followed by the gold tailings and lastly the clay.

	Percentage change in the shear strength parameters:	
Soil type:	**Δφ° (%)**	**Δc (%)**
Sand	-3.8	∞*
Clay	-21.2	7.7
Gold tailings	-23.1	23.1

* An infinitely large percentage increase – there is no % increase from a starting cohesion of 0. Rather the process resulted in apparent cohesion in the soil.

Overall, the impact calcite precipitation had on the angle of internal friction was inconclusive. This was due to the interaction of a variety of variables such as the particle shape and distribution of the soils, the relative density of the samples as well as strain hardening which occurred during failure and potentially the choice in sample geometry, test quantity and test boundary conditions. This said interaction resulted in a decrease in the shear strength parameters, contrary to the anticipated increase following the deposition of calcium carbonate crystals in the soil matrix. The gold tailings had the greatest decrease in friction angle, followed closely by the clay and lastly the sand. However, the trend in terms of the cohesion is clear, calcite precipitation was able to successfully cement particles of all three soils and thus increase the cohesion. Sand obtained the highest increase in cohesion from 0 to 20kPa, whilst tailings and clay obtained increases of 23.1% and 7.7% respectively.

4.5 Heavy metals analysis

Inductively coupled plasma mass spectrometry (ICP-MS) was carried out in order to determine the elements in the tailings samples. This was carried out to further examine the results of the urea, ammonium, and calcium fluctuations in the tailings system. As described in section 4.3, the tailings cylinders exhibited evidence of a slowing and eventual cessation of the hydrolysis of urea, followed by the precipitation of calcium carbonate. One of the factors explored in section 4.3, that has an impact on the success of the MICP process, is the presence of heavy metals (Hiroki, 1992; Rathnayake et al., 2013). Heavy metals are naturally occurring in low concentrations throughout the environment in soils, rocks, water, animals and plants (Chu, 2018; Duo et al., 2018; González Henao & Ghneim-Herrera, 2021). Tailings are comprised of crushed waste rock and the processing fluids used in the extraction of the relevant commodity (Lottermoser, 2010; Mpanza et al., 2020). In this case, gold tailings were obtained for use in this research. Therefore, the elements As, Cr, Cu, Fe, Mn, Ni, Pb, Zn, Ca, K and S were expected in trace and major concentrations in the soil specimens (du Plessis & Curtis, 2021; Mpanza et al., 2020). However, high concentrations or concentrations above the MIC of these heavy metals can have an adverse impact on the bacterial community proliferated within the specimens (Kim & Lee, 2019). Some heavy metals have also been identified as toxic irrespective of their concentration in soils, with the microbes exhibiting selective tolerance according to the element, and the specific bacterial culture (Ahmad et al., 2005; Yao et al., 2017). Hence, the average trace and major elements within the treated and untreated tailings samples in concentrations of parts per million (ppm) are summarised in **Figure 4-19** and **Figure 4-20** below.

Qualitatively, trace elements are defined as elements which are required in the biological upkeep of an organism. Thus, typically serving the function of cofactors in enzymatic activity (Banik et al., 2014; González Henao & Ghneim-Herrera, 2021). Quantitatively, trace elements can be described as an element at a concentration lower than 100 ppm within a given sample (Koller & Hosam, 2018). The concentrations including that of V, Zn, Ba and Ce were found to be equivalent, or significantly lower than the traces found in the instruments used to carry out the analysis. This was with the exception of Pb which was slightly higher. This is to be expected as lead is identified as one of the common major or trace elements found in gold tailings (du Plessis & Curtis, 2021). The concentrations, however, are typically so low that they have no impact on the organisms, other than the provision of essential nutrients (González Henao & Ghneim-Herrera, 2021). Therefore, the major elements with concentrations higher than 100 ppm in the soil samples are shown in **Figure 4-20** below.

