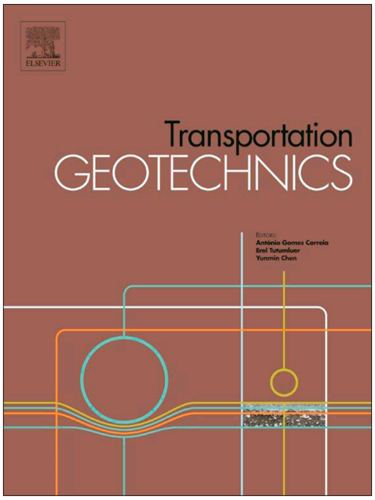

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Veshara Malapermal Ramdas, Prisha Mandree, Martin Mgangira, Samson Mukaratirwa, Rajesh Lalloo, Santosh Ramchuran

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REVIEW OF CURRENT AND FUTURE BIO-BASED STABILISATION PRODUCTS (ENZYMATIC AND POLYMERIC) FOR ROAD CONSTRUCTION MATERIALS

Veshara Malapermal Ramdas[a, b*], Prisha Mandree[b], Martin Mgangira[b], Samson Mukaratirwa[a], Rajesh Lalloo[b] and Santosh Ramchuran[a, b]

[a] *School of Life Sciences, University of KwaZulu Natal, Durban, 4041, South Africa*
[b] *Council for Scientific and Industrial Research, PO Box 395, Pretoria, 0001, South Africa*

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[*] Veshara Malapermal Ramdas: Current physical address: The CSIR Future Production, Chemicals, Meiring Naude Drive, Brummeria, Pretoria, 0001. Postal address: PO Box 395 Pretoria 0001 South Africa; Telephone number: +27842993794; Email: VMalapermal@csir.co.za; veshara@gmail.com

ABSTRACT

In situ soil modification is required in order to improve the primary engineering properties of the material to meet a road construction standard. Bio-stabilised soil is an environmentally friendly, cost-effective alternative to imported granular fills, concrete, costly hauling of materials or export to a landfill. In-service soil performance and required maintenance is highly dependent on methods of stabilisation, ranging from expensive mechanical stabilisation to chemical processes. As such, many alternative materials originating from bio-based sources are being explored as potential stabilising additives to improve weak subgrade soils (i.e., dispersive, erodible and collapsible soil, and soft or expansive clays). Some key solutions include the use of bio-derived enzymes, microbes, and polymeric additives to avert road failure caused by water penetration and/or erosion. The role of microbial substrate specialisation has been largely unexplored, since the level of research done on alternative stabilisers consists mostly of small *ad hoc* studies. In addition, research has focused on a reduction in permeability and an increase in compressive strength using enzymes and polymers, however, the complexity of these products and their implementation for a wide range of soil types and structural applications remain limited. Currently there is a need for more supporting research methodologies and systematic approaches on the implementation of bio-based materials for infrastructure development. This also includes the simplification of bio-based products for potential construction applications. This review provides (a) an overview of soil stabilisation techniques, (b) the primary challenges that lay ahead for future research in bio-based stabilisation products application in the road sector and (c) innovations to address the challenges of using modernised techniques in the road construction industry (i.e., weak subgrade and the required maintenance thereof, as well as the development of potential bio-based additives for unpaved road construction application).

Keywords: *Bacillus* species, biopolymers, soil stabiliser, road construction, soil stabilisation, bio-additives

1. INTRODUCTION

The growing urban populations and infrastructural expansion of developing countries are understandably creating an impetus for road development. Given the unprecedented pace and scope of these initiatives, it is vital to assess the potential consequences of large-scale road and transportation projects. New roads are vital pathways in the socioeconomic growth of developing countries. Thus poorly designed or implemented road projects can result in serious cost overruns, scarcity of natural resources, and negative environmental impacts (such as high global CO_2 emissions) (Alamgir et al. 2017). In practice, concrete construction of roads will always require a certain amount of maintenance and repair, albeit ironically still at a lower maintenance frequency requirement compared to asphalt roads. This is due to unexpected wear or weathering, micro crack formations, accidental overloading, increased porosity, or other phenomena related to specific changing environmental conditions resulting in early loss of functional properties (Jonkers et al. 2016). Ordinary Portland Cement (OPC), a typical civil engineering construction material is the most used material worldwide. However, production of cement is both highly energy consuming and environmentally unfriendly (Ivanov et al. 2015), with production reaching 10 000 million tonnes/year in 2013 and will increase by 100% within the next 40 years (Pacheco-Torgal and Labrincha 2013). Typically road projects generation of greenhouse gas (GHG) emissions occurs throughout its lifecycle phases (i.e., construction and operation, albeit to lesser extent maintenance and rehabilitation) (Alzard et al. 2019). As expected, the construction industry will continue to grow at a fast pace just to accommodate urban population that will increase to 6.4 billion by 2050 (Pacheco-Torgal 2016). Recent estimates on urban expansion suggest that by 2030 urban land cover will increase by 1.2 million km^2 (Seto et al. 2012). Therefore, the demand for alternative construction materials will also rise.

In developing countries, roads are expensive to develop and maintain. There has been a concerted effort in industry to reduce construction costs particularly in challenging ground conditions. However sustainable and environmentally friendly road development is a challenge to implement. According to the South African National Roads Agency Limited (SANRAL), the total asset value of South African roads is estimated at $200 billion (2014) with the value of the paved road network making up 80% of this (approximately $160 billion). New roads and vital repairs of existing roads typically cost $300-500 thousand per kilometre for a low volume paved rural road, while constructing and maintaining a heavy freeway structure can cost millions per kilometre (United States Dollar (USD)) (National Treasury 2015; Siyepu 2016). In developing countries, soil roads are constructed for low traffic volume areas such as access roads in rural areas or as estate roads. In South Africa (SA) there is ample evidence pointing to lack of adequate maintenance of current road infrastructure, as well as halted development of new roads. This is worsened by the persistent increase in traffic volumes as well as moisture damage caused by heavy rains. A lack of reliable road infrastructure also undermines prospects for future development. Unproclaimed roads, which are at best gravel roads that do not offer all-weather access is a prime example of sub-par infrastructure. According to Paige-Green (2008) these types of roads can have a significant negative impact on the economic well-being of the affected communities and also result in negative environmental impacts (i.e., soil lost due to erosion, dust clouds).

The construction industry is investigating new technologies such as, deep mixing, electro kinetics, nano-materials for sensors and sensing, as well as the use of micro-organisms for groundwater remediation (Wijeyesekera 2014). These have effectively advanced the road construction practice, resulting in a 'new' interdisciplinary approach to civil and geotechnical engineering asset management by integrating concepts and techniques from other disciplines. The development of microbiological processes for improvement of the soil properties is one of the more recent manifestations of this trend.

Bio-based exploration for use in construction is a potentially cost-effective, and an environmentally sustainable engineering approach to recondition and strengthen the existing road base, sub-base, or subgrade materials for an extended lifespan (Pei et al. 2015). Biological stabilisers such as *Bacillus* spp. produce a wide range of metabolites and eco-friendly additives that offer cumulative nature of benefits for stabilisation and compaction in structural applications (Samang et al. 2018), such as lowered cost, environmental and performance-based benefits. Several *Bacillus* spp. and their derivatives have been considered as potential alternatives to conventional chemical soil stabilisers, particularly in challenging ground conditions. This is clearly an excellent example of the application of multi-faceted green technologies promoting multidisciplinary research and collaboration between road/pavement engineering, green chemistry, biotechnology and geomicrobiology. It is important to pursue these greener technologies, which necessitates research and development for new eco-friendly materials as an alternative to conventional cement. The significance of harnessing geomicrobiological research (using novel bio-enzymes, biopolymers or waste, such as fly ash, to catalyse various reactions that stabilise *in situ* soil for road applications. This includes their mode/s of action) makes it possible to devise engineering solutions for temporary and/or permanent geotechnical engineering problems.

A significant economic incentive exists for developing new and innovative, yet environmentally safe bio-based product applications. The challenge is to find low-cost, high-volume applications of bio-based products for in-place soils, and to convert waste into value-added products. This approach could be used for numerous geotechnical applications such as dust suppression, dam control, wind soil-erosion control, earthquake liquefaction mitigation, construction of reactive barriers, and long-term stabilisation of contaminated soils. With respect to their mode of action, this presents an opportunity to discover novel micro-organisms through suitable assessment of their properties such as, secretion of one or more metabolic products, enzymes of interest, or biopolymers and investigate it as soil additives. It becomes clear that due to a lack of knowledge on bio-based soil stabilisation several areas of applied research need to be addressed in the biotechnology sector. Such as, appropriate upstream processes for biomass production, downstream processes to yield stable products (including the necessary *in-vitro* mechanisms and field testing), effectively formulated products and the appropriate application of each soil stabiliser under various environmental conditions. This is discussed in more detail in the paper.

1.1 CHALLENGES AND CONVENTIONAL STABILISATION METHODS USED BY THE CONSTRUCTION INDUSTRY

Soil stabilisation is defined as a process of improving the physical and engineering properties of a soil to achieve a predetermined target level of performance (Latifi et al. 2016). Stabilisation of *in situ* material is considered to be an important method for improving the strength and performance of the treated soil material, which contributes to prolonging the service life of the subsurface and results in a more economical design (Mgangira 2009a).

Bearing capacity of subsoil is one of the major site selection components in geotechnical design criteria. Once the bearing capacity of soil is deemed poor, the following options are considered (a) change the design to suit the site condition, (b) remove and replace the *in situ* soil; or (c) abandon the site. Abandoned sites linked to undesirable bearing capacities have dramatically increased, and the outcome of this is the scarcity of land and an increased demand for natural resources. The affected areas include those susceptible to liquefaction, those covered with high level clay material and organic soils which can be problematic because of low shear strength and high compressibility, contaminated land, as well as areas in a landslide (Makusa 2013). Problematic soil is recognised by its sudden volumetric reduction (i.e. collapsible soils), after exposure by various environmental conditions such as, increasing humidity, wind deposited sand or silt, or under water immersing conditions, hence altering cementation bonds between soil particles (Ayeldeen et al. 2017).

Drainage problems are caused by a lack of diversion of rainwater, which can lead to deterioration of the road structure or pavement surface. This is mostly due to moisture retention within the road prism and in particular within the subgrade soils relating to typical subgrade problems, as described in **Table 1** (Paige-Green 2008). The adverse effects of increase in moisture content on the soil behaviour have also been a major concern among geotechnical and pavement engineers. Soil possesses excellent performance at the optimum moisture content or below the optimum moisture content (dry-side of optimum) however, the strength of soils reduce significantly as the moisture content increases beyond the optimum (wet-side of optimum). Sensitive soils tend to swell with moisture content increase, particularly in areas of higher rainfall, as a result subsurface soil becomes too weak to support the pavement or road loads resulting in collapsed road surfaces and potholes (Stabnikov et al. 2011). The replacement of weak *in situ* soil is not always a good option, especially in pavement or road developments due to the ongoing construction cost conundrum in cash strapped countries, which is the typical situation in most developing countries.

An opportunity exists to improve unpaved roads in both urban and rural areas. Unpaved roads are defined as those with a surface coarse of unbound aggregate (gravel), where no binder or chemical additive is used (Netterberg and Paige-Green 1988). Good wearing coarse on unpaved or earth roads should have the ability to resist abrasive action of traffic, erosion by water and wind, freedom from excessive dust during dry weather and freedom from excessive slippage during wet weather. Over time of service, unpaved roads experience loss of the wearing coarse material, due to traffic as well as through surface material erosion caused by heavy rainfall. The fines content diminishes over time of service, resulting in loose coarse material which tends to accumulate. In traffic, loose coarse material induces fugitive dust, which affects air, soil and water quality, roadside flora and fauna, agricultural productivity, and road safety (Greening 2011). Additionally, it is well established that long-term exposure to traffic generated dust can be attributed to serious health problems from the effects of exposure to high concentration of airborne particulates. This loss of material causes road surface deterioration that requires re-gravelling to recover the serviceability of the road. Re-gravelling involves re-grading the road, transporting and spreading new aggregates on the affected road. Re-gravelling is environmentally unsustainable and financially not feasible as it increases maintenance costs. Therefore, the strategy by road engineers is to improve the engineering properties of the *in situ* materials with chemical treatment. The benefit of treated surface material (using alternative non-traditional stabilisers) is the reduction in maintenance costs. This is due to the reduction in gravel loss, and therefore reduction in frequency of grading and re-gravelling. Controlled gravel loss reduces dust loading and air-borne emissions which are hazardous to human health.

The cost savings and benefits, over the initial and long-term life cycle of the road, are apparent (Andrews 1999). Ongoing initiatives are therefore important for developing solutions to current road construction problems as they will contribute towards improved quality of life and safety, as a result of the reduction in traffic generated dust from the treated roads.