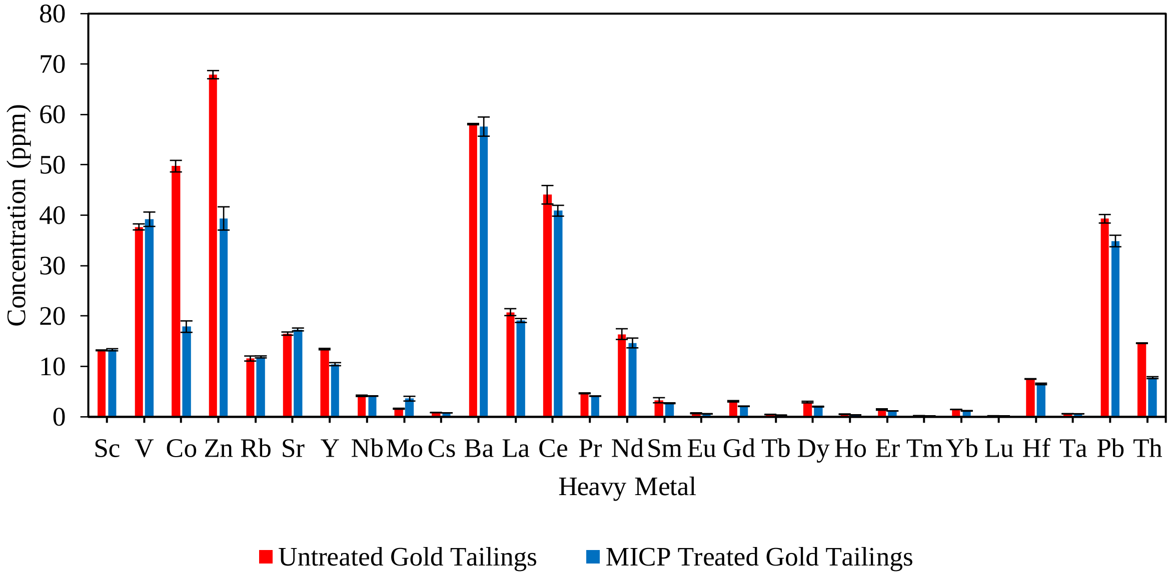

Figure 4-19: The concentrations in parts per million (ppm) of average trace elements found in samples of the untreated and MICP treated gold tailings are identified. Overall, slightly lower concentrations of the heavy metals were found in the treated gold tailings. Notably higher traces of V, Zn, Ba, Ce and Pb were observed within the trace elements in both untreated and treated soil. A trend of higher heavy metal concentrations in the untreated gold tailings and a reduction in the MICP treated gold tailings heavy metal concentration was observed.

Similarly to the trace elements indicated on **Figure 4-19** above, the major elements in **Figure 4-20** appear to decrease in the soils after MICP treatment with the exception of chromium (Cr), which shows a notable increase from untreated to treated soil. The reduction in heavy metal concentration could be a result of two processes; the physical removal of heavy metals with the erosion of soils which was observed during treatment dispensation or the chemical sequestration of the heavy metals by the microbial community in the soils. Based on the behaviour of the bacteria gleaned from the urea, ammonium and calcium fluctuations described in section 4.3, the latter might seem unlikely due to the reduction in urea hydrolysis, ammonium production and calcium usage efficiencies observed in tailings. These observations are indicative of a reduction in the bacterial activity over time in which would limit the likelihood of heavy metal sequestration (Dhami et al., 2013; Kang et al., 2014; Mugwar & Harbottle, 2016; Okyay et al., 2016). However, evidence of initial bacterial activity alongside the significant growth in the Cr concentration in the treated tailings shown in **Figure 4-20** is indicative of some developments in the soil.