Table 1 Most common subgrade soil problems adapted from Paige-Green (2008)

Soil type	**Key disadvantages &-typical damage to roads**	**Current methods for recognition & counter measures (physical/chemical)**	**Effectiveness of the standard practice for road application**
a. Expansive clays	Associated with movement of expansive soils (including both expansion on wetting up & shrinkage on drying out). Damages include: localised deformation/surface distress (i.e., unevenness, cracking) especially in areas with high seasonal rain fall.	Commonly identified as shale, this expansive material is notorious in Southern Africa. Derived from weathered rock such as, dolerite comprised of smectite clays. Smectite in subgrade is best identified by: field observation (expression of crackling dark, grey, or red soil), x-ray diffraction, & measure of Plasticity Index (PI). Most successful technique is to remove the expansive clay beneath the road structure & replace it with a raft of inert material. However, success has been achieved by wetting prior to construction fills, & placing uncompacted layers of sand, gravel/rockfill over the clay soil. Removal of “water loving” trees due to root moisture extraction (usually associated with arcuate and/or longitudinal cracking).	The expansive soils are often widespread & lateral making the counter measure costly including: • This would typically involve the costly excavation. • High cost of haulage of denser better compacting soil usually from far distances. • Expensive use of lime stabilisation to alter clay properties. The partial removal of the clay from the subgrade and replacing it with less active material remains the most effective. Including proper understanding of water movement in & around the road which relates to the swell potential of the pavement materials.
b. Collapsible soils	Condition in which ‘bridges’ of fine materials (usually clays or iron oxides) within a framework of coarse & harder particles (usually quartz) become weak when wet and results in road collapse under load due to the presence of voids.	Weathered granites and feldspathic sandstones result in kaolinite and quartz particles remain intact, mainly identified by: low density, > 60% mass comprised of fines with 0.075-2 mm range & < 20% is < 0.075 mm. Collapsible soil needs to be saturated then	To avoid road damages this soil needs to be highly saturated with water but, the solution is impractical in water limiting regions. Alternatively, the use of high energy impaction techniques using impact rollers with or without water. The implementation of these techniques in

	Collapsible soils result in rutting and uneven road surface.	wetted and heavily compacted to disrupt the collapsible fabric. An effective but more expensive method is to excavate the material.	the rainy season may significantly reduce construction costs.
c. Dispersive/erodible soils	Dispersive soils are those soils that, when placed in water, have repulsive forces between the clay particles that exceed the attractive forces. Flow of water in subgrade can lead to formation of cavities. Unpaved roads – risk of material loss due to the frictional drag of water flowing over the material exceeding the cohesive forces holding the material together.	Identifiable via multiple tests including: the exchangeable sodium percentage, cation exchange capacity, the crumb test, the double hydrometer test, the sodium absorption ratio & pH. Avoid its use in fills, remove & replace it in the subgrade; Manage water flows and drainage; treatment with lime or gypsum will allow the calcium ions to replace the sodium and reduce the problem.	Management of water flows and drainage in areas often neglected. However, an advantage of its CEC capacity, is that lime or gypsum may be used as a stabiliser treatment (i.e., ion exchange of Na^+ to Ca^{2+}).
d. Saline soils	Affected by the combination of specific cations and anions in the form of soluble salts. This can be a major problem on road projects where movement of soluble salts to beneath bituminous surfacing leads to weakening of the upper base & blistering; disintegration of surfacing.	For presence of salts pH and electrical conductivity tests. As soluble salt problems arise from the accumulation and crystallization of the salts under the road surfacing and in the upper base layer, minimisation of salts in the pavement layers and subgrade should be attempted.	Soluble salts, particularly sulphates, & their acids can also have a serious detrimental effect on the stability/durability of chemically stabilised materials &concrete. The addition of lime to increase the pH to more than 10.0 will suppress the solubility of the more soluble salts.
e. Karst areas	Associated with limestones, dolomites carbonate rocks, resulting from dissolution of the carbonate material by acid groundwater. In SA, a major issue for the road	The biggest challenge is identifying these karst areas, thus experienced specialists and road engineers are involved in landscape assessments. Control road drainage or sinkholes are	Adequate maintenance and storm water drainage is vital (i.e., there are different maintenance requirements as a function of material type) (Mgangira 2009a).

	networks that cross subsidence area and sink holes leading to serious failures (resulting to loss of life & costly amendments).	normally filled with waste rubble or grouted.	
f. Soft clays	Associated with fluctuating water tables causing low shear strengths, high compressibility, and low permeability resulting in time-related settlement problems.	Predominantly found on coastal areas, making it impossible to walk on (shear strength of 10-40 kPa). Embankments are constructed slowly layer by layer; or the use of geosynthetic products as separation layers and to facilitate and accelerate drainage. The use of the wide range of geosynthetic products as separation layers and to facilitate and accelerate drainage has contributed to improved construction over such areas.	The materials are associated with low shear strength, high compressibility, low permeability, also organic matter contents make it difficult to predict their properties. The long-term differential settlements require ongoing maintenance to provide an adequate performance of the road.

For improvement of soil structure and engineering properties conventional approaches include dynamic compaction, soil-cement stabilisation, soil-lime stabilisation, drainage of vegetation, geotextiles, as well as injecting synthetic materials such as micro-fine cement, epoxy, acrylamide, phenoplasts, silicates, and polyurethane into the pore space to bind soil particles together (Saleh et al. 2019). This is accomplished using a variety of chemicals, jetting, and permeation grouting techniques. It must be noted that these approaches create serious environmental concerns as all chemical grouts apart from sodium silicate may be toxic and/or hazardous (Bryson and El Naggar 2013).

In most civil engineering projects, it is difficult to obtain a construction site that will meet all design requirements without introducing ground modification. Therefore, the current practice is to modify the engineering properties of the native problematic soils to meet the design specifications (Ikeagwuani and Nwon 2019). This leads to using alternative (non-traditional/biochemical) stabilisers which are one of several methods of soil improvement. In terms of viability, this may create a viable substitute to cement but it is all dependent on cost. Non-traditional additives are diverse in their composition and in the chemical and physical manner in which they interact with soil (Rajoria and Kaur 2014). Unfortunately, little is known about their interaction with different geotechnical materials and their fundamental stabilisation mechanisms. Despite the lack of research, an environmentally friendly solution is still advantageous, as the larger construction companies are very conscious of their environmental footprint, and thus eager to invest in smart ‘green’ construction materials (Shehata and Poulos 2018).

2. DISCUSSION

2.1 THE USE OF BIO-BASED INNOVATIONS FOR POTENTIAL ROAD APPLICATION

Several micro-organisms and other existing bio-based products such as secondary metabolites, enzymatic and polymeric materials have been considered as potential alternatives to conventional chemical stabilisers for the development of sustainable road infrastructure (Ikeagwuani and Nwon 2019). Therefore, better understanding of these bio-based materials and technologies is essential for their greater implementation in construction. In line with this, the Council for Scientific and Industrial Research (CSIR) in South Africa is currently investigating a selection of appropriate bio-based material/s using new technologies for assessment to a given structural application (i.e. unpaved roads, pavements). Bio-based admixtures made via industrial fermentation processes employ bacteria and is gaining more attention due to a number of advantageous factors such as their fast biosynthesis rates, high yields in products of interest, rapid growth on inexpensive culture media available in bulk quantities, being open to genetic manipulation, and their are non-pathogenic traits (Pei et al. 2015).

There are at least eight various types of construction-related biotechnological processes, classified by the results of the microbial treatment of soil (**Fig. 1**) (Ivanov et al. 2015). Of these, two biological processes (as far as the fundamental characteristics of research techniques) stand out, (a) bioclogging, the production of pore-filling materials through biological means, to significantly reduce the hydraulic conductivity of soil or porous matrix and, (b) biocementation, the generation of particle-binding materials through microbial processes *in situ* so that the shear strength of soil can be increased (i.e., based on road industry requirements and aforementioned disadvantages of soil types in **Table 1**). This is further reinforced by DeJong et al. (2010) stating that these methods allow a holistic view and meaningful characteristics in the area of soil stabilisation. The micro-organisms best suited for these techniques (i.e., soil bioclogging, biocementation, and bioaggregation) are facultative anaerobic and microaerophilic bacteria, although anaerobic fermenting bacteria has been suggested (Ivanov et al. 2015).

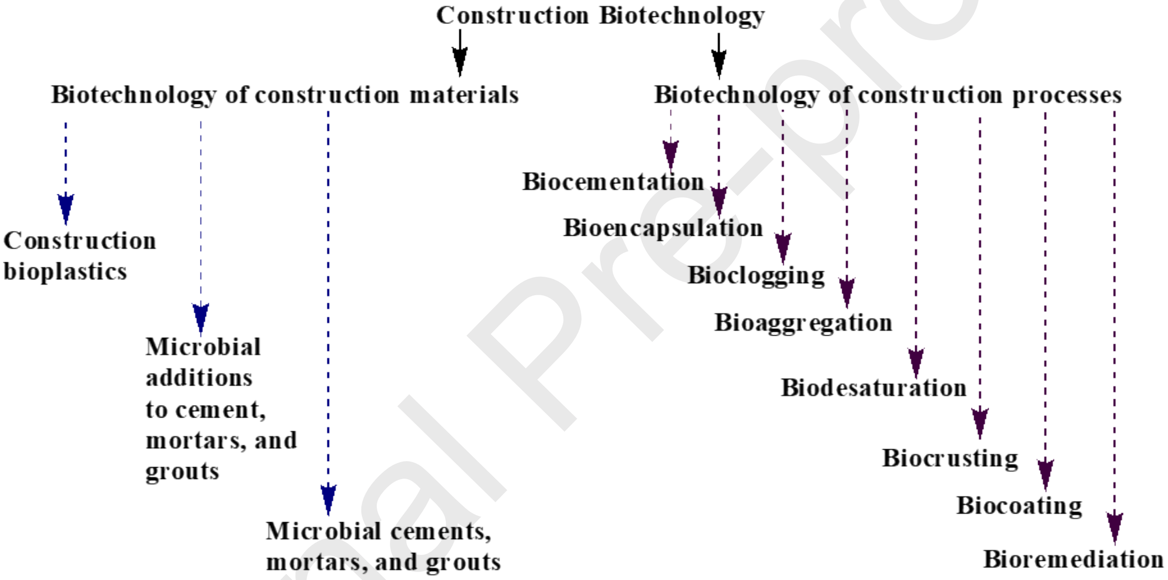

Fig. 1 Construction related microbial biotechnology

2.2 LARGE SCALE LABORATORY TESTING AND *IN SITU* DEMONSTRATIONS OF BIO-BASED ADDITIVES

2.2.1 Microbial biocement

One of the most studied methods is the precipitation of calcite uniformly within soils through biological action to elevate the pH that creates supersaturated conditions known as microbial induced calcium carbonate precipitation (MICCP), commonly referred to as microbial biocement (Oyediran and Ayeni 2020). For example, *Bacillus pasteuri* is an alkalophilic soil bacterium with a highly active urease enzyme that consumes urea and decomposes it into carbon dioxide (CO_2) and ammonia (NH_3). In the presence of water, NH_3 is converted to NH_4^+ and CO_2 equilibrates in a pH-dependent manner with carbonic acid, carbonate, and bicarbonate ions. The increase in pH provides the alkaline environment and the carbonate required for the reaction with Ca^{2+} and precipitation of calcite ($CaCO_3$), schematically shown in **Fig. 2**.

Cheng et al. (2013) demonstrated bio-enzymatic mediated improvement of soil using unconfined compressive strength (UCS) of MICCP treated samples before and after freeze-thaw (FT) cycling that showed less than 10% decrease on strength occurred from > 800 kPa (before FT cycle), for the OPC treated samples > 600 kPa (before FT cycle) and usually causes serious damage, as expected with ~ 40% decrease on strength. Shear strength investigated by Ng et al. (2012) using *B. megaterium* MICCP process found that the shear strength ratio of treated to untreated increased from 1.40-2.64. Compressive strength of at least 300 kPa was reported by van Paassen et al. (2010) similar to findings were reported by Nafisi et al. (2019) which show that the tensile strength varies between 210 kPa to 710 kPa depending on sand type and mass of carbonate. The author van Paassen et al. (2010) also reported a 60% reduction in the permeability of treated soils at ~ 100 kg/m^3 $CaCO_3$ precipitation however, with increasing concentration of the solution calcite crystal clogged pore spaces which reduced permeability between 50-99% using 1 M cementation solution (Ivanov et al. 2010), as the crystals formed at the contact points can maintain the connection of pores without restricting the pore water mobility. Even though these tests characterise the strength properties at large scale, triaxial test is recommended to test the response of biocemented soils toward the monotonic and cyclic loadings as it simulates the natural behaviour of soil in the field for road applications.

Cheng et al. (2013) reports mechanical behaviour of the biocemented sand in triaxial tests showed increase in effective shear strength (i.e., cohesion, frictional angle) with increase of $CaCO_3$ content at all degrees of saturation. At a lower degree of saturation, the precipitated crystals contributed to better improvement of cohesion using coarse sand than by improving the frictional angle. In comparison to fine sand, under the same saturation condition and similar $CaCO_3$ content, the coarse sand demonstrated a higher friction angle than the fine sand. The researchers explained this as follows: small soil particles' main effects include: (a) provide more interparticle contact points for MICCP process and (b) reducing the stress acting per particle contact. The MICCP process acts most efficiently at a particle contact just as cementation begins, and continued expansion of cementation around a particle contact has a decreased effect. By reallocating the $CaCO_3$ crystals to two contact locations (connection points) instead of one would be more effective due to increasing interparticle connections. Simultaneously, the contact stress decreases as a function of the particle radius squared. Therefore, smaller particles provide two compounding benefits: (a) more efficient MICCP and (b) lower particle contact stresses (Cheng et al. 2013).

Upon evaluation, we conclude, based on the experimental data that there is a similarity in test methods and these key criteria enable better selection of the best performing bio-additives of interest that meet technical specifications such as durability properties. Data from here can also be used as an input for the design of experiments for small- to large scale field testing using correct dosing strategies, curing times and soil clay content as further implications in terms of strength requirements that will need to be assessed (e.g. focus on materials in response to pressure, temperature or moisture).

According to van Paassen et al. (2010) an exponential relationship exists between the UCS value and the $CaCO_3$ content of the treated soils, revealing that despite having the same amount of $CaCO_3$ precipitated, the mechanical response can vary according to the mechanism of action of the $CaCO_3$ precipitation/crystals. Furthermore, the mode of action (MICCP) may be strain dependent and the microbes must possess strong urease activity to perform the reaction (van Paassen et al. 2010). An enhancement of the effect can be initiated since the bacterial cell wall is negatively charged; the bacteria attract more cations from the environment, including Ca^{2+}, to deposit on their cell surface. The Ca^{2+} ions subsequently react with the CO_3^{2-} ions, leading to the precipitation of $CaCO_3$ at the cell surface that serves as a nucleation site, the initial process that occurs in the formation of a crystal from a solution (Van Tittelboom et al. 2010). Several documented researches show several *Bacillus* spp. have the ability to precipitate calcium carbonate with structural properties, but a limited selection of micro-organisms highlights the triaxial and California bearing ratio (CBR) tests for potential road/pavement applications (**Table 2**).

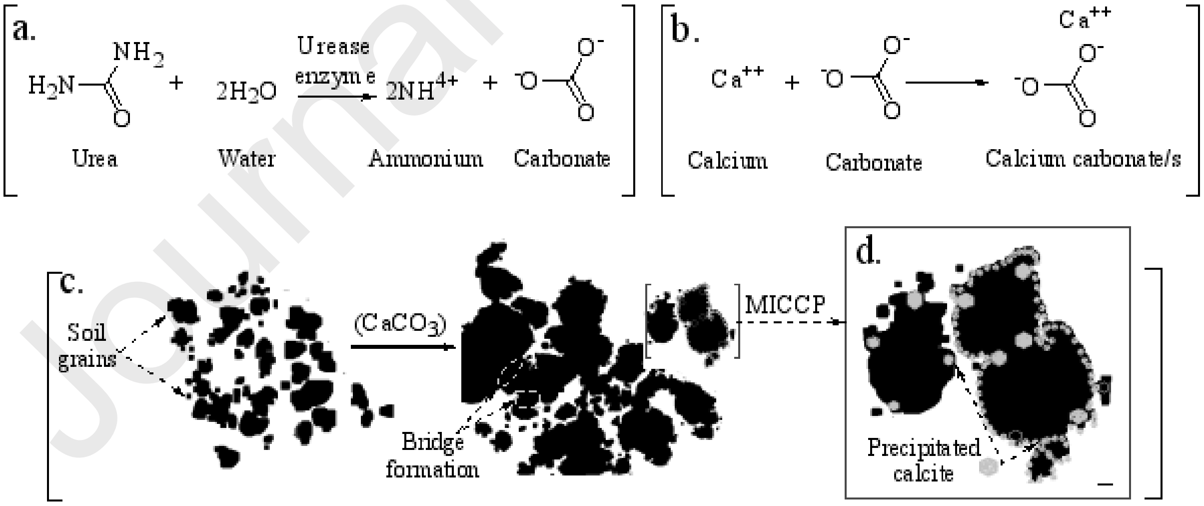

Fig. 2 Biocement is two-step process **a:** Phase I, bacteria ingest nutrients such as, sugar, nitrogen, & proteins with the growth of the bacterial population and enzymes produced from the bacterial species they hydrolyse urea components in the presence of water to form ammonium and carbonate ions which leads to **b:** Phase II, again the addition of nutrients such as calcium chloride, in the presence of calcium ions and nucleation sites on the soil particles, the carbonate ions react spontaneously with the

calcium ions to form calcium carbonate, c-d: the calcite precipitates/the cementing agent (produced by the bacteria) used to bind the soil particles together to increase strength and stiffness of the soil.

Table 2 *Bacillus* species related to microbial biocement

Preferable microbes come from Bacillacae family	**Key micro-organisms showing mechanical improvement of construction material**
B. flexus	Laboratory strength tests showed increase in compressive (>40%), flexural (>30%) & split tensile (>10%) strength. X-ray powder diffraction (XRD) show ureolytic properties of the organism (Roa et al. 2015).
B. sphaericus	Experimental results indicate polyurethane immobilised bacteria induced higher strength regain (60%) and lower water permeability coefficient (10−11 m/s) (Wang et al. 2012; Wang et al. 2014).
Sporosarcina pasteurii formerly known as B. pasteurii	Biocalcification mechanism showing potential construction application such as, dust control and strengthening of brick masonry (Sarda et al. 2009; Meyer et al. 2011). Triaxial tests indicate that the treated specimens exhibit a non-collapse strain softening shear behaviour, with a higher initial shear stiffness and ultimate shear capacity than untreated specimens. Samples treated showed great increment in unsoaked and soaked CBR gained more strength when soaked for hours compared to unsoaked (Ovediran and Ayeni 2020). CBR obtained in a study showed poorer outcome from CBR index treated (0.6) compared to untreated samples (2.5). These investigations are of interest to improve soil stability, to build roads and paths, and to restore monuments (Morales et al. 2019).
B. licheniformis	Calcium carbonate precipitation from the strain as a potential for sealing cement-based materials (Vahabi et al. 2015).
B. subtilis	Strength performance of microbial concrete mechanism on the principle of calcite mineral precipitation by alkali-resistant spore-forming bacteria. Its response showed autonomous improvement in self-healing process due to micro-crack formation (Rao et al. 2017).

MICCP is being established and lab/small scale field tested for numerous geotechnical and construction applications (DeJong et al. 2013). A fundamental concern to road engineers is that MICCP may result in crust formation resulting in impermeability and increased runoff, as well as increasing pH impact on flora and fauna, degradation of calcite due to acids in rainwater and sheet runoff specifically a concern for karsts (refer to **Table 1**). Therefore, rarely has microbial or enzyme bio-cement technology been evaluated at field scale under real-world conditions for road applications as indicated in **Table 3**.

Table 3 Bio-based construction-related applications

Potential application	**Reference**
a. Road construction and soil erosion prevention	(Ivanov et al. 2015)
b. Construction in sandy soil of channels, aquaculture ponds, or reservoirs	(Chu et al. 2013)
c. Sand immobilization and dust suppression	(Meyer et al. 2011)
d. Sand reinforcement in near-shore areas	(Raut et al. 2014)
e. Brick production	(Sarda et al. 2009; Dhami et al. 2012; Raut et al. 2014)
f. Crack remediation in concrete and rock and increase in durability of concrete structures	(Ghosh et al. 2005)
g. Concrete improvement	(Pacheco-Torgal and Labrincha 2013)
h. Concrete self-remediation	(Jonkers and Schlangen 2007)
i. Mortar modification	(Ghosh et al. 2005)
j. Consolidation of porous stone	(Jimenez-Lopez et al. 2008)
k. Bioremediation of weathered building-stone surfaces	(Achal et al. 2011)
l. Fractured-rock permeability reduction	(Cuthbert et al. 2013)
m. Encapsulation of soft marine clay to produce solid fill material	(Ivanov et al. 2015)

Innovation is required in the use of alternative materials and in construction techniques that enhance *in situ* materials and minimises environmental load. Therefore, the potential solution is to investigate using novel micro-organisms and their products to achieve a specific performance requirement (smoother, stronger, shock-resistant longer lasting road surfacing, and better road substructure). Some reports of biological stabilising techniques include those by Ghosh et al. (2005) and Raut et al. (2014) which shows multiple applications of biological agents in similar fields, such as liquefaction and erosion prevention, building settlement reduction, dam safety, slope stabilisation, impermeable and reactive barriers for groundwater protection, aquifer and energy storage, and carbon sequestration (Ivanov and Stabnikov 2016; Rehm 2010; Rajoria and Kaur 2014). However, the majority of the studies on microbial geotechnology for soil road construction present significant gaps in *ex situ* field-testing research that prevents the commercialisation of bio-based products. This presents an opportunity to conduct research in this field that can result in novel processes and products of value to this rapidly growing industry.

The use of bacteria and enzymes is employed in endeavours towards soil improvement. Alkaliphillic heterotrophic spore-forming bacteria appear to be suitable candidates for improving durability aspects of concrete as no other potentially detrimental metabolic by-products, such as ammoniums in the ureolytic pathway, are produced (Jonkers and Schlangen 2007). Moreover, alkaliphillic species of the genus Bacillus are not only adapted to be metabolically active in highly alkaline environments, but are also able to produce spores, (i.e., specific robust 'dormant' cells which survive being incorporated in the concrete matrix for self-healing). *Bacillus megaterium*, is an example of ureolytic bacteria that's able to adapt to soil environments (Jiang et al. 2016).

Bacillus subtilis, a common soil bacterium has been found to induce silica precipitation and other metals by binding it to the cell wall of the bacteria (Urrutia and Beveridge 1994). These studies show an exemplary approach of cell wall modification using *Bacillus* spp. via ethylenediamine to become electropositive to bind SiO_3^{2-} ion from the silicic acid. In line with this approach, cement induces calcium silicate hydrated (C-S-H) gel and calcium hydroxide in aqueous form after reacting with water during the primary reaction, the secondary hydration will proceed with the presence of pozzolans (i.e. siliceous and aluminous material) inside the concrete and produce an additional C-S-H gel that accelerate and enhance the strength of final concrete. In this case, micro-organisms may be used as an alternative method to produce a precipitation that possesses characteristics similar to that of the cement pozzolans which can be derived naturally from the process of biomineralisation from micro-organisms - a process that forms precipitation and fills the pores that enhanced the mechanical properties in concrete materials, such as strength and durability. Therefore, evidence of bacterial modification in the presence of silicate by reacting aqueous calcium hydroxide to form C-S-H and enhancements made to the compressive strength development in soil constitutes further investigation in this field (Afifudin et al. 2011).

2.2.2 Bioenzymes and their role in construction engineering

Recent experimental progress focuses on applications of protein engineering as a means for biochemical soil modification. More attention has been given to the use of bioenzymes as soil stabilising agents due to expansion in manufacturing capacity, the low cost, and relatively wide applicability compared to standard chemical stabilisers (i.e., hydrated lime, Portland cement, and bitumen), which are required in large amounts to stabilise soils (high associated cost). Innovative thought is provoked in observing the engineering prowess of the 'termite engineers' in the construction processes of their all-weather proof termite mounds (Wijeyesekera 2014). Ant saliva, full of enzymes is used to build soil structures, which are rock hard and meters high. These structures are known to stand firm despite heavy tropical rain seasons (Assam et al. 2016). With research developing within this direction, some of these biochemical solutions for soil stabilisation include enzymes such as peroxidases, phospholipase, cellulase, phytase, lipase, luciferase, and proteases (Ivanov et al. 2015; Das and Varma 2010).

A review of previous studies on enzyme-based soil stabilisation indicate enzymes have been used successfully to stabilise roads in Malaysia, China, India, and the Western USA despite the heavy traffic and high rainfall (Taha et al. 2013). Besides an increase in the strength and durability of the roads, a reduction in project cost has also been achieved. In Mendocino County, the California Department of Transportation has conducted several tests of a compaction additive based on enzymes. This natural product helped the road base to set very tightly, reducing dust and improving chip-seal applications. With air quality and water quality agencies requiring dust reduction, this is a potentially effective new product, cheaper than asphalt (Marasteanu et al. 2005).