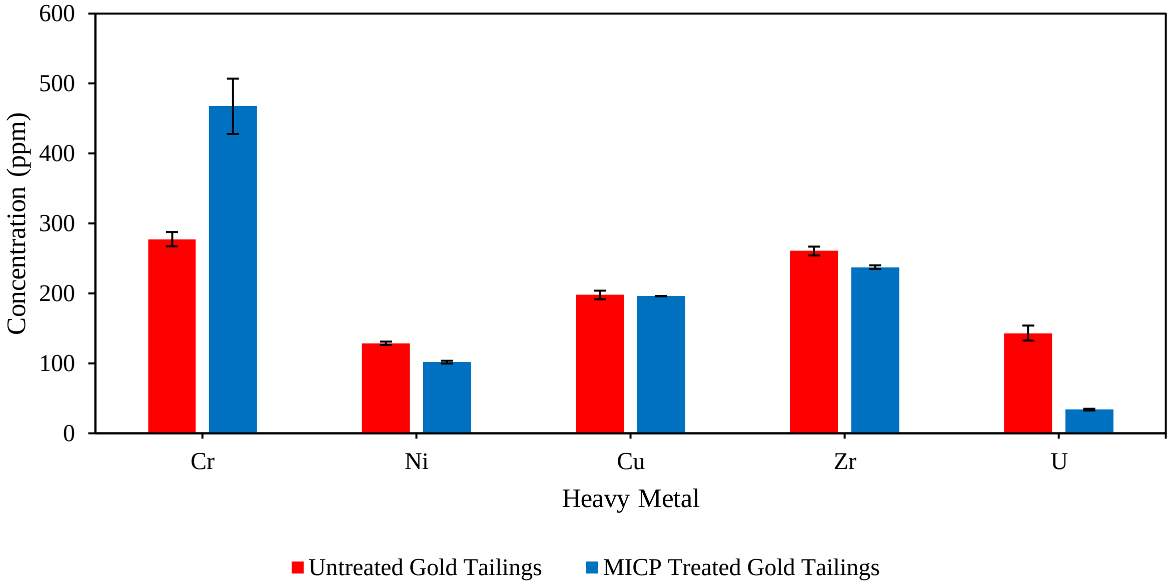

Figure 4-20: The concentrations in parts per million (ppm) of average major elements found in samples of the untreated and MICP treated gold tailings following destructive inductively coupled plasma mass spectrometry analysis are identified. Overall, slightly lower concentrations of the heavy metals were found in the treated gold tailings in comparison to the untreated soils apart from Cr. A notably higher concentration of Cr was observed in the MICP treated soil.

As described in section 2.4.7, microorganisms have various mechanisms to facilitate the removal or immobilisation of heavy metals in soils including but not limited to microbial remediation, biosorption, bioaccumulation, biotransformation, biomineralization and biodegradation amongst others (Banik et al., 2014; Zhang et al., 2020). The availability of additional ions in the soil following the injection of the cementation media could potentially allow for the precipitation or uptake of the available heavy metals out of solution or into the microbes in the soil.

As such, the biotransformation of Cr^{6+} to Cr^{3+} has been frequently observed in numerous bacterial strains. This alters Cr^{6+} to a less toxic, and less soluble form (Ameen et al., 2020; Banik et al., 2014; Cheung & Gu, 2007). Therefore, this process is likely the cause of the increase in Cr observed in the treated soil. The less soluble Cr^{3+} precipitates out of solution in the presence of aqueous ammonia (Birk, 2020). As described in Equation 1, urea is decomposed into ammonia and carbon dioxide in the presence of the urease enzyme. This aqueous ammonia is then ionized into ammonium in the presence of water. However, as shown in Equation 18 below, ammonia can also potentially react with Cr^{3+} and precipitate chromium hydroxide out of solution.

$$\mathrm{Cr^{3+} + 3NH_3 + 3H_2O \rightleftharpoons Cr(OH)_3 \downarrow + 3NH_4^+} \quad \text{Equation 18}$$

As seen in Equation 18 above, ammonia reacts with Cr^{3+}in the presence of water, which results in the precipitation of chromium hydroxide. Chromium hydroxide is described as a grey-green precipitate (Birk, 2020). A similar discoloration was observed in the tailings throughout the samples following treatment as shown in **Figure 4-21** below.