Products such as *Permazyme* and *EcoBric* are non-hazardous, and eco-friendly, which use low energy techniques for soil stabilisation that have weathered resistance approaching that of concrete. The advantages of competitive bioenzyme products (i.e. *Renolith*, *Fujibeton*, and *Terrazyme*) are that it is rapid, cost-effective, convenient to use, and utilises eco-friendly technology yielding a 'value added' product (Rajoria and Kaur 2014). Even though these enzyme-based soil stabilisers appear to have many advantages compared to conventional chemical stabilisers, it is unclear how these products work and under what conditions. The stabilising mechanisms of these products are limited in application, and their proprietary chemical composition makes it difficult to predict their long-term performance. According to Khan and Taha (2015) the reported formulation of *Terrazyme* is composed of ethoxylated alcohols, fermented vegetable extracts, and non-ionic surfactants (Khan and Taha 2015). *Permazyme* is composed of multiple enzymes, proprietary ingredients, and unspecified organic material, according to the manufacturer. Further laboratory investigation showed the presence of proteins and non-ionic surfactants in both preparation (AbouKhadra et al. 2018). The bioenzymes treatment of soil roads with a combination of different multi-enzyme formulation or in combination with other polymer compounds serves the purpose of waterproofing and hardening soil. Therefore, it becomes important to perform a research study that can give objective scientific support for the use of novel enzymes as a product to stabilise soils. Addition of enzymes to clayey soils provide a desirable reduction in the compaction effort necessary to reach target soil densities and improves resistance to freeze-thaw cycling (Rajoria and Kaur 2014). However, the recommended soil types are limited to 12-30% clay content, 18–30% cohesive fines and should have a plastic index between 2–10, and a max liquid limit of 30% (Jayathileka and Adikari 2007; Guthrie et al. 2015).

The idea of using enzyme stabilisation for roads was developed from the application of enzymes to products used to treat soil in order to improve horticultural applications. A modification to the process produced a material which is suitable for stabilisation of poor ground for road traffic. When added to a soil, the enzymes increase the wetting and bonding capacity of the soil particles. It is suggested that the enzyme/s react with soil molecules to form a cementing bond that stabilised the soil structure. At higher application rates the treated soil can be stabilised to form a dense, firm, hard, and water-resistant bond layer that may be used as a road surfacing (Lim et al. 2014).

Preliminary laboratory investigation of various enzyme products as a soil stabiliser originates from a common description: certain enzymes are adsorbed by the clay lattice, initiating a catalytic reaction (i.e. metal cation exchange mechanism). Cation exchange refers to an immediate reaction of the clay with the enzyme, within a few minutes of mixing, resulting in improved soil texture. They have an important effect on the clay lattice, initially causing them to expand and then to tighten. The enzymes can also be absorbed by colloids enabling them to be transported through the soil electrolyte media. The enzymes aid the soil bacteria to release hydrogen ions, resulting in pH gradients at the surfaces of the clay particles, which assist in breaking down the lattice. The change in the structure causes a decrease in the moisture sensitivity and increases the workability and constructability of the soil (Marasteanu et al. 2005; Rajoria and Kaur 2014).

Furthermore, an enzyme activity generally corresponds with element stabilisation. A number of enzymes require the presence of metal ions, such as calcium ions, for the maintenance of their stable and active structures. These ions are bound to specific binding sites on the surface of the molecules and restrict local flexibility and unfolding (Guncheva and Zhiryakova 2011). Enzymes require trace amounts of specific divalent metal ions such as Mn^{2+} for glucosylgydrolase, Zn^{2+} for phosphohydrolase, and Ni^{2+} for amidohydrolase enzymes as functional cofactors, which can be substituted by elements with similar ionic properties when present in excessive concentrations. In practice, the biochemical reactions are brought about largely through the catalytic contribution of enzymes and variable substrates that serve as an energy source for micro-organisms. Enzymes containing Ca^{2+} binding domains or other metal binding sites such as Co^{2+}, Mg^{2+}, and Zn^{2+} may act as a soil stabiliser by ion exchange, thus replacing the monovalent cations surrounding the surface of expansive clay soil particles and may decrease bound water molecules. This results in a stronger bond/clay lattice structure and particle cohesion (i.e. enabling clay particle flocculation) (Carvalho et al. 2013).

The utilisation of enzymes is unique in that it is one of the key present-day strategies towards environmentally benign energy– and material saving biochemical processes. The need is to identify more novel enzymes and to elucidate their modes of reactivity on various soil geochemistry and/or waste, such as fly ash. The potential of using enzymes to stabilise waste material raises the question of whether it can work synergistically. However, enzymes such as amylase, arylsulphatases, β-glucosidase, cellulose, chitinase, dehydrogenase, phosphatase, protease, lipase, and urease. have shown potential in related industrial applications (Ivanov et al. 2015; Das and Varma 2010).

Several reported stabilisation mechanisms termed enzyme induced calcite precipitation (EICP) are proposed (similar to the process microbial induced calcite precipitation (MICP)) such as bacterial lipases that was found to be activated by Ca^{2+}, and other metal ions. These properties make immobilised lipase an attractive option for soil stabilisation applications (Carvalho et al. 2013). These Ca^{2+} activators may increase adsorption of calcium on soil particles, thereby liberating metal ions to bond with clay soil particles. Enzyme products containing high amounts of silica (i.e. silica-immobilised lipase (Kumar et al. 2006) or calcium and sodium) is hypothesised to make the enzyme product act more like cement by forming hydrated calcium silicate when added to soil (Carvalho et al. 2013). Bio-phosphate cements are known as microbial induced deposition of phosphate. Alkaline phosphatase constantly hydrolyses phosphate monoester in bacterial solutions, thus PO_4^{3-} ions are obtained. $MHPO_4$, MPO_4 or $M_3(PO_4)_2$ are prepared by the reaction of PO_4^{3-} ions with metal cations (alkaline earth element Ba) and adopted to cement loose particles. The cementing mechanism of barium hydrogen phosphate particles by microbial precipitation can form large agglomerates with each other and interact with quartz sand to produce van der Waals bonds in sandstones. Bio-phosphate cement can bind loose sand particles into a bio-sandstone (Yu et al. 2015). Furthermore, the efficiency of soil enzymatic stabilisers for road applications highlights two prominent commercial preparations *Terrazyme* and *Permazyme.* Overall, the use of bio-enzymatic soil stabilisers reduces the amount of water required at the construction sites, minimise the need to haul materials and are promising additives to reduce the application of OPC. Factors such as, non-toxic formulation, manufactured through natural fermentation processes make enzyme products highly economical with considerable cost savings of 3-5 times when compared with conventional road construction methods (Taha et al. 2013).

The paper presents efficiency and limitations from studies such as, soil type, curing time, and method for application which significantly affect the effectiveness of the bio-enzymatic treatment. Peng et al. (2011) found under air-dried curing conditions UCS of enzyme treated soil were better in comparison to OPC because the cement particles cannot be fully hydrated under the air-dry conditions, in comparison to sealed curing conditions the UCS of the bio-stabilised soils were < 0.5 MPa. Marasteanu et al. (2005) conducted triaxial tests and determined the resilient modulus for two types of soils that were treated by two different enzymatic preparations (1 cc of enzyme per 5 L of the water), comprising of soil-1 96% of fines (75% of clay) and soil-2 60% of fines (14.5% of clay). The triaxial tests were performed on two confinement pressures (27.6 kPa and 55.16 kPa). The results showed that the soil treatment of enzyme-1 increased the shear strength of soil-1 and soil-2 by 9% and by 23%, respectively. In case of enzyme-2 treatment, the increases in shear strength for soil-1 and soil-2 was 31% and 23%, respectively. The resilient modulus tests with a deviatoric stress = 96.5 kPa and confinement pressure = 13.8 kPa, and the resilient modulus for the treated soil was between 86453.4 kPa and 5805.4 kPa which was greater than the mean of the untreated soil. Resilient modulus of soil is a common parameter defining the stiffness of a soil. For instance, at 48.3 kPa deviator stress, improvement in resilient modulus was noticed for soil-1 with enzyme-2 whereas the increase in resilient modulus was 55% to 85%. For soil-2, at the same deviator stress level the increase of resilient modulus reached 51% to 61% with enzyme-1 and 57% to 137% with enzyme-2.

According to AbouKhadra et al. (2018) enzymatic stabilisation introduced a significant improvement in UCS and CBR for the fine grained soils. In this study, improvement of the enzyme product as soil strength was normalised to that of untreated soil and represented by (CBR)r. For fine grained soil, the improvement in (CBR)r ranges from 2.75 to 4.5 times those values observed for non-treated soil and the best concentration was 2.5 g/L. Also, the soil strength increased with increase clay content therefore percentage fines important criteria. At 0.5% concentration *Permazyme* at 30-40% clay content achieved maximum (CBR)r (4) also the fines material UCS was 800-1200 kN/m^2. It is inferred from the results that using the enzymatic preparations significantly increased the strength of fine grained soil, the rate of improvement was found to be proportional to the clay content in the soil. Panchal et al. (2017) also found that using the correct dosages was fundamental such as, 700 mL dosage of *Terrazyme* enzyme solution which treated 1 m^3 soil, showed consistent increment in the CBR values of the soil of 9.80% from an initial value of 8.31% (unsoaked) and 4.19% (soaked) after the first week of curing, 13.43% after second week curing, and 14.80% after fourth week curing. However, significant variation was shown in CBR values with longer curing periods and increased dosages (900-1000 mL/m^3). This classification of bio-catalyst namely, *Terrazyme* (comprised of water, ethoxylated alcohols and matured vegetable concentrate) proved its efficacy in varying strength properties, consistency limits and various other geotechnical properties of fine grained soils. However, to acquire the best performance from this bioenzyme additive, soil gradation ought to be fulfilled with minimum clay content required for the reaction, sufficient active elements to react with metal cations resulting in a succession of breaking down of bonds as well as the advancement of a new system of links. The characteristics of the soil mixtures also decide the diversity in its breaking down process of the enzyme. In that study an essential element of the bio-catalyst is getting disregarded inadvertently, due to decline in strength improvement after longer curing periods. Therefore, as indicated by Thomas and Rangaswamy 2019 low concentrations of enzymes are enough for the soil improvement since they do not get attenuated in the regeneration of the enzyme catalyst after the reactions completed. The authors demonstrated an ideal soil combination, clay soil with the presence of 1% cement added and 0.06 mL/kg of *Terrazyme* which exhibited higher UCS. Initially, the strength of clay soil was 28 kPa, and improved to 127 kPa after introducing 1% cement and 0.06 mL/kg of the bioenzyme and it is about 354% higher than the UCS of untreated soil. This improved strength could be sustained with extended curing periods. These changes in the strength properties of soil matrix were supported by shifts of peaks in the FT-IR results, and the formation of additional C-S-H gel from the SEM images (Thomas and Rangaswamy 2019).

Most of the enzyme stabilisers on the market are imported with respect to SA, and have shown inconsistencies in performance, including frequent changes of names for the same product, which has led to their limited support in the local road construction industry (Moloisane and Visser 2014). Due to lack of innovative solutions using conventional approaches, the construction industry is exploring the use of biological methods to improve structural properties such as strength, volume stability, durability, and permeability in road construction applications. Due to the lack of performance, a gap has been identified to produce improved bio-stabilisers for the local market.

2.2.3 The role of biopolymers in construction engineering

For more than half a century fossil fuels have provided the resources for the polymer industry. However, the future scarcity of petroleum resources (Sorrell et al. 2012) is a fact that must be faced. Sustainability has now become important because of the significant depletion of fossil resources and major scientific and political players have encouraged the paradigm shift to 'bio-based material'. The twentieth century became the age of admixtures, the history of which started in the 1920s with the introduction of lignosulfonate, a biopolymer, for OPC concrete plasticisation, the first functional polymer used in construction on a large scale (Plank 2004). Recent years have seen a tremendous increase in the number of publications on using bio-based polymers (Chang and Cho 2019), however the fact is that these materials still constitute only a small fraction of the polymer industry. Biopolymers include polymers from agro-resources (polysaccharides, cellulose, starch, chitin, chitosan, and alginates), from micro-organisms by fermentation (polyhydroxyalkanoates, such as, polyhydroxybutyrate) and from biotechnology via conventional synthesis (polylactides (PLA), polybutadiene succinate, biopolyethylene (PE), polytrimethylene terephalate, poly-*p*-phenylene) (Averous and Pollet 2012). The reuse of agricultural and biomass waste will contribute to the environmental advantages of biopolymers opposed to traditional petroleum-based polymers (Hottle et al. 2013).

Biopolymers are naturally existing polymers that often have polymeric carbohydrate structures. The types currently used in the construction industry include, xanthan, guar gum, welan, succinoglucan, curdlan, chitosan, sodium gluconate, and gellan gum. These are used in dry-mix mortars, wall plasters, self-levelling under layers, or injection grouts to improve viscosity, water retention, set retardation and flowability (Plank 2004). The biopolymer b-1,3/1,6-glucan has been shown to aggregate soil particles and subsequently enhance the overall soil compressive strength. As well as, to have a positive effect on the compactability, Atterberg limits and swelling index of treated soil and a negative effect on the consolidation coefficient. A small amount of biopolymer has a significant effect in reducing soil erosion by enhancing soil inter-particle cohesion (Fatehi et al. 2018). Studies on xanthan and guar gum by Ayeldeen et al. (2017) show remarkable soil strengthening for road sector application. Whereas, Chang et al. (2015) used two thermos-gelation biopolymers (gellan gum and gaur gum), which enable hydrogen bonds to enhance the strength and durability of sandy and clayey soils. While time was not a crucial parameter, temperature played an important role in soil treatment. Investigation is still required as the soil improvement degree differs according to polymer types, soil types and compositions, doses, and curing conditions.

These studies provide an eco-friendly solution to improve the geotechnical behaviour (strength) of different types of soil, so this may resolve the need to provide complex conditions regarding microbial injection methods (Chang et al. 2016). The economic feasibility of soil–biopolymer matrix interactions and related soil strengthening mechanisms, in the field, may be analysed in comparison to ordinary Portland cement (OPC). According to Chang and Cho (2019), the cost of utilising biopolymers such as gellan gum for soil treatment remains relatively high compared to conventional soil treatment (OPC). They showed that the total cost to treat one ton of soil with gellan gum via thermal treatment is approximately $25–30 USD, in comparison to the current cost ($10 USD) of 10% cement treatment. However, recent analyses of economic feasibility and prospects show that the use of biopolymers for construction purposes is becoming more feasible as a result of the current rapid growth of the global biopolymer market. Findings also demonstrate that biopolymers that are of high-tensile strength (e.g. bioplastic PLA) have strong potential to replace cement as a soil treatment material within the context of environment-friendly construction and development (Babu et al. 2013; Muniyasamy et al. 2016).

There is a growing need for utilisation of economical stabilisers on locally available soils (*in situ*) that are cost-effective and environmentally friendly. Great potential exists for the development of bio-based stabilisers, however further research is required for testing of the concept and development of the product prototypes for application on the *in situ* construction materials. **Table 4** provides a preliminary guide on cost and benefits of soil stabilisers. The data shows current products on the market and the potential development of an integrated and validated process for the potential pilot manufacture of the desired products to meet techno-economic requirements. Several factors may be considered for further investigation into cost benefit ratios such as, material availability, equipment, technology viability, duration of construction, cost of hauling, reduction in carbon footprint and manpower (Chang et al. 2016; Chang and Cho 2012; Larson et al. 2013; Chang et al. 2015b; DeJong et al. 2006; Maclaren and White 2003; Velde and Kiekens 2002; Stocks-Fischer et al. 1999; Bang et al. 2015). A comparative analysis between the various stabilisers was limited at this stage, but a techno-economic feasibility study may be performed to fully understand the advantage of bio-stabilisers over conventional products. Moreover, cost versus performance is a requirement for upscaled technologies and is also important for the determination of product feasibility or alternative applications.

Table 4 Bio-based *in situ* soil stabilisers cost benefit analysis

Method of bio-based stabilisation	**Usage requirements (L/m^3)/(concentration)**	**Performance effect (Compressive strength (MPa))**	**Main consumables**	**Method for road application**	**Price for soil treatment [1]**
Ordinary Portland cement (OPC)	10% cement content to the weight of soil.	2 [2] (Chang et al. 2015b)	Cement, water	Deep mixing, injection, grouting, direct mixing spray.	60-100 USD/tonne (Chang et al. 2015b; Chang and Cho 2012)
Chemical stabiliser	10% content to soil mass.	0.8 [3] (Chang et al. 2016)	Chemically synthesised	Injection, grouting, Spray.	200 USD/tonne (Chang et al. 2016)
Enzymatic stabiliser product	0.033 L/m^3	2.5 (Peng et al. 2011)	Multienzyme formulation, surfactants	Sprayed on the soil (water truck).	5 – 6 USD/tonne [4] (Specialised Protection Products, Gauteng, South Africa)
Polymeric stabiliser	14-24 L/m^3	2-4.3 (Chang et al. 2015b; Chang and Cho 2012)	Biopolymers, water	Direct mixing, injection, spraying exo-cultivated polymers.	28 [5] – 250 [6] USD/tonne (Chang et al. 2015b; Chang and Cho 2012)
Microbial biocement	1×10^7 cells/mL [7]	1.8 (shear strength) (DeJong et al. 2006)	Micro-organisms, urea, starch, nutrient	Surface spraying (urea broth & Ca^{2+} solution); Subsurface	60 – 90 USD/100 L [8] (DeJong et al. 2006)

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[1] 2016-2019 cost estimates.

[2] 10% OPC content to soil mass (0.3-0.4% mix with water/0.1 kg OPC).

[3] Acrylics (polymers chemically synthesised).

[4] Enzyme product coverage area for 20 L (1 L/cube, ~ 500-800 meter per tanker depending on the depth of the road); based on estimated cost from a manufacturer and average of wet clay soil density 2300 kg/m^3.

[5] Xanthan gum (0.5% to the mass of soil).

[6] β-glucan biopolymer (0.5% to the mass of soil) mixed with residual soil.

[7] $CaCO_3$ at a rate at $1\text{-}7\times10^{-9}$ g $CaCO_3$ precipitate cell^{-1}.hr^{-1} (33 g of calcite/L).

[8] Cost of 100 L of nutrient solution with an activity of 10 mM urea.min^{-1} activity.

			solution.	injection; mechanically mixed in soil.	
Benefits	**Industrial production bioreactor (Yes/No)**	**Eco-friendly additives**	**Biodegradable**	**Soil competitiveness**	**Sustainable (longevity for unsealed roads)**
Ordinary Portland cement (OPC)	No	+	+	Coarse and fine grained soils	+++
Chemical stabiliser	No	+	++	Coarse grained soil	+++
Enzymatic stabiliser	Yes	+++	+++[9, 10]	Fine soils with at least 20% clay content	+++
Bio-polymer stabiliser	Yes	+++	+++[9, 10]	Coarse & fine grained soil	+++
Microbial biocement	Yes	+++	+++[9, 10]	Coarse grained soil	++

Key: *+ poor, ++ average, +++ good*

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[9] Reduce GHG (able to store carbon which would otherwise be oxidised to CO_2).

[10] Possible use with material remediation.

2.2.4 Micro-level analysis to understand the mechanism of interaction of soil stabilisers

Non-traditional additives are used to improve the engineering properties of subgrade soil. The assessment of these products, based on a combination of *in situ* standard performance testing, determines the macro-level strength characteristics. However, studies show limitations in predicting the expected performance on a macro-scale (Mgangira and Ndibewu 2010). Thus, there's a need to identify more complementary methods in the design. As such, using micro-structural details for the determination of a mechanism of action may contribute to identifying the interaction between the product and the soil particles.

Physico-chemical bonding mechanisms of stabilised material induced by stabilisation processes are advantageous for geotechnical engineering applications. Mechanistic tactics namely, bioclogging, bioaggregation and biocementation may be identified via micro-level analysis. Latifi et al. (2016) show the mechanisms of pore filling and particle bonding by cementitious products through the following techniques: X-ray powder diffraction (XRD), Field Emission Scanning Electron Microscope (FESEM), Energy-Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared Spectroscopy (FTIR), Brunauer, Emmet and Teller (BET) surface area analysis and particle size analysis (PSA), and using a laser diffraction approach. In conjunction with these tests, UCS tests show an improvement in strength with calcium based additives such as, SH-85, a commercial product (Latifi et al. 2013). Micro-level analysis such as, Field Emission Scanning Electron Microscopy (FESEM) can be used to determine a morphological representation to assess the prototype bio-additives for *in situ* soil stabilisation (Mgangira and Ndibewu 2010). Since the mode/s of action of biological stabilisers are not mutually exclusive, and bio-additive/s may function with one functional effect, or a combination thereof, it is thus important to identify the level of laboratory performance testing necessary for determination of soil-particle interaction, that complement large scale tests.

2.3 ADVANTAGES OF BACILLUS SPECIES IN INDUSTRIAL APPLICATIONS

The problems often faced in road construction is the quality of the soil subgrade, more specifically, whether or not the subgrade has a low bearing capacity, causing premature distress and failure of the newly constructed road. Methods showing a solution to overcome this problem include the bio-stabilisation with microbes, as shown in a recent study by Samang et al. (2018), in which they demonstrated improvement of the bearing capacity of subgrade soil using *B. subtilis*. The *Bacillus* organism class will form the first line of intervention towards a solution for the challenges mentioned. The advantages of the upstream and downstream processing for biomass production is outlined in **Fig. 3** (Maharajh et al. 2008).

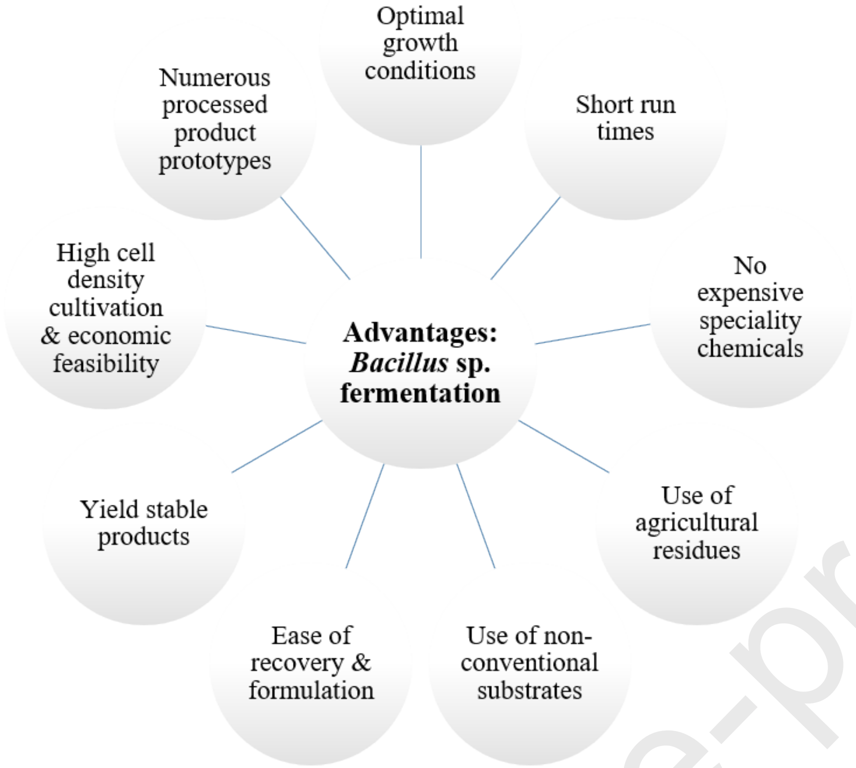

Fig. 3 Advantages of *Bacillus* spp. fermentation

The proposed mechanism is that *Bacillus* spp. (i.e. *B. subtilis*, *B. cereus*) produce hydrolase enzymes, such as lipases, which work in combination with added ionic stabilisers that may increase the strength and the number of ionic bonds present on the surface of the soil-clay particles. The result is the formation of a more highly weathered, less-expansive clay structure. Four major classes of polymers are produced by bacteria: polysaccharides, polyesters, polyamides and inorganic polyanhydrides (such as polyphosphates). The biopolymers and the enzymatic stabilisers are proposed to form polymer-soil matrices due to hydrogen and ionic bonds between the soil-clay particles. In this case the enzymes will have a dual role in polymer stabilisation, as they form both inorganic and organic catalysts with the natural polymers, to strengthen soil structural properties. The result is a soil material that is much less sensitive to moisture, more workable, and can be compacted to a better particle-interlock state by equipment and traffic forces. Better particle-interlock means higher internal friction and improved bearing capacity (Firoozi et al. 2017).

The importance of *Bacillus* spp. (particularly *B. subtilis*) has been shown in their production of surfactants, such as glycolipids, polysaccharide-protein complexes, fatty acids, phospholipids as well as lipopeptides. These compounds enable greater surface and interfacial tension reduction, lower critical micelle concentration, emulsification properties, and tolerance to ionic strength. Recent trends in soil research also suggest the stabilising activity of extracellular polymeric substances (EPS) produced by *B. subtilis* and *B. licheniformis*. EPSs can bind toxic metals, promote the dissolution of minerals and enhance aggregation associated with biofilms. When released into a solution, they can react with other solutes and soil-particle surfaces and may contribute to the stabilisation of microbial-derived organic carbon in soil. More generally, such microbial products can be expected to enhance the binding capability of unstable/weak subsoil particles and is discussed in more detail. **Fig. 4** shows fermentation components as potential material for testing.