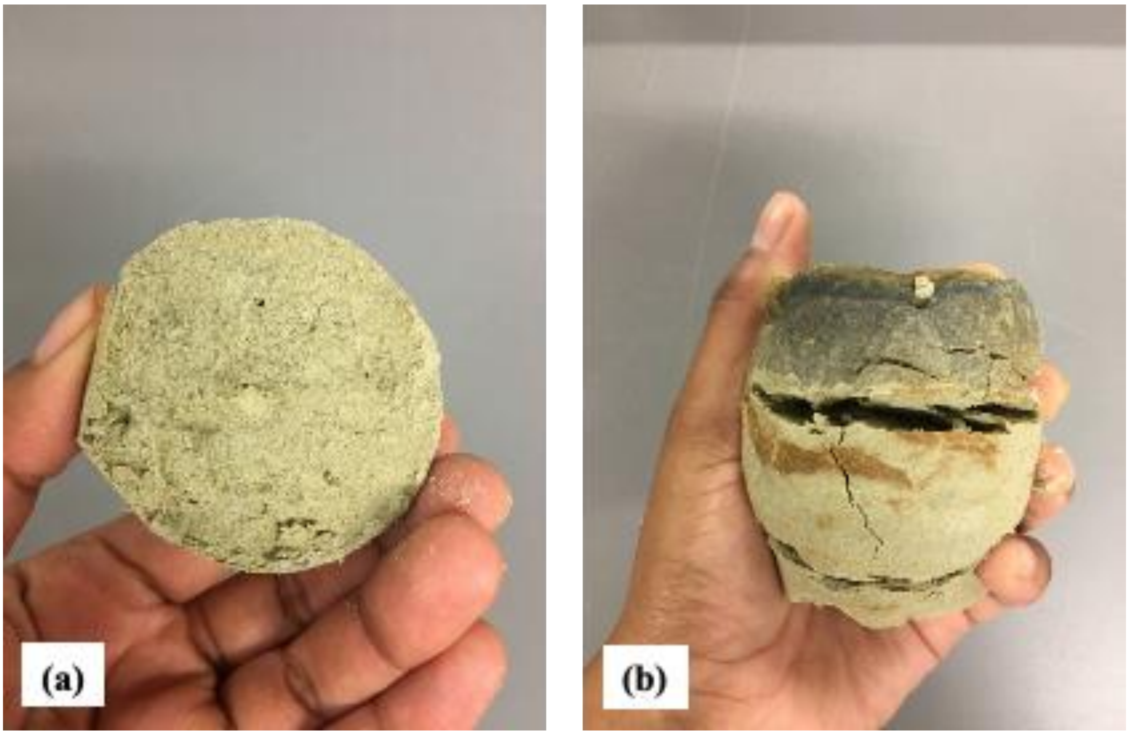

Figure 4-21: The colour change observed in the tailings in the untreated and treated specimens. **(a)** The colour of the untreated tailings was a lighter, pale green colour with minimal variation throughout the specimens. **(b)** The change in the colour of the soil was discovered following the removal of the sample from the treatment cylinder. Streaks of grey-green discoloration were seen frequently throughout the soil specimens.

Therefore, it is highly likely that the grey-green traces or flecks seen in **Figure 4-21** are solid chromium (III) hydroxide deposits. This would explain the increase in Cr concentrations observed in the tailings following treatment as shown in **Figure 4-20** above. Hence the growth in the Cr concentrations in the MICP treated tailings observed is attributed to the precipitation of chromium (III) hydroxide out of solution in the presence of ammonia. This precipitation of additional solids into the soil structure of the tailings is in line with the decrease in porosity indicated in **Figure 4-3**. The observed reduction in porosity confirms the possibility of additional deposits of solids in the tailings. However, the other observation made during experimentation was the runoff of soil particles in the clay and tailings columns as well as the retention of the injection channels following treatment as shown in **Figure 4-4** in section 4.1.3. Therefore, it is worth considering that the proposed precipitation and minor erosion are occurring concurrently in the tailings specimens. This would explain the reduction in porosity as well as the grey-green deposits witnessed in the tailings and the decrease in particle density. This erosion would also explain the general trend seen in **Figure 4-19** and **Figure 4-20** of a decrease in heavy metal concentrations in the tailings following treatment in both the trace and major elements respectively. The erosion resulting from the flow of treatment media through the tailings specimens was likely abetting the removal of trace and major heavy metals attached to the eroded soil particles.