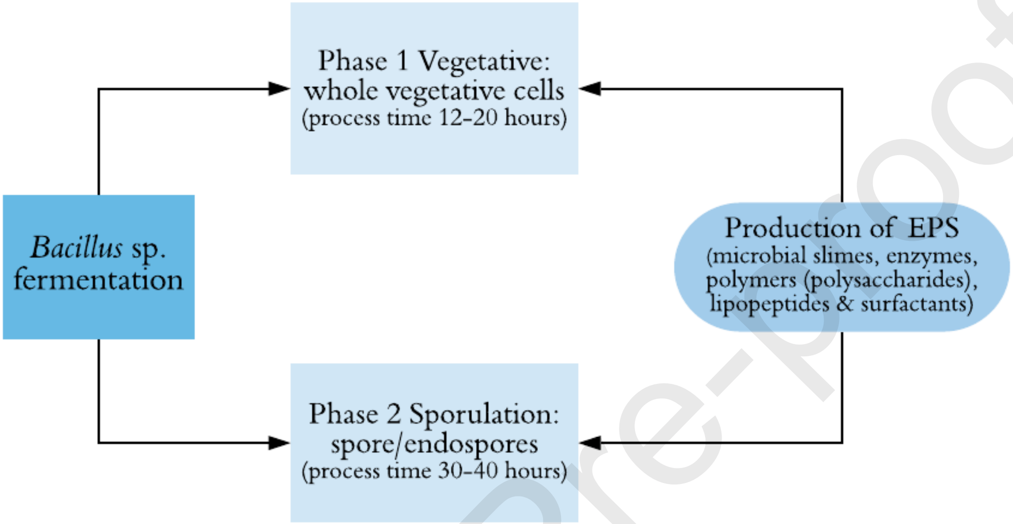

Fig. 4 Typical representation of fermentation material for potential structural testing

Key: *EPS – Extracellular polymeric substances*

Components of *Bacillus* spp. ferment that are well known is bacterial extracellular polysaccharides produced under excess carbohydrates or other water-soluble sources of carbon over a source of nitrogen. The food-processing wastes or sub-products such as corn glucose syrup, cassava glucose syrup, and molasses with Carbon:Nitrogen ratio >20 are used for industrial production of bacterial water-insoluble polysaccharides (Portilho et al. 2006). Extracellular polysaccharides form an integral part of biofilm formation (Limoli et al. 2015), biofilms are communities of bacterial cells living in a sticky, self-produced extracellular matrix on either a liquid or solid surface. The ability to form biofilms, like many bacterial phenotypes, is a result of bacteria differentiating into transcriptionally distinct cell types. *B. subtilis* is a model organism whose cellular differentiation capabilities have been well characterised in this regard (Joshi et al. 2016).

The EPSs either remains attached to the cell surface or gets released into the extracellular medium in the form of slime, comprising of a mixture of polysaccharides, amino sugars, proteins, teichoic, and nucleic acids. EPS in the form of homopolysaccharides or heteropolysaccharides and can be decorated with other residues such as phosphates, sulfates, N-acetyl-amino sugars, and acetyl groups. Bacteria producing different kinds of polysaccharides are reported in the literature that exhibit flocculation activity, namely, *B. licheniformis* and *B. polymyxa* (Spanò et al. 2013; Sardari et al. 2017). The EPS is the 'construction material' of the biological active biofilm matrix and possesses a multifunctional role for gel formation, flocculation, emulsification, absorption, film formation, and protection (Costa et al. 2016).

The most suitable candidates that show potential in producing extracellular polysaccharides to bind soil particles and fill in soil pores belong to the *Bacillus* spp. (Schallmey et al. 2004). Research shows growth of polymer producing bacteria in soil, their associated geotechnical applications potentially acts as a long-term stabiliser (Mikutta et al. 2011). Thus, understanding the chemical signals that induce biofilm formation and extracellular polymeric production in bacteria has broad relevance. Various *Bacillus* spp. showed the highest flocculation activity within 2 days of cultivation. The polysaccharide present on the cell surface was released into the culture medium that binds kaolin clay particles, resulting in the flocculation. (Kumar et al. 2004). Once released into soil, EPS is subjected to potential aggregation with polyvalent metals or sorption to mineral surfaces, thus leading to the enrichment of microbial-derived carbohydrates and proteins in the clay fraction or in high-density mineral organic associations. In addition, EPS could increase the hydrophobicity of the bacterial cell surfaces and also neutralise the surface charge of the cells (Pacwa-Płociniczak et al. 2011). A study of four bacterial strains, used for inoculation of quartz sand packed in columns with continuous supply of a medium, show that only slime producers greatly reduce the saturated hydraulic conductivity of sand (Vandevivere and Baveye 1992). Sewage sludge of municipal wastewater treatment plants, which is a waste microbial biomass, produce in quantities of several million tonnes a year could also be used as a source of cheap microbial polymers (Ivanov and Stabnikov 2016).

The application of biological approaches to geotechnical engineering requires the basic understanding of the mechanisms of the common problematic materials such as, their recognition and quantification. This necessary in order to implement counter measures to the detrimental effect on road structures when incorporating such materials, previously identified in section 1.1 **Table 1**. These are problems that are often faced in road construction such as soft soil subgrade with a low bearing capacity. Bio-stabilisation (i.e., bacteria utilisation) methods are hypothesised to overcome the problems such as, soft clay. Studies using *B. subtills* slurry showed that the maximum CBR value tested on soft soil was 39% using a 6% bacterial solution and 7 day curing period which was 13 times than that obtained for the untreated soil. However, with increasing concentration of the bacterial treatment (8-10%) the CBR value declined. This preliminary evaluation showed the use of bacteria solution of *B. subtilis* on soft soil significantly increased the bearing capacity of soft soil as a potential subgrade layer, but dependent on obtaining the correct dosage. Bearing capacity improvement was also shown with high tensile polymers produced by these microbial components (Samang et al. 2018). A two-pronged approach/mechanism was identified by this method of biological treatment which includes a synergistic effect, (a) microbial injection and by-product precipitation and may be introduced as a new type of soil binder, the dual effect from *B. subtills* based on (b) biofilm formation in the presence of soil clay minerals, induces attraction forces between micro-organisms and soil particles that later develops into a larger irreversible binding properties (Ma et al. 2017).

2.4 ASSESSING THE PERFORMANCE EFFECT (MODES OF ACTION) OF ADDITIVES FOR STRUCTURAL APPLICATIONS

Soil material such as, expansive clay is extensively located worldwide and is a great source of damage to the road infrastructure, due to changes in its volume when water content varies. There are a number of chemical products used for soil strength improvement, commonly referred to as stabilisers or additives (Jones and Ventura 2004). The mechanism to stabilise these soils occur using traditional additives such as cement, lime, and fly ash. The mechanism of action is outlined below:

Additives with calcium oxide produce the flocculation of the layers of clay by the substitution of the monovalent ions by the Ca^{2+} ions. This balances the electrostatic charge of the layers of the clay and reduces the electrochemical charges and electrochemical forces of repulsion between the layers of the clay minerals. The adhesion of the particles of clay into flocs occur, giving rise to a soil with improved engineering properties (a more granular structure, lower plasticity, greater permeability, and above all lower expansibility) (Seco et al. 2011; Tastan et al. 2011; Sukmak et al. 2013).

Even though weak subsoil material replacement can be reduced, the result remains environmentally unsustainable. Thus, bio-based techniques for upgrading roads cost-effectively, to an appropriate standard have been implemented in many countries recently. These techniques optimise the use of local *in situ* materials. However, limited literature is available on the direct structural performance/effect (modes of action) of commercial products comprising of enzymes, polymers or other proprietary biological road additives. This is particularly problematic in the road sector as the use of these products may be selective in soil type and complicated in their mechanism/s or application rates. It has been historically observed in South Africa, that civil/road engineers are reluctant to implement or to effectively standardise these liquid chemicals in road sector applications (Van Veelen and Visser 2007).

A significant amount of research and development (R&D) is accessible to propose mechanisms of non-traditional additives (Katz et al. 2001; Ayeldeen et al. 2017; Alazigha et al. 2018; Fatehi et al. 2018), although challenging to predict, as a majority of these studies conclude with several limitations, including application rates, concentrations, plasticity index and soil properties, thus making it difficult to standardise functional aspects regarding the mechanism of non-traditional additives. On the contrary, even though there’s the lack of fundamental research Mgangira (2009b), Moloisane and Visser (2014) and Van Veelen and Visser (2007) have demonstrated a national standardised (*ex situ*) approach to the determination of strength characteristics of both polymer- and enzyme-based additives. This approach infers the modes of action and associated mechanisms may be attributed or demonstrated by a pre-defined strength criterion and to the performance effect of key bio-molecules (i.e. resistance to: compression, erosion, abrasion, and water absorption), as described below:

- **Bio-stabiliser effect: resistance to compression load**

The capacity to resist applied stress is an indication of structural integrity and quality of a given material. Investigating the compression load infers testing the material's resistance to deformation and is therefore an important assessment of material strength to ensure the long-term durability and stability. For example, uniaxial compression test of biocemented sand (calcite) shows an increase in compressive strength. According to recent finding by Gao et al. (2019) the improvement in this material durability is sufficient to fulfil geotechnical applications. In addition, auxiliary additives such as, fly ash, lignosulfonate, and biopolymers have all indicated improvement in compression strength (Chang et al. 2015a; Fatehi et al. 2018). Supported research at laboratory scale and field applications show that compression testing is a key design criterion for quantifiable assessment of the effect of a bio-based stabiliser/additive on performance.

- **Bio-stabiliser effect: resistance to erosion**

Surface erosion occurs once the shear pressure applied by moving fluids exceeds a critical value. This surface erosion often leads to material failures of geo-structures. Several techniques have been used to increase the soil erosion resistance, including chemical methods. Ham et al. (2018) shows improvement of surface erosion resistance by using microbial biopolymers, altering the mechanical and the hydrological behaviour of soils, such as inter-particle bonding, stiffness, strength, and permeability by the insoluble biomaterials. Potential mechanisms of biomaterials to enhance erosion resistance include, (a) gel like EPS (polymers – hydrophobic properties) that fill the pores of soils and cause bio-clogging by reducing hydraulic conductivity; (b) grain coating and bridging by film-like biopolymers increases the apparent cohesion and erosion resistance, as reported in previous literature (Chang et al. 2015a); and (c) the biocement method is also suitable for the control of soil erosion induced by hydraulic scour. These observations are also consistent with Alazigha et al. (2018), MacLaren and White (2003), Chang and Cho (2019) which use gellan gum, xanthan, lignosulfonate, and cement to increase resistance to erosion. Biopolymers and clays as inclusion in sands play a similar role in erosion control behaviour, increasing the apparent cohesion of the soils and reducing the permeability and void ratio. The erosion test is one of several tests that are used to assess the material's resistance to running water, simulating rainy conditions, a preliminary test necessary for further assessment (field studies).

- **Bio-stabiliser effect: resistance to abrasion**

The dominating form of deterioration on unpaved or earth roads depends on whether it is during the wet conditions or dry conditions. Good wearing course on these roads should have the ability to resist abrasive action of traffic and erosion by water and wind, freedom from excessive dust during dry weather and freedom from excessive slipperiness during wet weather (Netterberg and Paige-Green 1988). All unpaved and earth roads, when dry, suffer from surface abrasion loss resulting in dust generation caused by a reduction in adhesion between the soil particles. The movement of loose material when there is insufficient binding power of the material to keep the surface intact, leads into corrugations under traffic action, and ravelling of the road surface (Visser and Hudson 1983). The high levels of dust created from these unpaved roads reduces visibility and hinders traffic safety. The unpaved road network makes up a significant portion of the overall road network in SA, and in most developing countries and more than 90% of these roads are in rural areas (Burrow et al. 2016). Thus, unpaved roads provide access for the majority of the population and directly support social and economic growth. The problems associated with unpaved roads include, low standards, poor riding quality, increased erosion in wet weather, and large quantities of dust that is generated by moving vehicles and the wind. Contributing reasons, according to (Moloisane and Visser 2014) is that many unpaved roads do not have the ideal range and distribution of particle sizes to give a good load-bearing capacity when wet (coarser particles) or sufficient plastic capability (clay) when dry to prevent material from breaking loose.

Historic data shows that approximately 3 million tonnes of dust was generated on SA unpaved/unsealed roads per year in 2004. The estimate was that two-thirds of this dust resettles on the road and the remaining million tonnes are permanently lost, leading to a negative environmental impact and rapid deterioration due to loss of material (Jones and Ventura 2004). The lost material requires replacement and frequent maintenance, due to the development of potholes and ruts, contributing to a decrease in durability and strength properties of unpaved roads. Replacement of gravel material is unsustainable as natural resources are being depleted. A potential solution to this ever-increasing problem is the use of soil stabilisers (additives). As previously discussed road materials can be improved using traditional additives such as cement and lime, however, studies on ionic additives (i.e. enzymes and sulfonated products) suggests a cost-effective and practical solution to ongoing problems (Moloisane and Visser 2014). An investigation into dust suppressors, termed dust palliatives (e.g. SoilSeal, Aggrebind product) which are agents applied to the road surface, to prevent the formation of dust clouds and their associated environmental impact is therefore important.

- **Bio-stabiliser effect: resistance to water absorption**

Unpaved roads are more vulnerable to weather conditions, especially during rainy seasons as soil material can expand as well as lose cohesiveness, with increased porosity and possessing a higher permeability. Chemical/biochemical stabilised soils may have adequate compressive strength under dry conditions; but they will lose their strength under adverse moisture content. The amount of water absorbed by the stabilised soil is therefore of importance in this case. A key factor in reducing soil permeability is the presence of additives (polymers – hydrophilic properties) in soil which reduce the pore volume and increase the viscosity of pore water, and thereby decreasing soil permeability. Field experiments have been conducted using a process of bioclogging and can be utilised to tackle seepage problems such as the reduced leakage volume of earth dikes and dams through the biofilm method (Blauw et al. 2009). In another field experiment, the biocement grouting method was applied for ground reinforcement and remediation of heavy metal-contaminated soil water seepage and wind forces (Cuthbert et al. 2013). Therefore, water repellent agents are considered an effective method of preventing moisture damage in construction materials.