A notable decrease in uranium (U) was observed in the major elements following the treatment of the tailings. High uranium content has been found in gold tailings in the Gauteng region and the rest of South Africa, where uranium is recovered from the production and beneficiation of gold ore (Mpanza et al., 2020; Winde & Walt, 2004). A significant proportion of uranium exists as adsorbed ions on minerals (Alam & Cheng, 2014). This uranium then migrates from the tailings into proximal groundwater and surface waters typically through the dissolution or desorption of uranium from soil solids (Alam & Cheng, 2014; Winde & Walt, 2004). This process is contingent on a number of factors including the redox potential, soil moisture levels, microbial activity and pH, where the uranyl ion is stable at pH levels above 2.5 (Gavrilescu et al., 2009). One of the most effective pathways for the distribution of heavy metals into adjacent fluvial systems is through flowing surface water. Regarding the aqueous transportation of U however, a significantly lower migration in surface water systems was observed in comparison to groundwater systems (Winde & Walt, 2004). This higher immobilisation of U in surface water is attributed to co-precipitation of U with calcium carbonate and iron or manganese compounds. The bottom sediments of the stream water confine the U from groundwater sources, hence the higher immobilisation in the flowing water systems is observed (Winde & Walt, 2004). Therefore, it is likely that the reduction in U following the treatment for the gold tailings was a result of the desorption of the compound into the treatment media which left the system through the effluent. Concerning co-precipitation, **Figure 4-20** above indicates that U concentrations in the treated gold tailings were significantly lower than the untreated gold tailings. On this basis, it can be assumed that co-precipitation was likely not occurring, but solely the flushing of the U compound during treatment through the pore water and out of the system.

In related research, the toxicity of copper on Sporosarcina pasteurii was investigated (de Oliveira et al., 2021). Relatively low levels of copper with an MIC between 0.2–0.5 mM (~13–32 mg/L) have been found to inhibit the growth of the *Sporosarcina pasteurii* bacteria and in other cases the cell density began to decrease once an MIC of 128 mg/L was reached (de Oliveira et al., 2021; Mugwar & Harbottle, 2016). The presence of copper was thus found to supress the efficiency of the MICP process in the soil in the formation of the bio-solids (de Oliveira et al., 2021). Copper is therefore one of the likely culprits for the limitation in the completion of the MICP process observed in the urea, ammonium and calcium fluctuations explored in section 4.3. However, due to the presence of a host of other heavy metals at high concentrations a MIC test would need to be carried out to identify the exact heavy metals responsible.

Overall, the interaction of the systems with the heavy metals exhibited a negative impact on the bacterial community in the gold tailings as evidenced by the systems response to urea hydrolysis, ammonium production as well as calcium carbonate precipitation. This limited the efficacy of the MICP treatment in improving the shear strength parameters of the soil in conjunction with the erosion of the particles during treatment. The specific heavy metal proving to be the most toxic to *Sporosarcina pasteurii* was not identified in this research. Therefore, the minimum inhibitory concentration (MIC) of each heavy metal in the gold tailings would need to be determined in order to conclusively identify the specific compound as well as concentration inhibiting the growth of the bacteria. With this said, the results have shown an interaction of various factors, which impacted the efficacy of the MICP treatment process in each soil. This includes erosion during treatment, the characteristics of the soil particles themselves in terms of relative density and strain hardening and the presence of potentially toxic heavy metals which likely inhibit the microbial community in the gold tailings. In spite of this, the sand and the gold tailings exhibited the most promising results. This was to be expected from the sand, which is a commonly and successfully used soil in MICP research. This was also expected from the clay, which showed equally promising chemical results in this research. The chemistry indicated that the gold tailings were not as successful as the clay and sand soils in calcium usage. However, the shear strength results indicated that clay achieved the least significant change in shear strength parameters and overall stability. Therefore, particle size once again dominates as an influencing factor in the efficacy of MICP treatment. Related research similarly postulated that the inhibition of the MICP treatment is only partially due to the presence of heavy metals and is likely a direct consequence of the small particle size of the copper mine tailings used (de Oliveira et al., 2021). This is due to a number of factors which include reduced permeability and porosity with decreasing particle size which in turn results in limited movement of treatment media through the soil, reduced availability of oxygen to the bacteria and less freedom of movement for the bacteria through the soil (de Oliveira et al., 2021; Frederickson et al., 1991).

5. Conclusions and recommendations

Microbially Induced Calcite Precipitation (MICP) is an emerging bio-mediated technology which has been successfully applied in research and in practice as a sustainable soil improvement method. MICP uses the urease enzyme to breakdown urea into carbonate ions which combine with free calcium ions forming calcium carbonate, acting as a bio-cement. MICP treatment has been proven to increase shear strength and decrease porosity in soils, however its application in soil improvement outside erosion mitigation in granular soils remains limited. This research focused on injecting MICP treatment to increase shear strength and decrease porosity in sand, clay, and gold tailings below surface depth. Results indicated successful cementation evidenced by an infinite increase in apparent cohesion in sand and an increase in gold tailings and cohesion in clay of 30% and 8.3% respectively. A general decrease in the sand, clay, and gold tailings of 3.8%, 21.2% and 23.1% in the internal friction angle was observed. The porosity of clay and tailings decreased by 20.6% and 7.3%, whilst sand increased by 3%. Furthermore, the comparison between shear strength and porosity should be explored in future studies concerning which parameter has the most significant impact on the overall stability of the soil.

The inoculated soils were compacted into nine stainless steel cylinders and treated with the cementation media. Each cylinder exhibited unique behaviour in terms of treatment dosage which potentially had the domino effect of introducing greater variability in the effluent concentrations of urea, ammonium, and calcium. Although a variation was observed in terms of the said concentrations for each cylinder's effluent, the general trends identified were clear. The sand exhibited the greatest calcite precipitation as well as the most significant improvement in the shear strength parameters of specific gravity, and apparent cohesion. This was followed by the gold tailings, which is similarly coarse grained. However, the prevalence of heavy metals in the gold tailings likely limited the process over time. Although preferential flow paths and inhomogeneous cementation was observed throughout the sand and gold tailings samples, overall, an improvement in the shear strength parameters was observed, with the exception of the angle of internal friction. Due to the atypically high friction angles obtained, it is recommended that further investigation into the calibration of the load cell and its impact on the friction angle results is carried out. The injection method was limited in terms of the erosion of soil particles during treatment, particularly in the sand sample which exhibited an increase in porosity. This was identified as the likely cause of the reduction in porosity in the sand samples observed following treatment. The injection method did however exhibit promising results in terms of the depth of treatment that can be achieved at a laboratory scale in sand and tailings as cementation was observed throughout the length of each cylinder.

Thus, in the results, the interaction of various factors, which impacted the efficacy of the MICP treatment process in each soil was observed. This included erosion during treatment, the characteristics of the soil particles themselves in terms of relative density and strain hardening and lastly, the presence of potentially toxic heavy metals which likely inhibited the microbial community in the gold tailings. In spite of this, the sand and the gold tailings exhibited the most promising results. This was to be expected from the sand, which is a commonly and successfully used soil in MICP research. This was also expected from the clay, which showed equally promising results with regard to urea, ammonium, and calcium fluctuations in this research. The gold tailings however, were not as successful as the clay and sand soils in calcium usage, urea decomposition and ammonium production. Nonetheless, the shear strength results indicate that clay achieved the least significant change in shear strength parameters and overall stability. Therefore, the results suggest that the particle size had a dominant role in influencing the efficacy of MICP treatment.

Overall, the reaction of the sand, clay, and tailings to MICP agreed with the consulted literature whereby the larger the particle size is, the greater the efficacy of the cementation of the soil particles. Therefore, it can be concluded that the use of MICP as a soil improvement technique for treatment depths greater than surface level, is feasible for scalable implementation in gold tailings and sands.

With this in mind, further research into the interaction of the bacteria with the heavy metals identified in the gold tailings is recommended. This is pertaining to the MIC of the heavy metals as well as determining the toxicity of specific heavy metals in the gold tailings to the bacteria used. The possible bioremediation of chromium from the gold tailings as a result of its co-precipitation during the completion of the MICP process is another promising avenue of investigation in related research. This will highlight the broader range of applications for MICP using *Sporosarcina pasteurii* bacteria and gold tailings and fully encompass the limitations of these methods. In addition, the stress-strain behaviour of the soils during triaxial testing and the interaction of the relative density and the particle morphology of the soils should be investigated. This is because of the complex behaviour that was observed particularly in the untreated soils which resulted in uncharacteristically high angles of internal friction. Examining this, was beyond the scope of this investigation, and it is therefore recommended for further research.

A challenge in this research was maintaining consistency in each of the nine cylinders. From the outset, each cylinder exhibited unique behaviour in terms of treatment dosage which potentially had the domino effect of introducing greater variability in terms of urea, ammonium, and calcium concentrations. The general trends of behaviour however were similar across the specimens. Therefore, it is suggested that more cylinders for each soil type are investigated in the possibility of further investigation. This would aid in quantifying the impact variability in treatment dosages has on the efficacy of MICP. Lastly, to further investigate the compatibility of MICP with fine grained soils, a longer treatment duration should be explored. This is primarily due to the increasing time required for the treatment media to permeate throughout a fine-grained soil, as well as the movement of the bacteria throughout the soil sample.

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Appendix A

Analytical methods and sampling

Calcium sampling procedure:

1. Pipette 1 mL and 2 mL of the influent and effluent samples respectively into three graduated 15 mL vials each.
2. Pipette DI water to the 10 mL graduation in each of the six vials tubes to achieve a 1:5 and a 1:10 dilution ratio for the influent and effluent samples respectively.
3. Mix the samples in a vortex mixer and following this, filter each mixed specimen through a 0.22 µm pore syringe filter.
4. Pipette the filtered samples into specimen containers, seal and refrigerate pending the TFG analysis.

Ammonium sampling procedure:

1. Pipette 1 mL of the influent and effluent sample into three graduated 50 mL volumetric flasks each.
2. Continuously add 0.1 M HCl into the flask to acidify the samples until a pH range below 3 reached.
3. Pipette DI water to the 50 mL graduation in each of the three flasks. From each of the volumetric flasks, pipette 0.5 mL of diluted sample into three 15 mL graduated vials. In each of these vials, pipette additional DI water to the 10 mL mark to achieve a final 1: 1000 dilution ratio.
4. Mix the samples in a vortex mixer and following this, filter each through a 0.22 µm pore syringe filter.
5. Pipette the filtered samples into specimen containers and refrigerate pending the TFG analysis.

Urea sampling procedure:

1. Pipette 1 mL of the influent into three graduated 50 mL volumetric flasks.
2. Continuously add 0.1 M HCl into the flask to acidify the samples until a pH range between 7 – 8 is reached.
3. Pipette DI water to the 50 mL graduation in each of the three flasks. From each of the volumetric flasks, pipette 0.5 mL of diluted sample into three 15 mL graduated vials.
4. In each of the vials, pipette 40 µm of Jack-bean urease followed by DI water to the 10 mL mark to achieve a final 1:1000 dilution ratio.
5. Leave the specimens to rest for 1 hour, allowing for the hydrolysis of urea.

6. Mix the samples in a vortex mixer and following this, filter each through a syringe filter with 0.22 µm pore size filter paper.
7. Pipette the filtered samples into specimen containers, seal and refrigerate pending the TFG analysis.

Appendix B

Triaxial Data

https://drive.google.com/drive/folders/1r_bzXGfCIQ5yU4CmaHCYtEYjm_m3IV9h?usp=sharing