Recently, a field application of the bio-augmentation – enzyme induced precipitation technology was investigated by Hodges and Lingwall (2020). They used three large scale field test sites which focused on mitigation of road surface erosion and dust suppression. One of the test sites with weathered shale showed that the bio-stimulators possessed potential for mitigation of surface erosion prevention. This was due to the soil properties containing a clay content opposed to the sandy soils. In their study the main 'take-away' was its several shortcomings in terms of field implementation, such as if the soil has large concentrations of salts, microbial biomineralisation may not occur at all (i.e., large amounts of salt kill bacteria if chlorine levels exceed that to which the bacteria have tolerance). By analysis of this case study, two research questions for future bio-stabiliser product development and performance was suggested, (1) will an alternative formulation (using bacterial carriers) for the installation of microbial based bio-stabiliser treatment on-site be ideal (opposed to live cultured bacterial suspensions on-site, or transported from laboratories); and (2) will longer curing times such as 28-days be optimal as this is directly dependent on strength gains and positively affects sorption, coating, and the aggregation effect of the ingredients by the stabiliser.

The aim of this literature survey was to address challenges in road construction. CSIR (SA) has identified various mechanisms of action for improving the strength properties of construction materials by using microbial components and produced metabolites (i.e., bio-enzymatic, surfactants, ionic, and polymer soil stabilisation), refer to **Table 5** for a summary of bio-mediated treatments. This allows for proper isolation and assessment of strains based on their ability to produce unique compounds of interests, for achieving a specific performance requirement, such as workability, mix-ability, binding and compaction resulting in longer lasting road surfacing, improved road substructure and reduction of ongoing maintenance requirements. The research is important as it outlines important processes involved for the design and development of soil stabilisation products from *Bacillus* spp. and related bio-additives for road construction application. These bio-stabiliser products are representative of whole cell bacterial suspension, fermentation components and biopolymers which aim to address challenges in road construction. At present research focusing on developing novel products from *Bacillus* spp. Ferment is limited. Therefore, the purpose of this literature research was to obtain and evaluate the potential use of fermentation material such as active whole cell, biopolymers and 'sticking' agents produced by microbes in the soil stabilisation processes. A summary of key focus areas for development of bio-stabiliser product research:

- Development of medium- to high-throughput screening to assess key structural characteristics of biological components/fractions and hence to select the top performers (Ramdas et al. 2020).
- Large scale demonstration of functional components and verified selection of candidate fractions.
- Showing the mode of action (i.e. structural criterion including: resistance to abrasion, erosion, water absorption and compression load) of the candidate prototypes.
- Testing of top prototypes in real world road subsurface studies. Laboratory simulated tests are to be used to demonstrate performance, alternatively the use of the Heavy Vehicle Simulator is recommended.

Table 5 Microbial bio-treatment types related to construction-biotechnological processes

	Admixture	**Biochemical origin**	**Mechanism**	**Descriptive features**
Whole cell bacteria & spores	Whole cell bacterial slurry; Dry biomass or liquid formulation.	*Bacillus* spp. e.g. *B. flexus*, *B. pasteurii*, *B. licheniformis*, *B. subtilis*, *B. cereus.*	Process of metabolically mediated calcium carbonate precipitation; bioclogging and biocementation process.	Microbial pore filling material, termed 'Biocement', water entering freshly formed concrete cracks that will activate the bacteria which in turn will seal micro-cracks.
Bacterial cell walls	Bound extracellular substances (polysaccharides & proteins) to cells walls.	Aerobic cultivation of *Bacillus* spp. such as *B. subtilis.*	The cell wall of the Gram-positive *B. subtilis* contains peptidoglycan as the major component, similar chemical structure to polymers commonly used in construction.	Microstructural filler for concrete: increasing compressive strength of construction materials (i.e. concrete) & decreasing its porosity; reduce segregation & bleeding of materials.
Bioenzymes	Immobilised/liquid formulation.	*Bacillus* spp. hydrolytic enzymes e.g. lipases, ureases, proteases, phosphatases and phytases	Involves cationic exchange capacity (CEC), a clay-water effect & an interaction of enzymes; the electrical double layer of clay particles neutralises the charge of the particle & breaks down the clay lattice causing pore-water dissipation.	Reduction in the compaction effort necessary to reach target soil densities & improves resistance to freeze-thaw cycling. The enzyme regeneration property is an important consideration for long term stabilisation of soil (increasing strength over time).
Extracellular polymeric substances (EPS)	Mixture of polysaccharides, amino sugars, proteins, teichoic & nucleic acids.	*B. pumus*, *B. megaterium*, *B. cereus*, *B. flexus*, *B. subtilis*, *B. licheniformis*, *B. subtilis*, & *B. polymyxa*	Polyelectrolytic EPS contain various ionisable functionalities: carboxyl, phosphoryl, amino & hydroxyl groups, they may modify the charge & hydrophilicity of the mineral surfaces, thereby conditioning surfaces for bacterial attachment, colonisation & biofilm formation.	The use of bacterial EPS may result in flocculation & agglomeration, also as hydrophobic agents to act as water erosion repellents. Due to their interplay of effects direct use of microbial components/EPS (enzymatic, polymeric, protein) may improve road construction materials.

3. LIMITATIONS IDENTIFIED IN CURRENT LITERATURE

Numerous experimental and demonstration formats have been constructed for performance testing by companies using their own additives. However, very little has been reported, especially with regards to cost and benefits. This has led to suppliers of road additives not being able to provide sufficient information to road authorities and engineers to make a decision on the appropriate use of these products, which would instead use conventional stabilisers rather than implementing a more expensive design (Jones and Ventura 2004).

The most widely available liquid stabilisers are enzyme based, usually verified by laboratory performance testing (Boutique et al. 2007). For example Taha et al. (2013), Rajoria and Kaur (2014) describes the improvement in the engineering properties of the treated soils using enzyme based products. Field tests reported by Velasquez et al. (2006) show good performance of enzyme treated soils and Van Veelen and Visser (2007) have also reported significant improvement in CBR values of enzyme-treated material in field test panels. Whereas, testing by Mgangira (2009a) show variation in performance in the use of enzyme products for several soil types. The inference from these studies shows a clear lack of significant, consistent improvements in the structural properties of treated material. Several of these studies also do not cover the microstructural details that may be responsible for the mechanisms that contribute to the interaction between the product and the soil particles, and the accompanying bio-geotechnical engineering properties. As previously outlined, the characteristics of the treated materials at a micro-scale can lead to an understanding of the expected performance of the materials at the macro-scale. It also infers the potential of modifying the products in order to provide significant improvements in the stability of treated soils (Mgangira and Ndibewu 2010) as well as select the correct stabiliser for the intended purpose in a consistent manner.

A more controlled testing on the performance of bio-stabilisers is needed for unpaved road application, prior to *in situ* field testing. The initial testing programme should focus on demonstrating the effectiveness of the various microbial derived bio-additives in terms of bonding performance at small scale. This test design should inform process development of standard large scale product prototype production. The mechanical properties of biocemented sand should be understood before large scale applications, as the stabilising mechanisms of these products are limited in application especially in predicting their long-term performance and maintenance. Thus, investigating various environmental factors prior to field applications which affect the long-term durability and biodegradability of a product, such as investigating ground conditions with rapid fluid flow is important. The current biological approach requires specific parameters to be addressed for acceptable performance of bio-based additives. These include, ideal temperature, pH, concentration, electron donor, electron acceptors, the rate of nutrients (Ca^{2+}), and metabolites. A study showed the *in situ* microbial biopolymer formation appears to be less effective in enhancing the erosion resistance, compared to other biochemical agents, because the quantity of biopolymer produced by bacteria is fairly small (less than 2% pore saturation). Ham et al. (2018) suggests re-designing this bio-stimulation strategy.

This review on biological based additives for structural applications identifies the significance of an entirely new value chain that is being established. This presents a challenge for previously unassimilated industries like the construction industry. It is evident today that several joint ventures have been formed between biotechnology, geochemistry and civil engineering; this integration will leverage potential synergies. The research strategy on bio-stabilisers is imperative to ensure that bio-stabilised materials for the future construction and maintenance of unpaved roads meet acceptable level of service for all road users, within the limited budget available to road authorities, while protecting the environment in terms of level of dust generation and gravel loss.

4. RECOMMENDATIONS FOR FUTURE STUDIES

In this paper, several microbes and microbial consortia involved in the study of bio-based stabiliser techniques as well as, alternative manufacturing methods for *in situ* soil stabilisation are presented. *Bacillus* spp. production of various components, in the fermentation and dual functioning, makes it a superior candidate for an investigation into its structural properties. This indicates viable alternatives to chemical or traditional additives for many current uses of earthen construction materials. However, more supporting data is required to demonstrate the superiority, even though the theoretical argument through mechanism description/definition previously stated clearly demonstrates this.

The development of bio-stabiliser soil additives/products for the construction industry requires a systematic approach in the design of the experiments. These techniques include targeted isolation of micro-organisms, followed by medium- to high-throughput screening to a set of pre-defined strength criteria that is associated with commercially relevant desirable characteristics. The use of large scale *ex situ* tests to select stabilising additives from large numbers of isolates are expensive, time consuming, material limiting and not easily achievable. It is thus critical to perform extensive *in-vitro* evaluation and selection processes to reduce the number of isolates, biological fractions, and material costs. This pre-assessment in conjunction with large scale strength tests (i.e., brush test, erosion test, and trial compression testing) has basis and will provide an overall indication of the bio-stabiliser/s strength characteristics as previously proposed in Rauch et al. (2002). A step-wise process is provided for potentially establishing new bio-based stabilisers for road application:

- Development of a Bio-stabiliser for application on selected *in situ* materials.
- Study of the effect of Bio-stabilisers on soil microstructure.

- Elucidation of Bio-stabiliser-soil interaction mechanisms.
- Establish the application of Bio-stabiliser in road construction under South African conditions in a field trial.
- Compare the technology – technical, economical and environmental impact against other commercial benchmark products.

5. OTHER ENVISIONED APPLICATIONS – ENVIRONMENTAL

World-wide there's a strong drive to facilitate environmentally sustainable– low-carbon economy. The need is to identify ways that contribute to this transition. Finding value in solid waste in the preparation of soil based construction material is one of the several technically feasible and cost-effective solutions for waste management. In South Africa, 54.425 tonnes of waste is generated daily with this amount being the 15th highest world-wide. It is not surprising that South Africa's landfill areas are rapidly running out of space (Chvatal and Smit 2015). The development a novel bio-based technology includes exploring alternative applications for bio-based products. One such application is the potential for improvement of the mechanical properties of weak soils with the addition of by-products and waste material of industrial origin such as fly ash.

Numerous waste materials can be turned into valuable products and this is a proposed step in the right direction, as these techniques optimise the use of local materials. The utilisation of high energy waste material such as ash, plastic, paper or agricultural waste (i.e. sugarcane bagasse) as a binding agent can address the numerous challenges faced in soil stabilisation, by focusing on enhancing the particular physical and mechanical properties of a soil (i.e. improved resistance to erosion or abrasion, hydraulic conductivity, and durability against repeating wetting and drying, as well as for environmental revitalisation). Knowledge on the needs in the construction industry, and the symbiotic relationship between research and product development is paramount in creating these new opportunities for waste utilisation. The challenge is to find low cost but high-volume applications, and to convert waste into value-added products.

5.1 Industrial wastes as proposed auxiliary additives for *in situ* soil stabilisation

- **Ash waste**

Bottom ash is collected from the boilers (i.e. a coarse, granular, incombustible by-product of coal combustion), and fly ash is obtained from the gas washers (i.e. pulverised coal burned in the bottom of boilers, most of these unburned material is caught in the flue gas) (Le et al. 2018). Ash is currently a major problem, approximately 46 million tonnes of fly ash is produced globally per annum (Gomes et al. 2020). Bottom ash is composed of both organic and inorganic fractions, whereas fly ash consists of more organic content. The heavy metals within fly ash are not biodegradable; they can only be transferred from one chemical state to another, which changes their mobility and toxicity. The technical utilisation of ash has potential for more advanced uses (i.e. deep soil application), however gaps exist in terms of toxicity, and leaching into the environment (Kumpiene et al. 2008). One proposed solution to counteract this is the use of micro-organisms. Micro-organisms can influence metals in several ways, (a) some forms of metals can be transformed either by a redox reaction or by alkylation; (b) metals can also be accumulated by micro-organisms by metabolism-independent (passive) or by intracellular metabolism-dependent (active) uptake; (c) micro-organisms can influence metal mobility indirectly by affecting pH; and (d) by producing or releasing compounds which change mobility of the metals (Igiri et al. 2018). The proposed bio-molecules (i.e. EPS) derived from *Bacillus* spp. and/or in synergy with other green composites (**Fig. 4**) are postulated as potential carriers/stabilising agents of ash waste.

- **Sugarcane bagasse**

Sugar cane bagasse is a solid waste with economic importance, produced as a by-product from the sugar manufacturing industry in enormous quantities (South African sugar mills produce 24.7 million tonnes of sugarcane annually, each 10 tonnes of sugarcane crushed produces 3 tonnes of bagasse) (Muniyasamy et al. 2016). Bagasse is the fibrous residue remaining after extraction of the cane juice from sugarcane. Sugarcane bagasse consists of approximately 50% cellulose, 25% hemicellulose, and 25% lignin. Whereas, sugarcane bagasse ash consists of silica rich material that can play the role of an effective pozzolans leading to enhanced pozzolanic reactions resulting in better performing building materials (James and Pandian 2017). The economic importance of these solid waste materials has been realised with several applications such as, adsorbents, filters, ceramics, briquettes, bricks, and soil modifiers. In addition, the use of bioplastics (i.e. PE), a recent development from bagasse and maize stalks may have a future role in road construction due to their permeability properties, remediation of soil and high tensile strength (Muniyasamy et al. 2016). With regards to the biodegradable aspect of bio-based materials, the tests are complex and subject to the environment in which the testing is completed, however standards are available, (i.e., American Society for Testing and Materials (ASTM) and International Organization for Standardization (ISO)) to adopt, and test green composite materials in soil environments, to manage their end-life cycle. It is notable for construction purposes, that not all bioplastics are biodegradable but instead recyclable (see **Fig. 5**) (Muniyasamy et al. 2013).

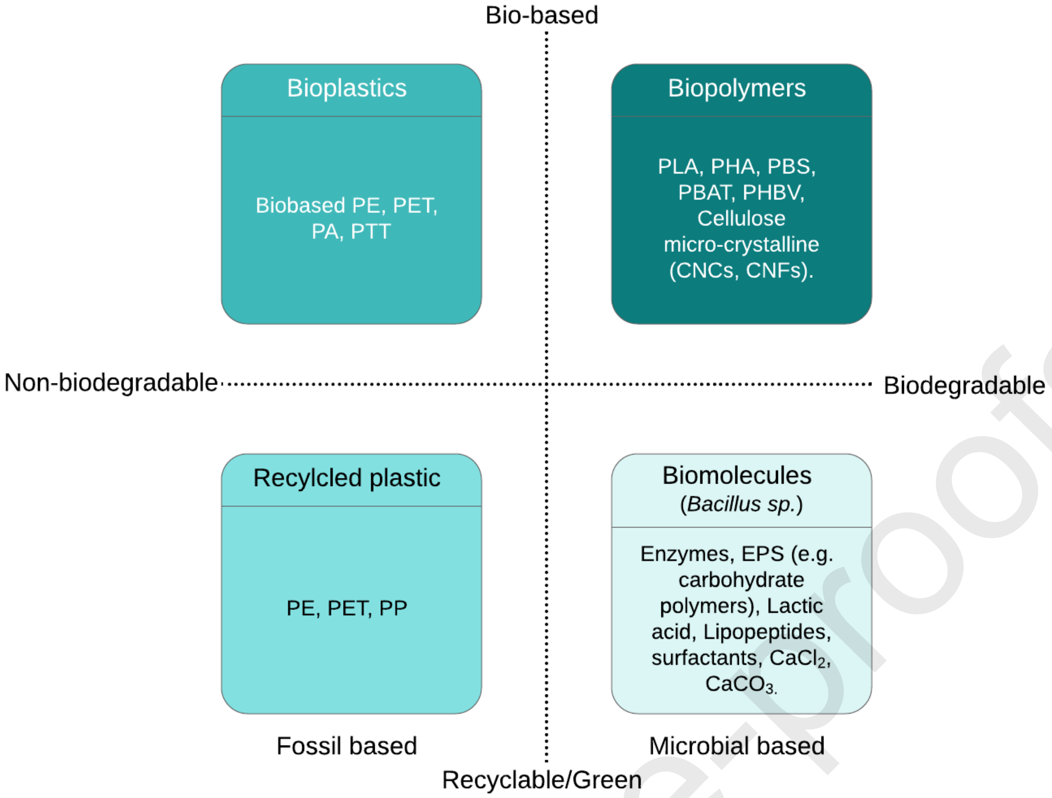

Fig. 5 Classes of bio-materials and alternative waste proposed for the improvement of structural properties

Key: *PE – Polyethylene, PET – Polyethylene terephthalate, PA – Polyamide, PTT – Polytrimethylene terephthalate, PLA – Polylactic Acid, PHA – Polyhydroxyalkanoate, PBS – Polybutylene succinate, PBAT – Polybutylene adipate terephthalate, PHBV – Poly(3-hydroxybutyrate-co-3-hydroxyvalerate), CNCs – Cellulose Nano crystals, CNFs – Cellulose Nano fibres, PP – Polypropylene, EPS – Extracellular polymeric substances*

- **Lignin-based waste**

Lignin is a by-product of paper and timber industry. Similarly, wood is composed of cellulose, hemicellulose, lignin, and extractives, while lignin is resistant to biodegradation in an anaerobic environment and can remain in a landfill for exceedingly long periods of time (Ramage et al. 2017). Lignin is the most abundant organic polymer in existence on earth after cellulose. Sustainable reuse options for lignin in civil engineering applications, such as road embankments and dam foundations or use of lignin as a stabilisation additive for soil may be viable options for the reuse of this bio-based organic by-product (Zhang et al. 2017). Lignosulfonate (LS) (i.e. industrial by-product) is also being investigated as a soil stabiliser (Ta'negonbadi and Noorzad 2017). In this research by Ta'negonbadi and Noorzad (2017), improved soil properties was induced by a polymer with lignin-base that included hydrophilic groups, comprising of alcoholic hydroxyl sulfonate, phenyl hydroxyl, and hydrophobic groups consisting of carbon chains. The stabilisation effect of the LS-treatment leads to reduction in Plasticity Index (PI) of the soil, improved stiffness and UCS of the soil without any brittle nature. The increase in strength properties is attributed to the electrostatic reaction that occurs between the LS-water mixture and soil particles resulting in the PI changes which could be due to the alteration of the lattices of the soil minerals (i.e., decrease in crystalline size of the double layer thickness of clay minerals) and better flocculation properties of the soil (i.e., formation of stable soil aggregation), this in effect improves the plasticity and the stabilised soil strength. Since LS is a by-product of other processes (i.e., produced annually in large quantities (50 million tonnes) worldwide), they are relatively cheap and can be competitive with other chemical stabilisers (Zhang et al. 2017).

6. CONCLUSION

In summary, the construction industry is developing new opportunities involving the use of biological processes and products to modify the structural properties of subpar soils (i.e., strength, volume stability, durability, and permeability) through innovative engineering techniques. The bio-based stabilisation technology such as bio-stabilisers has the potential to reduce the use of conventional options such as cement and lime, whose production processes results in a higher environmental load/GHG emissions and therefore preserve the environment and natural resources. Enzymes and polymers have been long introduced into the market as soil stabilisers. However, the application of these imported eco-friendly products to improve the engineering properties of weak soil is not readily adopted in countries such as, South Africa. Furthermore, novel biopolymers or ‘sticking agents’ of microbial origin are currently not manufactured in South Africa. In addition, these complexities and limitations can be circumvented by recognising that *Bacillus* spp. represents unique and exclusive assemblages of diversity. The development of a bio-stabiliser product from an indigenous strain (*Bacillus* spp.) could therefore address this gap. Microorganisms and their products such as secondary metabolites, enzymes, endospores, and extracellular polymeric substances have been considered as interesting alternatives to conventional chemical soil stabilisers for the development of sustainably road infrastructure. The primary challenges with this bio-based approach is microorganism selection and bioprocess optimisation to obtain maximum yields of the desired product for testing and use. The extracellular components, consisting of EPS, enzymes, and biopolymers, are hypothesised to create a process of cross-linking with various soil particles via various interactions. This in essence, has the potential of improving the strength properties by improving shear strength and compressibility of soils. It is therefore essential that the active ingredient contained in the product is produced at a high concentration during fermentation for an efficacious product. Adoption of the bio-stabiliser technology has the potential to improve road infrastructure delivery and therefore contribute toward socio-economic development. By using bio-stabilisers, *in situ* waste utilisation, such as fly ash, may be possible in construction, which reduces waste to landfill. The application of the proposed technologies and products are envisioned to improve strength properties of *in situ* materials and reduce permeability characteristics. This may result in improved subgrade quality and marginal materials for potential application in structural pavement layers and for wearing coarse on unpaved roads. The stabilised soil may also be suitably used for constructing pavement layers of low volume, rural (unpaved) roads, bicycle tracks, and pedestrian walkways. Of interest are the other areas of opportunity beyond normal soil stabilisation such as, compressed earth blocks (CEB blocks), promoting low technology manufacturing from waste material and soil. This will help in ensuring effective implementation and proper use of natural resources as well as, bring about transparency against the intrusion of reinforcing physical processes or the use of hazardous chemicals in soil to create artificial binding. Therefore, in view of the enormous challenges and rising demand for sustainable alternative solutions, novel biological interventions (i.e. bioenzymatic, and biopolymer soil stabilisation) is the logical way forward to improve conventional soil stabilisation methods.

7. COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: The authors declare that they have no conflict of interest.

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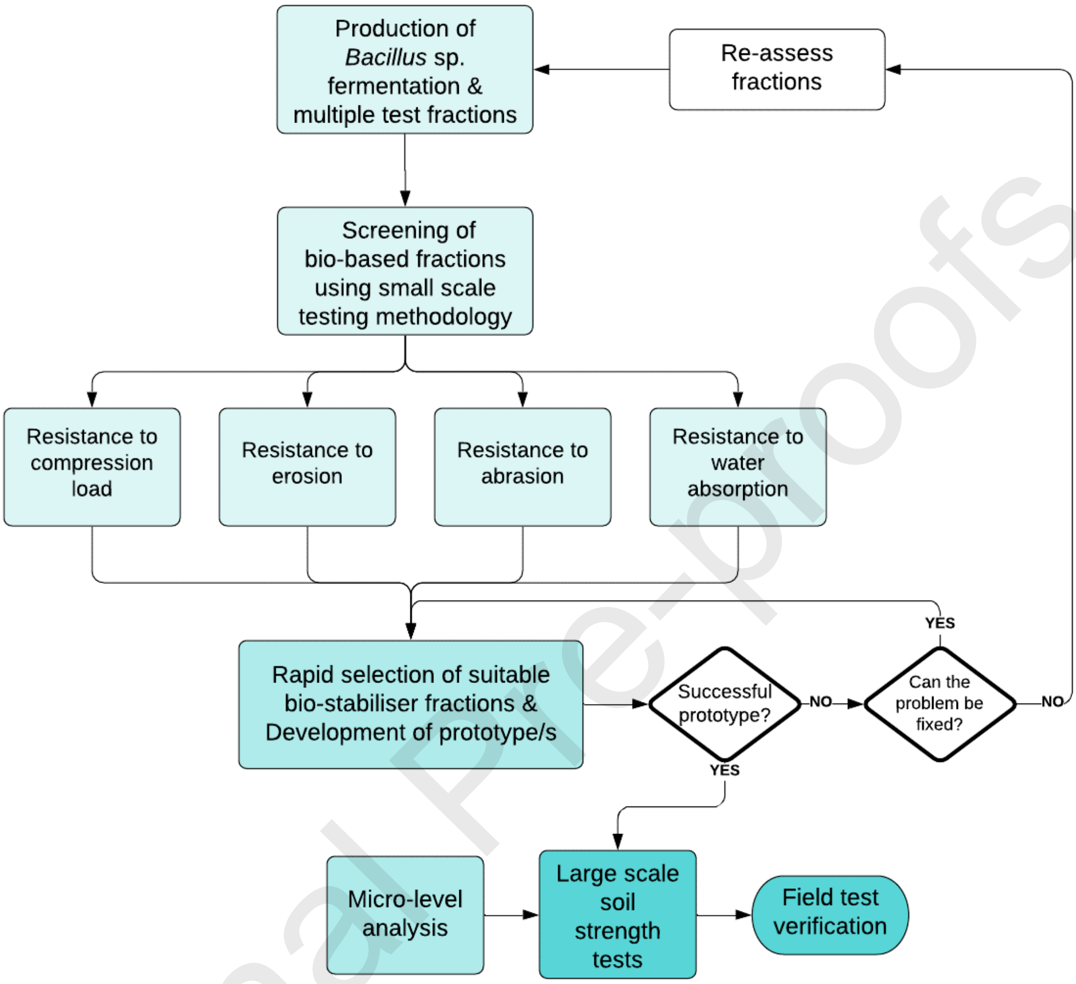
Graphical abstract



RESEARCH HIGHLIGHTS

- Investigation of novel biological components for road construction application.
- Large scale demonstration of functional components of best bio-based additives.
- Macro-structural criterion includes resistance to: abrasion, erosion, water absorption & compression stress.
- Evaluation of the mode of action of bio-materials using micro-structural techniques.
- Industrial wastes as auxiliary additives for *in situ* soil stabilisation.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: