

MICROBially INDUCED GELATION FOR REDUCTION OF PERMEABILITY OF GRANULAR MATERIALS

Field of Invention

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The current invention relates to a gelation method for producing a bio-gel material that can prevent or reduce the seepage of water through a granular material, such as sand and/or soil (e.g. sandy soil).

10 Background

The listing or discussion of a prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

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Seepage control in sand is a critical issue in geotechnical engineering. A higher seepage rate would cause geo-hazards like piping and seepage erosion. The traditional countermeasures for seepage include installation of cut-off walls, and injection of chemicals such as cement and polymers into the pore space of sand to slow down the water flow. However, these methods have shortcomings. Firstly, chemical grouting for seepage control is usually expensive as compared to other methods. Secondly, in some cases, the use of chemical grouting may change the pH of the soil. Lastly, cement grout has a high viscosity and thus requires a high pressure to inject. Some bioclogging methods using microbially induced calcium precipitation (MICP) processes have also been developed (L. Van Paassen *et al.*, Scale up of BioGrout: a biological ground reinforcement method. In *Proceedings of the 17th international conference on soil mechanics and geotechnical engineering*. Lansdale IOS Press, **2009**, 2328-2333). However, this approach generates ammonia which may not be tolerable depending on the nature of the projects. Another biological approach is to use biofilms or extracellular polymeric substance (EPS, P. Baveye *et al.*, *Crit. Rev. Environ. Sci. Technol.* **1998**, 28, 123-191). However, in this method, it may take a couple of weeks or even months for the microorganism to produce enough biomass to significantly reduce the permeability of sands (H. J. Dupin & P. L. Mccarty, *Environ. Sci. Technol.* **1999**, 33, 1230-1236; K. Seki, T. Suko & T. Miyazaki, Bioclogging of glass beads by bacteria and fungi. In *Trans. 580 World Congr. Soil Sci. Symposium*. **2002**, 1244-1-1244-8; and D. Seifert & P. Engesgaard, *J. Contam. Hydrol.* **2007**, 35 93, 58-71).

An alternative way is to directly inject low concentrations of polysaccharides (i.e., alginic acid, alginate, xanthan, and gum karaya) into the ground to form solid polymeric gels with soluble metal ions in the pore space (K. Y. Lee & D. J. Mooney, *Prog. Polym. Sci.* **2012**, 37, 106-126; and K. Wen *et al.*, *Constr. Build. Mater.* **2019**, 207, 174-180). However, the formation of the hydrogel can take place instantly once the polysaccharide contacts the metal ion and thus, it is difficult to distribute the gelling materials in soil homogeneously. Hence, no satisfactory methods have been proposed so far to effectively reduce the permeability of sandy soil at a low unit cost.

Therefore, there exists a need to discover new methods to effectively reduce the permeability of granular materials.

Summary of Invention

Aspects and embodiments of the invention will now be described by reference to the following numbered clauses.

1. A process of forming an organic barrier layer in a porous material in need thereof, the process comprising the steps of:

(a) providing a porous material in need thereof and a bio-gelation mixture, the bio-gelation mixture comprising:

water;

a polysaccharide suitable for crosslinking;

glucose; and

a bacterial population suitable for converting glucose to a carboxylic acid; and

(b) injecting the bio-gelation mixture into; or

spraying the bio-gelation mixture onto a surface of

the porous material to form an organic barrier layer comprising the polysaccharide crosslinked by a plurality of calcium ions, wherein the plurality of calcium ions is obtained from reaction of the carboxylic acid with a calcium ion source, where the calcium ion source is present in the porous material and/or is added as an additional component within the bio-gelation mixture.

2. The process according to Clause 1, wherein the bio-gelation mixture comprises:

water;

a polysaccharide in a concentration of from 1 to 10 g/L;

glucose in an amount of from 0.5 to 5 g/L;

a bacterial population suitable for converting glucose to a carboxylic acid which is provided as a bacterial suspension having a bacterial density (OD_{600}) of from 0.15 to 0.95 in an amount of from 1 to 50 mL/L; and

a calcium ion source provided in an amount of from 0 wt%, or in an amount sufficient to provide from 0.5 to greater than 1 wt% in a volume of the porous material that is to form part of the organic barrier layer.

3. The process according to Clause 2, wherein the bio-gelation mixture comprises:
water;

a polysaccharide in a concentration of from 2 to 4 g/L, such as about 3 g/L;

glucose in an amount of from 1 to 3 g/L, such as about 2 g/L;

a bacterial population suitable for converting glucose to a carboxylic acid which is provided as a bacterial suspension having a bacterial density (OD_{600}) of from 0.3 to 0.8 (e.g. about 0.60) in an amount of from 5 to 30 mL/L, such as about 15 mL/L; and

a calcium ion source provided in an amount of from 0 wt% to an amount sufficient to provide from 0.8 to 1.8 wt% in a volume of the porous material that is to form part of the organic barrier layer.

4. The process according to any one of the preceding clauses, wherein the porous material in need thereof is one or more of the group consisting of a sand, and a soil, optionally wherein the porous material in need thereof is a sand (e.g. Ottawa sand and/or Coral sand).

5. The process according to any one of the preceding clauses, wherein the porous material in need thereof comprises from 0.5 to 1.8 wt%, such as from 0.8 to 1.5 wt%, such as from 0.86 to 1 wt% of a calcium ion source.

6. The process according to any one of the preceding clauses, wherein the calcium ion source is $CaCO_3$, optionally wherein, when the $CaCO_3$ forms part of the bio-gelation mixture, it has a diameter of less than or equal to 50 μm .

7. The process according to any one of the preceding clauses, wherein the polysaccharide suitable for crosslinking is an alginate, optionally wherein the alginate is sodium alginate.

8. The process according to any one of the preceding claims, wherein the bacterial population suitable for converting glucose to a carboxylic acid is derived from activated sludge.

9. The process according to Clause 8, wherein the activated sludge is incubated in an aqueous medium comprising yeast and KH_2PO_4 at pH 7 for about 24 hours and is then centrifuged to provide a bio-mass pellet for resuspension and formation of the bacterial population.

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10. The process according to any one of the preceding clauses, wherein the bio-gelation mixture is used immediately after its formation, where the bio-gelation mixture is formed by mixing the polysaccharide suitable for crosslinking, glucose, the bacterial population suitable for converting glucose to a carboxylic acid and, when present, the calcium ion source in water.

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11. The process according to any one of Clauses 1 to 9, wherein the bio-gelation mixture is used after a period of from 1 to 24 hours after its formation, where the bio-gelation mixture is formed by mixing the polysaccharide suitable for crosslinking, glucose, the bacterial population suitable for converting glucose to a carboxylic acid and, when present, the calcium ion source in water.

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12. The process according to any one of the preceding clauses, wherein in step (b) of Claim 1, the formation of the organic barrier layer occurs over a period of from 1 hour to 4 days, such as from 2 hours to 2 days, such as from 3 hours to 38 hours.

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13. The process according to any one of the preceding clauses, wherein in step (b) of Claim 1, the porous material in need thereof has a temperature of from 0 to 60 °C, such as from 5 to 50 °C, such as from 10 to 40 °C, such as from 15 to 37 °C, such as from 20 to 25 °C.

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14. The process according to any one of the preceding clauses, wherein the permeability of the organic barrier layer is in the order of from 1×10^{-8} to 1×10^{-5} m/s, such as from 1×10^{-7} to 1×10^{-6} m/s.

15. The process according to any one of the preceding clauses for mitigating wave erosion of a beach or a shoreline, the process involving:

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(i) spraying the bio-gelation mixture onto a surface of a porous material in need thereof to form the organic barrier layer;

(ii) placing a porous material on top of the formed organic barrier layer; and

(iii) subjecting the porous material placed on top of the formed organic barrier layer

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to microbially-induced calcium carbonate precipitation to form an inorganic barrier layer on top of the organic barrier layer.

16. The process according to any one of clauses 1 to 14 for mitigating wave erosion of a beach or a shoreline, the process involving:

(ia) injecting the bio-gelation mixture into a porous material in need thereof at a defined depth to form the organic barrier layer, with a portion of the porous material undisturbed on top of the formed organic barrier layer; and

(iia) subjecting the undisturbed porous material on top of the formed organic barrier layer to microbially-induced calcium carbonate precipitation to form an inorganic barrier layer on top of the organic barrier layer.

17. The process according to any one of the preceding claims, wherein the process does not involve a step of microbially-induced calcium carbonate precipitation process before or during steps (a) and (b) of Claim 1.

Drawings

FIG. 1 depicts (a) set-up for the laboratory test; and (b) set-up for the field implementation.

FIG. 2 depicts the microscopic view of the bio-gelation process.

FIG. 3 depicts the particle size distribution curve of the sand used in this study.

FIG. 4 depicts the variation of the dynamic viscosity of the bio-gelation medium and the permeability of sand at different alginate concentrations under a constant hydraulic gradient of 0.1.

FIG. 5 depicts the laboratory testing system. 1: Water tank; 2: Load cell; 3: Soil sample; 4: Load Frame; 5: Motorized actuator; 6: Data logger; 7: Pneumatic Controller; 8: Air compressor; 9: GDS controller (for permeability test); 10: GDS controller (back pressure control); 11: Control computer; 12: Extension cap; and 13: Bender element.

FIG. 6 depicts the isotropic consolidation curve of hydrogel compared with loose Ottawa sand treated with or without bio-gelation method.

FIG. 7 depicts the change of the shear wave velocity of hydrogel during isotropic consolidation test versus loose Ottawa sand.

FIG. 8 depicts the undrained stress-strain behaviour of hydrogel at an effective confining stress of 100 kPa.

FIG. 9 depicts the relationship between the volume percentage of hydrogel in the pore space and Skempton's coefficient (B-value).

FIG. 10 depicts the change in the coefficient of permeability during the incubation period in Test PT06 (with 3 g/L alginate concentration).

FIG. 11 depicts the change in the coefficient of permeability with different alginate concentration.

FIG. 12 depicts the change in the coefficient of permeability in the seepage tests under different hydraulic gradient.

FIG. 13 depicts the set-up of the quicksand model test.

FIG. 14 depicts the change in the permeability with applied hydraulic gradient in the bio-gelled medium loose sand samples.

FIG. 15 depicts the measured critical hydraulic gradient in bio-gelled sand samples.

FIG. 16 depicts the coefficient of permeability of bio-gelled sand measured at a hydraulic gradient of 0.25.

FIG. 17 depicts the change in the pH value in the sands with 1% calcium content during the bio-gelation period.

FIG. 18 depicts the bio-gel barrier for the prevention of seawater erosion.

FIG. 19 depicts the bio-gel barrier for the deep excavation project as waterproofing curtain.

FIG. 20 depicts the bio-gel barrier for preventing seepage erosion in earth dams.

Description

It has been surprisingly found that some or all of the problems identified above may be solved through the use of a method that can make use of a calcium ion source present within the sand and/or soil to be treated, which method is also amenable to supplementing or supplying the calcium ion source if this is required by the soil/sand being treated. This process surprisingly enables the gelation reaction to be controlled, allowing for the formation of an organic barrier layer in a manner not previously possible. In this process, the injection/spraying of a polysaccharide solution is followed by an *in-situ* microbial acidification process which gradually dissolves a metal compound to release the metal ion required for the formation of hydrogel (i.e., it dissolves a calcium ion source to release calcium ions). The major advantage of this bio-gelation process over other chemical gelation methods is the ability to allow the polysaccharide solution to be well distributed before the hydrogel is formed in the pores of soil/sand. Thus, there is great potential to use this bio-gelation method for seepage control in soil, sand, and other porous materials. Moreover, through injection (or spraying) instead of grouting, the disturbance to the soil by the bio-gelation process disclosed herein is minimised to the extent that it can be essentially ignored as a factor.

Thus, in a first aspect of the invention, there is provided a process of forming an organic barrier layer in a porous material in need thereof, the process comprising the steps of:

(a) providing a porous material in need thereof and a bio-gelation mixture, the bio-gelation mixture comprising:

water;

a polysaccharide suitable for crosslinking;

glucose; and

a bacterial population suitable for converting glucose to a carboxylic acid; and

(b) injecting the bio-gelation mixture into; or

spraying the bio-gelation mixture onto a surface of the porous material to form an organic barrier layer comprising the polysaccharide crosslinked by a plurality of calcium ions, wherein the plurality of calcium ions are obtained from reaction of the carboxylic acid with a calcium ion source, where the calcium ion source is present in the porous material and/or is added as an additional component within the bio-gelation mixture.

In embodiments herein, the word “comprising” may be interpreted as requiring the features mentioned, but not limiting the presence of other features. Alternatively, the word “comprising” may also relate to the situation where only the components/features listed are intended to be present (e.g., the word “comprising” may be replaced by the phrases “consists of” or “consists

essentially of"). It is explicitly contemplated that both the broader and narrower interpretations can be applied to all aspects and embodiments of the present invention. In other words, the word "comprising" and synonyms thereof may be replaced by the phrase "consisting of" or the phrase "consists essentially of" or synonyms thereof and *vice versa*.

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The phrase, "consists essentially of" and its pseudonyms may be interpreted herein to refer to a material where minor impurities may be present. For example, the material may be greater than or equal to 90% pure, such as greater than 95% pure, such as greater than 97% pure, such as greater than 99% pure, such as greater than 99.9% pure, such as greater than 99.99% pure, such as greater than 99.999% pure, such as 100% pure.

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As will be appreciated, the organic barrier layer is formed from at least two components. That is, it may include:

- crosslinkable polysaccharides crosslinked together by calcium ions; and
- the porous material.

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As will be appreciated, the crosslinkable polysaccharides crosslinked together by calcium ions form around the pre-existing porous material particles, ensuring that the organic barrier layer is a mixture of these materials.

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When used herein, the term "calcium ion source" refers to any material that is readily digestible by an acid, and in particular, a carboxylic acid and which can then release calcium ions for the formation of crosslinks between polysaccharide molecules that are suitable for crosslinking. Examples of suitable calcium ion sources include, but are not limited to CaHCO_3 and, more particularly, CaCO_3 , and combinations thereof. The calcium ion source may be inherently present in the porous material or it may be introduced into the porous material as part of the bio-gelation mixture. In addition, it may be advantageous in some circumstances to add a calcium ion source to the bio-gelation mixture even if the porous material inherently incorporates a calcium ion source. This may be because the amount of the calcium ion source inherently present in the porous material is not present in an amount sufficient to enable formation of the organic barrier layer. For example, a supplementary calcium ion source may be used when the amount of the calcium ion source present in the porous material is less than 0.5 wt% in a volume of the porous material that is to form part of the organic barrier layer. Of course, it may be desired to include supplementary calcium ion source, even if there is a sufficient amount of the calcium ion source in the porous material (e.g., to improve the durability of the organic barrier layer or to improve the mechanical properties of the organic barrier layer).

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In particular embodiments that may be mentioned herein, the calcium ion source may be CaCO_3 . As will be appreciated, CaCO_3 is minimally soluble in water (0.013 g/L at 25 °C). While this is not an issue when the porous material includes CaCO_3 in an amount sufficient to form the desired organic barrier layer without supplementation, this fact needs to be accounted for when CaCO_3 is to be used as a supplemental calcium ion source within the bio-gelation mixture. As such, in embodiments where the calcium ion source is CaCO_3 and is provided as part of the bio-gelation mixture, it may be provided in the form of particles that remain suspended within the bio-gelation mixture. For example, when the calcium ion source is CaCO_3 and is provided as part of the bio-gelation mixture, it may be provided in the form of particles that have a diameter that is less than or equal to 50 μm . As will be appreciated, CaHCO_3 is relatively soluble in water and so it may be provided in any suitable form.

When used herein, the term “polysaccharide suitable for crosslinking” refers to a polysaccharide that can be crosslinked by a calcium ion. Therefore, it generally refers to a polysaccharide that comprises functional groups that are capable of being (or is provided with functional groups) in an anionic form, such as carboxylic acid groups or carboxylate groups, and the like. For example, the polysaccharide suitable for crosslinking may be an alginate. More particularly, the polysaccharide suitable for crosslinking may be sodium alginate.

When used herein, the term “porous material” refers to any suitable material that can support the formation of an organic barrier layer. Examples of suitable porous materials include, but are not limited to a sand, a soil, and combinations thereof. In particular embodiments that may be mentioned herein, the porous material may be a sand. For example, the porous material may be Ottawa sand and/or Coral sand.

In certain embodiments, the porous material may be one that does not contain any or contains an insufficient amount of a calcium ion source. When used herein, “an insufficient amount of a calcium ion source” may refer to a porous material that has less than or equal to 0.5 wt% of a calcium ion source in its make-up, such as less than or equal to 0.8 wt% of a calcium ion source in its make-up, such as less than or equal to 0.86 wt% of a calcium ion source in its make-up, such as less than or equal to 1 wt% of a calcium ion source in its make-up. In other embodiments, the porous material may be one that contains a sufficient amount of a calcium ion source. For example, the porous material may have from 0.5 to 1.8 wt%, such as from 0.8 to 1.5 wt%, such as 0.86 to 1 wt% of a calcium ion source.

For the avoidance of doubt, it is explicitly contemplated that where a number of numerical ranges related to the same feature are cited herein, the end points for each range are intended

to be combined in any order to provide further contemplated (and implicitly disclosed) ranges. Thus, the ranges cited above are explicitly intended to cover:

from 0.5 to 0.8 wt%, from 0.5 to 0.86 wt%, from 0.5 to 1 wt%, from 0.5 to 1.5 wt%, from 0.5 to 1.8 wt%;

- 5 from 0.8 to 0.86 wt%, from 0.8 to 1 wt%, from 0.8 to 1.5 wt%, from 0.8 to 1.8 wt%;
from 0.86 to 1 wt%, from 0.86 to 1.5 wt%, from 0.86 to 1.8 wt%;
from 1 to 1.5 wt%, from 1 to 1.8 wt%; and
from 1.5 to 1.8 wt% of a calcium ion source.

- 10 As noted above, the bio-gelation mixture requires the presence of a bacterial population that can convert a suitable sugar (e.g. glucose) to a carboxylic acid (e.g. gluconic acid, saccharic acid or, more particularly, pyruvic acid). Any suitable bacterial population source that can conduct this conversion may be used. For example, one source for the bacterial population may be activated sludge. The activated sludge may be treated in any suitable way to provide
15 the bacterial population suitable for converting glucose to a carboxylic acid. For example, the activated sludge may be incubated in an aqueous medium comprising yeast and KH_2PO_4 at pH 7 for about 24 hours and is then centrifuged to provide a bio-mass pellet for resuspension and formation of the bacterial population. It will be appreciated that a similar treatment can be used to obtain a suitable bacterial population from other suitable sources.

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Glucose is used as a source for the generation of carboxylic acids that digest the calcium ion source material and release calcium ions to form crosslinks with the polysaccharide material. As will be appreciated, any other suitable sugar molecule that can be subjected to metabolism to form such carboxylic acids may be used in glucose's place. Alternatively, these suitable
25 sugar molecules may be used in addition to glucose. Further, the combination of more than one (e.g. 2, 3, or 4) of such suitable sugar molecules, whether also in combination with glucose or not, is contemplated. All of the above possibilities are intended to be covered by the current application.

- 30 As noted hereinbefore, the bio-gelation mixture may be injected into the porous material or it may be sprayed onto a surface of the porous material. For the avoidance of doubt, it is contemplated that both spraying and injecting may be used in a single method in a case where one, or more particularly, more than one (e.g. two, three or four) organic barrier layers may be required. For example, in a situation where three organic barrier layers are required, with a
35 first organic barrier layer formed on the surface of the porous material, a second organic barrier layer formed at depth "A" within the porous material and a third organic barrier layer formed at depth "B" within the porous material, then spraying may be used to form the first

organic barrier layer, and injectors configured to deliver the bio-gelation mixture at depths “A” and “B” may also be used so as to form all three organic barrier layers simultaneously.

Any suitable bio-gelation mixture may be used in the invention, provided that it includes the components listed above (or suitable substitute materials). For example, the bio-gelation mixture may comprise:

water;

a polysaccharide in a concentration of from 1 to 10 g/L;

glucose in an amount of from 0.5 to 5 g/L;

a bacterial population suitable for converting glucose to a carboxylic acid which is provided as a bacterial suspension having a bacterial density (OD_{600}) of from 0.15 to 0.95 in an amount of from 1 to 50 mL/L; and

a calcium ion source provided in an amount of from 0 wt%, or in an amount sufficient to provide from 0.5 to greater than 1 wt% in a volume of the porous material that is to form part of the organic barrier layer.

As noted hereinbefore, a calcium ion source only needs to be included if the porous material to be treated has an insufficient amount of a calcium ion source inherently present in its make-up. The amount of the calcium ion source that may be added to the bio-gelation mixture may be calculated based on equation (5) below.

In more particular embodiments of the invention that may be mentioned herein, the bio-gelation mixture may comprise:

water;

a polysaccharide in a concentration of from 2 to 4 g/L, such as about 3 g/L;

glucose in an amount of from 1 to 3 g/L, such as about 2 g/L;

a bacterial population suitable for converting glucose to a carboxylic acid which is provided as a bacterial suspension having a bacterial density (OD_{600}) of from 0.3 to 0.8 (e.g. about 0.60) in an amount of from 5 to 30 mL/L, such as about 15 mL/L; and

a calcium ion source provided in an amount of from 0 wt% to an amount sufficient to provide from 0.8 to 1.8 wt% in a volume of the porous material that is to form part of the organic barrier layer.

As will be appreciated, any suitable amount of calcium ion source may be included in the bio-gelation mixture and it is contemplated that amounts over 1.8 wt% may be used if desired by the skilled person.

The bio-gelation mixture may be used (i.e. sprayed and/or injected) immediately after its formation. Alternatively, the bio-gelation mixture may be used (i.e. sprayed and/or injected) after a period of from 1 to 24 hours after its formation. The bio-gelation mixture may be formed by mixing the polysaccharide suitable for crosslinking, glucose, the bacterial population suitable for converting glucose to a carboxylic acid and, when present, the calcium ion source in water. The bio-gelation mixture is considered to be formed once a suitable homogeneous mixture of all of the components has been established. That is, all materials that are intended to be dissolved in water have dissolved and all components that are not intended to dissolve have formed a homogeneous suspension within the bio-gelation mixture.

As will be appreciated, the term “immediately” may refer to the initial use of the bio-gelation material shortly after its formation (e.g. within 50 minutes, within 30 minutes, within 20 minutes, within 10 minutes, within 5 minutes, within 1 minute etc.).

While it is possible to make use of the bio-gelation mixture immediately after its formation, it is also possible to delay the use of the bio-gelation mixture. This is because the bacterial population suitable for converting glucose to a carboxylic acid requires a period of time to conduct this conversion. As such, it is possible to wait for a period of time (such as from 1 to 24 hours) before one uses the bio-gelation mixture. As noted in the examples (see FIG. 10), the bio-gelation mixture may not start to generate sufficient amounts of the carboxylic acid that can digest the calcium ion source until a period of at least 25 hours (e.g. at least 26, 27, 28, 29 etc. hours) has passed since its formation. As such, the delay in making use of the bio-gelation mixture also applies to bio-gelation mixture that incorporates a calcium ion source too. As will be appreciated, the speed that the bacterial population works may be influenced by the ambient temperature and so the skilled person will appreciate that the amount of time that one can age the bio-gelation mixture before use will depend on the ambient conditions.

Given the ability to age the bio-gelation mixture before its application to the porous material, it may be possible to control the period of time between the application of the bio-gelation mixture to the porous material in step (b) of the process described above and the formation of the organic barrier layer. For example, the formation of the organic barrier layer may occur over a period of from 1 hour to 4 days, such as from 2 hours to 2 days, such as from 3 hours to 38 hours following the application of the bio-gelation mixture to the porous material in step (b) of the process described above.

As the process described herein may be used outside, it will be appreciated that the porous material will have a temperature that is influenced by the ambient temperature of the

environment. For example, the temperature of sand during the day in a hot country (e.g. Dubai) may be significantly higher than the ambient temperature of the air. As such, the temperature of the porous material may be from 0 to 60 °C, such as from 5 to 50 °C, such as from 10 to 40 °C, such as from 15 to 37 °C, such as from 20 to 25 °C.

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As will be appreciated, the resulting organic barrier layer will be less permeable than the porous material alone. For example, the permeability of the organic barrier layer may be in the order of from 1×10^{-8} to 1×10^{-5} m/s, such as from 1×10^{-7} to 1×10^{-6} m/s.

10 There are many potential commercial applications of the process described herein. For example, the process may be used for mitigating wave erosion of a beach or a shoreline. This process may involve:

(i) spraying the bio-gelation mixture onto a surface of a porous material in need thereof to form the organic barrier layer;

15 (ii) placing a porous material on top of the formed organic barrier layer; and

(iii) subjecting the porous material placed on top of the formed organic barrier layer to microbially-induced calcium carbonate precipitation to form an inorganic barrier layer on top of the organic barrier layer.

20 Alternatively, the process may involve:

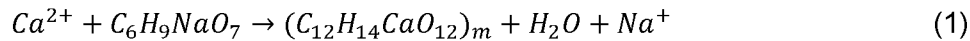
(ia) injecting the bio-gelation mixture into a porous material in need thereof at a defined depth to form the organic barrier layer, with a portion of porous material undisturbed on top of the formed organic barrier layer; and

(iia) subjecting the undisturbed porous material on top of the formed organic barrier
25 layer to microbially-induced calcium carbonate precipitation to form an inorganic barrier layer on top of the organic barrier layer.

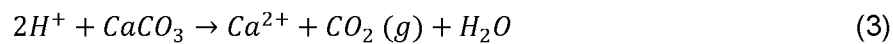
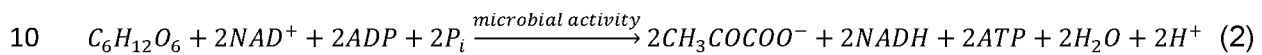
As will be appreciated, the process described herein does not require one to generate calcium carbonate *in situ* in a separate step before the formation of the organic barrier layer. This is
30 because the porous material may inherently include a sufficient amount of a calcium ion source to render this unnecessary. Alternatively, even if there is not a sufficient amount of the calcium ion source in the porous material, the use of a separate step of microbially-inducing calcium carbonate precipitation is still rendered unnecessary because the bio-gelation mixture can include the desired amount of calcium carbonate instead. Thus, the process disclosed
35 herein does not involve a step of microbially-induced calcium carbonate precipitation process before or during steps (a) and (b) of said process.

Bio-gelation refers to a process in which polysaccharide is activated to form gel. In this study, sodium alginate ($C_6H_9NaO_7$) was selected as the polysaccharide and calcium ions were used as an activate agent. When the two elements are put together, the sodium ions are exchanged by the calcium ions and alginate acids are crosslinked by the calcium ions to form the hydrogel.

5 The reaction formula is given below:



Calcium ions can be introduced by dissolving $CaCO_3$ using acid (H^+) generated using anaerobic microbial acidification of Glucose ($C_6H_{12}O_6$) or other carbon sources as shown below:



15 As noted herein, the bio-gelation of soil and/or sand may generally have three steps, as described by reference to an embodiment of the invention.

First, prepare the bio-gelation medium. Raw materials of the bio-gelation medium contain glucose, alginate (i.e. a polysaccharide), and activated sludge (i.e. a bacterial population). The bio-gelation medium may also include a calcium ion source if needed. The sodium alginate and glucose can be purchased from the market. The activated sludge can be obtained from a local wastewater treatment plant. The activated sludge may then be incubated in a medium containing 10 g/L yeast and 2 g/L KH_2PO_4 under neutral condition ($pH = 7$) for 24 hours. After incubation, the liquid culture of the bacteria may be centrifuged at a speed of 6000 RPM to collect the enriched bio-mass. For further usage, the collected biomass may be suspended in 9% NaCl solution, and the OD_{600} value of the suspension may be adjusted to around 0.6. Said suspension may then be stored in a fridge at 4 °C. It is believed that the substrate concentrations may have a significant impact on the gelation effect. The optimum concentrations for the maximum gelation efficiency may be 3 g/L of sodium alginate (i.e. a polysaccharide), 2 g/L glucose and 15 mL/L of the bacterial suspension having a OD_{600} value of 0.6.

Table A below provides a range of concentrations for each substrate. Concentrations approaching the lower limit may reduce the treatment efficiency, whereas concentrations approaching the upper limit may induce extra cost with marginal improvement in the treatment efficiency. It is also suggested that the sodium alginate (i.e. polysaccharide) concentration

should not exceed 10 g/L because it would increase the viscosity of the treatment solution, which may limit the treatment depth possible.

Table A Some suggested upper and lower limits for components in the bio-gelation treatment solution

Component Name	Suggested limit	Upper limit	Suggested Lower limit	Recommended value
Sodium Alginate	10 g/L		1 g/L	3 g/L
Glucose	5 g/L		0.5 g/L	2 g/L
Bacteria Suspension	50 mL/L		1 mL/L	15 mL/L
Bacteria Density	OD ₆₀₀ = 0.95		OD ₆₀₀ = 0.15	OD ₆₀₀ = 0.60

Second, inject the prepared bio-gelation mixture into the soil and/or sand. For a laboratory test, a water tank may be used to flush the medium through the soil and/or sand sample, as is shown in FIG. 1(a). For field implementation, rows of injection wells (cylinders in FIG. 1(b)) may be used to flush the medium through the soil and/or sand. The volume of the treatment solution that needs to be injected to the soil and/or sand layer for bio-gelation treatment may be at least 1.5 times the pore volume of the soil and/or sand layer, that is:

$$V_{tr} = 1.5 \cdot V_{pr} = 1.5 \cdot e / (1 + e) \cdot V_s \quad (4)$$

Where V_{tr} is the volume of the treatment solution, V_{pr} is the pore volume of the sand layer, V_s is the total volume of the sand layer, e is the void ratio.

If the soil and/or sand layer does not contain any calcium content, it is required to add some calcium carbonate powder (or other calcium ion source) to the bio-gelation treatment solution prior to the injection. The mass of the calcium carbonate to be added in per cubic meter of the treatment solution can be calculated as:

$$M_C = 10G_s/e \quad (5)$$

Where M_C is the mass of the calcium carbonate (unit: kg) in per cubic meter of the treatment solution, G_s is the specific gravity of the sand particles in the sand layer, e is the void ratio. Equation (5) ensures at least 0.8% (e.g. at least 1%) of calcium carbonate by weight is presented in the pore space of the sand layer, which is enough to promote the bio-gelation process. It is preferred that the mean particle size of the calcium carbonate powder should not be higher than 50 μm , and a mixing machine should be operated on site to homogeneously mix the calcium carbonate powder with the bio-gelation medium.

Third, after injection, wait for from 36 to about 48 hours for the gelation process to complete (this assumes addition immediately after the bio-gelation mixture is formed. As mentioned hereinbefore, this addition step may be delayed if desired). In this step, the microorganism will consume the glucose and produce acids. With the presence of calcium carbonate in the soil, these acids will dissolve the calcium carbonate and produce free calcium ions in the pore water. The calcium ions will then crosslink the alginate to form the hydrogel. The bio-gelation process is illustrated in FIG. 2.

The current process can be used to drastically change the permeability of sand. For example, as shown by FIG. 10, the permeability of standard 20-30 Ottawa sand can be reduced by three orders of magnitude after one and a half days' treatment (where the bio-gelation mixture is applied shortly after its formation). The costs of the materials used in the process disclosed herein is low (at around the cost of 5 to 6 USD per cubic meter of porous material to be treated), which is far lower than the costs associated with conventional methods.

As noted hereinbefore, another advantage over conventional methods is that the organic barrier layer will only be produced after a certain duration of incubation, which can be controlled to be a few hours to one to two days, so as to allow sufficient time for the injection and distribution of the bio-gelation medium in the soil/sand before the gel can be formed. This means that the bio-gelation mixture can be pre-prepared and shipped to the site of use if desired.

The bio-gelation process disclosed herein can be used for mitigating wave erosion of a beach or shoreline in conjunction with a microbially-induced calcium carbonate precipitation (MICP) method (see FIG. 18(a)). As shown in FIG. 18(a)(i), the first 30 centimetres or so of a sand layer may be excavated, and the bio-gelation mixture can then be sprayed onto the remaining sand to form an organic barrier layer. After that, the excavated sand can be distributed over on the organic barrier layer and an MICP solution can then be sprayed into the sand (FIG. 18(a)(ii)). Owing to the impermeable bio-gel layer, the MICP treatment solution will be retained in the sand layer on top of the organic barrier layer, resulting in the more efficient formation of an inorganic barrier layer (FIG. 18(a)(iii)), which will allow the formation of the final wave erosion barrier depicted in FIG. 18(b), which has an organic barrier layer **100** and an inorganic barrier layer **110** on top of the organic barrier layer **100**. Moreover, since the MICP treatment solution contains free calcium ions, the durability of the organic barrier layer may be enhanced, as the inorganic barrier layer would provide a source of calcium ions to strengthen the crosslinking of the organic barrier layer.

For projects with shallow or deep excavation into a sand and/or soil layer (such as for reclaimed land), the process described herein can be used for constructing waterproofing curtains as depicted in FIG. 19. In this case, a hole **1000** is dug which may be deeper than the underground water level **1010**. Given this, the process described herein may be used to form an organic barrier layer **1020** around the perimeter of the hole **1000**, followed by the insertion of a sheet pile wall **1030** and suitable reinforcements or shelving **1040**.

Further, around 90% of dam collapses are related to overtopping and seepage failure. The process disclosed herein can be used as a seepage control barrier for earth dams, as shown in FIG. 20.

Further aspects and embodiments of the invention will now be described by reference to the following non-limiting examples.

Examples

Materials

Sodium alginate (W201502), glucose (G8270), ethylenediaminetetraacetic acid (EDTA), yeast, KH_2PO_4 , NaCl and CaCl_2 were purchased from Sigma-Aldrich. The activated sludge was obtained from a local wastewater treatment plant (Singapore). Standard Ottawa sand was purchased from Engineering Laboratory Electronics (S) Pte Ltd.

Analytical techniques

Particle size distribution

The distribution of particle size was determined based on ASTM Standard "Standard test methods for particle-size distribution (gradation) of soils using sieve analysis" (ASTM D6913/D6913M-17). A standard sieving set described in the standard above was used.

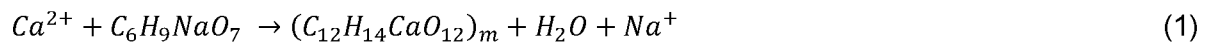
Gravimetric water content

The gravimetric water content was determined based on ASTM Standard "Standard test methods for laboratory determination of water (moisture) content of soil and rock by mass" (ASTM D2216-19).

Example 1. Bio-gelation process

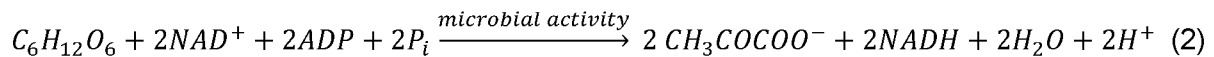
Bio-gelation refers to a process in which polysaccharide is crosslinked to form gel. In this study, sodium alginate ($\text{C}_6\text{H}_9\text{NaO}_7$) was selected as the polysaccharide and calcium ions were used

as a crosslinking agent. When the two elements are put together, the sodium ions are exchanged by the calcium ions and alginate acids are crosslinked by the calcium ions to form the hydrogel. The reaction formula is given below.



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Calcium ions can be introduced by dissolving $CaCO_3$ using acid (H^+) generated using anaerobic microbial acidification of glucose ($C_6H_{12}O_6$) or other carbon sources as shown below:



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The bio-gelation of soil generally has three steps. Firstly, the bio-gelation medium was prepared at the pre-determined alginate concentration (i.e., 3g/L) with necessary nutrition source (i.e., glucose) and microorganism. The microorganism is used for promoting the bio-acidification process and is introduced to the medium in a liquid form. Secondly, the prepared bio-gelation medium was injected into the soil. For the laboratory test, a water tank was used to flush the medium through the sand sample as shown in FIG. 1(a). For the field implementation, rows of injection wells (cylinders in FIG. 1(b)) are needed to flush the medium through the soil. Thirdly, 36 ~ 48 h was taken for the gelation process to complete. In this step, the microorganism will consume the glucose and produce acids. With the presence of calcium carbonate in the soil, these acids will dissolve the calcium carbonate and produce free calcium ions in the pore water. The calcium ions will then crosslink the alginate to form the hydrogel in the end. The bio-gelation process is also illustrated in FIG. 2. The difference between the proposed bio-gelation and chemical gelation is that by producing calcium ions through a microbial acidification process, a bio-gelation process can be controlled by adjusting bacteria activity and substrate concentrations to give sufficient time for reagents to be well-distributed in sand. For the bacteria and substrate concentrations used in this study, the time delay in reaction can be controlled within 24 ~ 48 hours. It should be noted that the proposed bio-gelation will only work for sand with the presence of a small amount of calcium carbonate. A preliminary study by the inventors (K. D. Wang, Dynamic Properties of Bio-mediated Sand. Ph.D. Qualifying Examination Report, **2018**, Nanyang Technological University, Singapore) showed that 1% calcium carbonate in sand could sufficiently promote the bio-gelation process. This is not a problem for river or sea sand that normally contains shell fragments in it. For sand without calcium, a MICP process (J. T. Dejong *et al.*, *Géotechnique* **2013**, 63, 287-301) can be adopted to generate 1 to 2% calcium carbonate in sand particles through one-time MICP treatment as was adopted in Cheng *et al.* (L. Cheng, Y. Yang & J. Chu, *Microb. Biotechnol.*

2019, 12, 324-333). Alternatively, direct mixing of calcium carbonate such as eggshell and limestone powder with sand can be used if feasible.

Determination of specific gravity

- 5 The specific gravity was determined based on ASTM Standard “Standard test method for relative density (specific gravity) and absorption of coarse aggregate” (ASTM C127-15).

Determination of calcium content

- 10 The calcium content was determined by acid digestion method (S.-G. Choi *et al.*, *J. Mater. Civ. Eng.* **2017**, 29, 06017015).

Determination of maximum void ratio

- 15 The maximum void ratio was determined based on ASTM Standard “Standard test methods for minimum index density and unit weight of soils and calculation of relative density” (DOI: ASTM D4254-16), and the minimum void ratio was determined based on ASTM Standard “Standard test methods for maximum index density and unit weight of soils using a vibratory table” (ASTM D4253-16e1).

Characterization

- 20 Standard Ottawa sand mixed with 1% coral sand by weight was used in this study. The particle size distribution curve and physical properties of the mixed sand are shown in FIG. 3 and Table 1. The coral sand used had a calcium content of 85.8% and was crushed and sieved to be in the same range of particle sizes as Ottawa sand. Thus, the soil used in this study initially had a 0.86% calcium content.

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Table 1. Typical engineering properties of the sand used in this study.

Description	Mean size (mm)	Typical specific gravity	Calcium content (%)	Maximum void ratio	Minimum void ratio
ASTM C778- 12 Graded sand mixed with 1% coral sand	0.4	2.65	0.86	0.795	0.503

Example 2. Preparation of bio-gelation medium and hydrogel

Preparation of bio-gelation medium

The bio-gelation medium contains glucose, alginate, and activated sludge. A mixed bacterial culture was used for the bio-acidification process in this study, which was obtained from a local wastewater treatment plant (Singapore) and established with 10% activated sludge as inoculums. Detailed cultivation of the bacteria can be found in Cheng *et al.* (L. Cheng, Y. Yang & J. Chu, *Microb. Biotechnol.* **2019**, 12, 324-333). Briefly, the activated sludge was incubated in a medium containing yeast (10 g/L) and KH_2PO_4 (2 g/L) at a neutral condition ($\text{pH} = 7$) for 24 hours. After incubation, the liquid culture of the bacteria was centrifuged at a speed of 6000 RPM to collect the enriched biomass. For further usage, the collected biomass was suspended in 9% NaCl solution, the OD_{600} value of the suspension was adjusted to around 0.6, and then stored it in a fridge at 4 °C. It should be noted that the substrate concentrations would have a significant impact on the gelation effect. The optimum concentrations for the maximum gelation efficiency can be determined as 3 g/L sodium alginate, 2 g/L glucose and 15 mL/L bacteria suspension at OD_{600} value of 0.6. The OD_{600} value of the bacteria solution used in this study was 0.673 after being diluted by 100 times. To ensure a homogenous distribution of the bio-gelation medium in the sand sample, the volume of the prepared bio-gelation medium for each test was 500 mL, which is equivalent to around 6 pore volumes of the sand sample. The glucose concentration was fixed at 2 g/L and 1% volume of liquid culture of the bacteria was added to the medium to promote the bio-acidification process. The alginate concentration in the bio-gelation medium varied from 0 to 5 g/L.

Preparation of hydrogel

To form the hydrogel, CaCl_2 (50 mL, 0.5 M) solution was mixed with alginate solution (50 mL) at each time. Compared with the alginate concentration, the calcium ions in the liquid are excessive. After mixing, the hydrogel was instantly formed and the formed gel was extracted from the solution by tweezers. The hydrogel was then gently wiped by a piece of tissue to absorb the water on its surface.

Example 3. Characterization of bio-gelation medium and hydrogel

Dynamic viscosity test

One concern was whether the bio-gelation medium would be injectable into soil. To address this concern, the viscosity of the bio-gelation medium at various alginate concentrations (prepared in Example 2) was measured by flushing the medium through a sand column using a constant-head permeameter. This method, according to Ejezie *et al.* (J. O. Ejezie *et al.*, *Géotechnique* **2021**, 71, 561-570), would be more accurate for pore fluid viscosity

determination in coarse soil than the use of a rheometer. All the tests were conducted at a room temperature of 25 °C.

Liquid batch experiment

- 5 A preliminary liquid batch experiment was conducted at alginate concentrations of 1, 3, and 5 g/L by following the preparation of hydrogel protocol in Example 2, to investigate the basic properties of the hydrogel after the formation of the hydrogel.

Wet density measurement

- 10 The hydrogel was put into a measuring cylinder with a precision of $\pm 0.5 \text{ mm}^3$ stood on a digital balance with a precision of $\pm 0.01 \text{ g}$ for the measurement of its volume and weight at a room temperature of 25 °C. Thus, the wet density of hydrogel can be calculated.

Dry density measurement

- 15 The dry density of the hydrogel was measured after drying it in an oven at 50 °C for 24 h. Similar to the procedure described in the measurement of wet density, the oven-dried hydrogel was put into a measuring cylinder and a digital balance for determining its volume and weight. The dry density of the hydrogel thus can be calculated.

20 Results and discussion

- The results are shown in FIG. 4 where the shaded zone illustrates the range of the alginate concentrations used in this study. As can be seen in FIG. 4(a), under a hydraulic gradient of 0.1, the dynamic viscosity of the bio-gelation medium ranged from 0.89 to 4.35 mPa·s in the shaded zone, and the corresponding permeability of sand varied from 8.7×10^{-4} to 1.84×10^{-4} m/s as shown in FIG. 4(b). Hence, the bio-gelation medium for this study is low in viscosity and can be injected into sand.
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- The gravimetric water content of the hydrogel was measured after drying it in an oven at 50 °C for 24 h. The test result is shown in Table 2, where the wet density, gravimetric water content, and dry density of hydrogel were not affected by the change in alginate concentration. However, the hydrogel enrichment ratio, which is defined as the ratio of the volume of the formed hydrogel to the total volume of the mixed solution, experienced an increase with an increase in alginate concentration. Table 2 also indicates that the wet density of hydrogel is close to the density of water. Since all the sand samples were initially fully saturated in this study, the formation of hydrogel would have little effect on the density of the soil.
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Table 2. Main properties of hydrogel formed with different alginate concentration.

Alginate concentration (g/L)	Wet density (g/cm ³)	Gravimetric water content (%)	Dry density (g/cm ³)	Enrichment ratio (%)
1	1.048	293.7	1.238	23.5
2	1.053	303.6	1.236	52.4
3	1.051	301.8	1.242	85.3

Example 4. Tests set-up and procedures**5 Test set-up**

Permeability tests were conducted on sand treated using the bio-gelation method. The stability of hydrogel in the bio-gelled sample was also investigated. All the tests were performed in the triaxial testing system shown in FIG. 5. The base of the cell chamber and the motorized actuator (5) were fixed to the load frame (4) as illustrated in FIG. 5. The motorized actuator had a displacement range of 100 mm and could be programmed to conduct monotonic or cyclic tests under either load-controlled or deformation-controlled loading mode. The load transducer (2) was immersed in the chamber and an extension cap (12) was attached to the top cap of the sample (3) for extension tests. The cell pressure was provided by an air compressor (8) and was regulated by a pneumatic controller (7). Compressing air instead of water to generate cell pressure could avoid pressure fluctuating during cyclic loading, especially under a high loading frequency (i.e., ≥ 1 Hz). The back pressure was controlled by a GDS controller (10). A water tank (1) was used to flush the treatment solution through the sample and a pair of bender elements (13) was employed to monitor the change of compressive and shear wave velocity during the incubation period. All the test parameters were set, monitored, and collected by the control computer (11).

Test procedure

The general test procedure adopted in this study is summarized as follows:

- a) Mix 1% sieved coral sand with standard Ottawa sand by weight.
- b) Prepare a standard sand sample (H:100 mm; D:50 mm) using a moist tamping method (K. Ishihara, *Géotechnique* **1993**, 43, 351-451) and apply a 20-kPa cell pressure to hold the sample in the triaxial cell.
- c) Flush the sample with pure CO₂ for 20 minutes, followed by deaired water for another 30 minutes to saturate the sample.

- d) Flush the bio-gelation medium from the bottom to the top of the sample using the water tank if bio-gelation is required. Otherwise, flush the de-aired water.
- e) Apply a back pressure of 10 kPa to the sample.
- f) Simultaneously increase the cell pressure and back pressure to the predetermined value (a rate of 0.1 kPa/s) while keeping the effective confining stress to 10 kPa.
- g) Check B-value and compressive wave velocity (V_p).
- h) If the B-value is higher than 0.95 and V_p is higher than 1500 m/s, the sample is then consolidated to an effective confining stress of 100 kPa; If not, the sample will be discarded.
- i) Wait for 48 hours for the completion of the bio-gelation process.
- j) Check the existence of hydrogel by checking B-value.
- k) Measure the coefficient of permeability with a constant water head difference.
- l) Determine the residual calcium content in the pore water and sand sample via EDTA titration and acid washing method (S.-G. Choi *et al.*, *J. Mater. Civ. Eng.* **2017**, 29, 06017015), respectively.

Permeability test

Nine permeability tests were conducted in this study. The test parameters are listed in Table 3. Tests PT01 to PT06 were conducted for determining the permeability of bio-gelled sand with different alginate concentrations, whereas Tests PT07 to PT09 were conducted for investigating the stability of hydrogel indicated by the change of the permeability under an upward seepage condition.

In these tests, an additional GDS controller (9 in FIG. 5) was connected to the top valve of the sample to generate a water head difference. The alginate concentration is the only variable in the first five tests and the permeability coefficient was measured only after the completion of the bio-gelation process. In Test PT06, the coefficient of permeability was continuously measured every 10 minutes during the incubation period to examine the bio-gelation process. The sand sample in all the tests was consolidated to 100 kPa before the permeability test. At the start of the permeability test, the GDS controller connected to the up valve was set to target a lower pressure value than that of the GDS controller connected to the bottom valve, so that an upward seepage can be generated through the sample. It should be noted that the water head of Test PT01 was lower than other tests because the permeability of clean sand was much higher than that of the bio-gelled sand. The water head of Test PT06 was continuously adjusted by the self-developed controller software to fit the change of the permeability coefficient during the measurement period. In Tests PT07 to PT09 for examining the stability of hydrogel under seepage conditions, the hydraulic gradients of 0.1, 0.35 and 0.7

were exerted on the sand samples treated with 3 g/L alginate concentration, and the permeability of sand was continuously monitored for 15 days.

Table 3. Test parameters for permeability tests.

Sample ID	Void ratio	Alginate concentration (g/L)	Cell pressure (kPa)	Back pressure_up (kPa)	Back pressure_Bot (kPa)	Remarks
PT01	0.723	0	400	298	300	Clean Ottawa sand
PT02	0.717	1	400	295	300	Ottawa Sand
PT03	0.722	3	400	295	300	mixed with 1% coral sand by weight
PT04	0.718	5	400	295	300	
PT05	0.719	7	400	295	300	
PT06	0.716	3	400	Variable	300	Continuous monitor
PT07	0.720	3	400	299	300	15 days of seepage
PT08	0.718	3	400	297	300	
PT09	0.717	3	400	293	300	

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Example 5. Behaviour of hydrogel

The compressibility of the hydrogel was investigated by an isotropic consolidation test conducted by the triaxial machine by following the protocol in Example 4. The 0.5 M CaCl₂ and 5 g/L alginate solutions were separately injected through the two bottom drainage holes to form the hydrogel in the sampling mould by following the protocol in Example 2.

Results and discussion

The formed hydrogel was measured to have a height of 99.7 mm and an average diameter of 49.3 mm, and the initial water content was measured to be 308.3%. Starting from an effective confining stress (σ'_c) of 20 kPa, the hydrogel was gradually isotropic consolidated to $\sigma'_c = 500$ kPa, after which the σ'_c was unloaded to 100 kPa and then the sample was subject to undrained shearing. It should be noted that during initial consolidation, the gel concentration would increase as the water was drained out from the hydrogel. However, the concentration

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of hydrogel only increased from 0.355 g/cm³ to 0.399 g/cm³ when the effective confining stress (which was also the effective consolidation stress σ'_v for isotropic consolidation) was increased from 20 kPa to 500 kPa. Such a slight increase in the gel concentration is negligible on the compressibility behaviour of hydrogel. FIG. 6 shows the $e - \log \sigma'_v$ curve of pure hydrogel under isotropic consolidation. For comparison, the $e - \log \sigma'_v$ curves of loose Ottawa sand with or without bio-gelation under isotropic consolidation are also shown in FIG. 6. The slope of normal consolidation line (λ) of the hydrogel was found to be much higher than that of the sand. Furthermore, the hydrogel manifested a purely nonlinear elastic behaviour during unloading stage which is different from the behaviour of soil. The isotropic consolidation behaviour of bio-gelled sand is similar to that of clean sand as shown in FIG. 6, where the bio-gelled sand has around 1.7% volume percentage of hydrogel occupied in the pore space. Hence, a small amount of hydrogel present in the pore space does not change the compressibility of sands. From FIG. 6, the slope of normal consolidation line (λ) and the slope of unloading line (κ) of the loose Ottawa sand used in this study can be determined as 0.018 and 0.005.

The shear wave velocity (V_s) of hydrogel was also measured using bender elements and the results are shown in FIG. 7 together with the V_s of loose Ottawa sand. It can be seen that the shear wave velocity of hydrogel was much lower than that of loose sand and it did not increase much with the increase in the effective consolidation stress. On the other hand, the V_s of loose Ottawa sand increased considerably with increasing effective consolidation stress. The results of pure hydrogel under undrained shearing are also shown in FIG. 8. It can be seen that the hydrogel had a negligible shear strength of only around 3 kPa under an effective confining stress of 100 kPa. The Young's Modulus of the hydrogel at $\sigma'_c = 100$ kPa was determined to be 1.422 MPa according to the stress-strain response. At the peak, there was a sudden collapse in the deviator stress (FIG. 8(a)) which was observed with the sudden jump in the excess pore water pressure after point A. Therefore, the pure hydrogel formed in this study did not have much shear resistance.

In terms of compressibility, the bulk modulus (K) of hydrogel can be calculated by Equation 6:

$$K = \frac{1+e}{\kappa} \cdot p' \quad (6)$$

At a fully saturated condition under an effective confining stress of 100 kPa, the void ratio of the hydrogel was 2.44, and the unloading slope κ was 0.088. Hence, the bulk modulus of hydrogel at $\sigma'_c = 100$ kPa was calculated to be 3.91 MPa.

By knowing that the bulk modulus of hydrogel is distinctly different from that of water ($K_w = 2.2$ GPa), the amount of hydrogel in the bio-gelled sand may be estimated through the measurement of the Skempton's coefficient, B. Based on the equation proposed by Wissa (A. Wissa, *J. Soil Mech. Found. Div.* **1969**, 95, 1063-1073) and ignoring the flexibility of the testing system, the relationship between B-value and the pore fluid compressibility (C_f) can be expressed as:

$$B = \frac{1}{1+n(C_f/m_v)} \quad (7)$$

Where n is the porosity of soil and m_v is the volumetric compressibility coefficient of soil. For the loose sand tested in this study, $n = 0.42$ and $m_v = 2.31 \times 10^{-5} \text{ m}^2/\text{kN}$. Under fully saturated condition, the compressibility of pore fluid can be calculated as:

$$C_f = X \cdot C_{gel} + (1 - X) \cdot C_w \quad (8)$$

Where C_{gel} is the compressibility of hydrogel, C_w is the compressibility of water, and X is the percentage of hydrogel occupied in the pore space. Note that the compressibility is the inverse of bulk modulus.

The B-value versus the volume percentage of hydrogel occupied in the pore space was then plotted in FIG. 9, in which the experimental data points generally followed the trend of the theoretical curve based on Equation 8. FIG. 9 also illustrates that the production of hydrogel increased with increasing alginate concentration, which is in accordance with the liquid batch experiment results. Therefore, the hydrogel produced using the bio-gelation method is much more compressible than sand and thus, it does not affect the isotropic compression behaviour of sand after it is bio-gelled.

Although the existence of hydrogel can be checked by measuring the permeability coefficient (L. Cheng, Y. Yang & J. Chu, *Microb. Biotechnol.* **2019**, 12, 324-333), performing B-check is simpler and has minimal disturbance to the sand sample in a triaxial test. Moreover, due to the formation of hydrogel, the pore water pressure response will be affected, which can be evaluated by the B-checking method as well. Hence, the B-value was measured after the completion of the bio-gelation process to check the existence of hydrogel in this study.

Example 6. Permeability test

The permeability test of the samples (prepared in Example 2) was performed by following the protocol in Example 4.

Results and discussion

FIG. 10 presents the variation of permeability of a bio-gelled sand sample with time in the permeability test PT06. The sand sample was treated by 3 g/L alginate concentration. The permeability was automatically measured every 10 minutes during the incubation period by adjusting the back pressure through a controller software to create an appropriate water head difference for measuring the permeability. It can be seen from FIG. 10 that the permeability reduction was gradual from 7.32×10^{-4} to 6.83×10^{-5} m/s in the first 28 hours, and then the permeability reduced drastically to 5.37×10^{-7} m/s in the next 10 hours. Therefore, most of the hydrogel was formed between the 28th to 38th hour during the incubation period, which suggests that the permeability of the sand can be drastically reduced by three times of magnitude after one and a half days' incubation.

The coefficient of permeability measured from Tests PT01 to PT05 after the completion of the bio-gelation process is shown in FIG. 11. It can be seen that there is a drastic reduction in the permeability once the sand is bio-gelled. The coefficient of permeability was reduced from 8.13×10^{-4} m/s for clean sand to 9.12×10^{-7} , 1.79×10^{-7} , 7.11×10^{-8} and 3.11×10^{-8} m/s for sand samples treated using 1, 3, 5 and 7 g/L alginate concentration, respectively. Taken together, the test results presented has shown that the coefficient of permeability can be reduced to the order of 10^{-8} m/s after a single round of bio-gelation treatment. Therefore, it can be concluded that the higher alginate concentration, the higher hydrogel production and the lower the permeability of the bio-gelled sand. Further, the formation of hydrogel can be delayed to up to 28 hours after the injection and thus, this method allows sufficient time for the distribution of reagents in soil when it is used in the field, and can be used for seepage control in sand.

Example 7. Stability of the hydrogel

One concern with the use of hydrogel is whether the material and its effect on the stress-strain responses of sand is stable particularly when there is a seepage flow. The stability of the hydrogel (prepared in Example 2) was investigated under seepage conditions by following the protocol in Example 4.

Results and discussion

The stability of hydrogel was investigated under seepage conditions with a hydraulic gradient of 0.1, 0.35 and 0.7 in Tests PT07, PT08 and PT09, respectively. The three samples tested were prepared under identical conditions. The hydraulic gradient was applied by introducing a constant pressure difference between the top and the bottom of the sample. During the test, the coefficient of permeability was measured at a time interval of 12 hours during the 15-day

seepage period. As shown in FIG. 12, the bio-gelled sand has an initial coefficient of permeability of around 3.36×10^{-7} m/s and the permeability remains almost unchanged under a hydraulic gradient of 0.1. The permeability slightly increased when the samples were flushed under a hydraulic gradient of 0.35 and 0.7 for 15 days. Specifically speaking, the coefficient of permeability only changed a small amount to 4.66×10^{-7} and 7.18×10^{-7} m/s under a hydraulic gradient of 0.35 and 0.7, respectively, after 15 days of seepage flow. The above test results show that the hydrogel used was stable for at least 15 days under seepage conditions with a hydraulic gradient as high as 0.7.

10 Example 8. Quicksand model tests

A series of quicksand model tests was conducted to investigate the use of hydrogel (prepared in Example 2) for improving the critical hydraulic gradient of clean sand.

15 Test set-up

The test set-up is illustrated in FIG. 13. The test set-up 200 includes a column 210 comprising gravel 211 and a sand column 212, a tank 220 for collecting liquid for permeability measurement, a burette 230, a vertically slidable water tank 240 comprising a water tank 241 and a slideway 242, a water reservoir 250 comprising a pump 251, where the column 210, burette 230, the vertically slidable water tank 240, the water reservoir 250, and the pump 251 are hydraulically connected to one another by pathways 260, 270 and 280.

In FIG. 13, the water level of the burette 230 is equivalent to that of the water tank 241, and the hydraulic gradient is calculated as:

$$25 \quad i = \frac{\Delta h}{H_s} = \frac{h_2 - h_1}{H_s} \quad (9)$$

Where Δh is the water head difference between water tank and sand column, h_2 is the reading of burette which reflects water level of the water tank, h_1 is water level above the sand sample, and H_s is the height of the sample.

30 The height of the water tank was initially set to make the hydraulic gradient equal to approximately 0.05 and was then gradually raised until it reached the critical hydraulic gradient (i_{cr}). The permeability was measured after each adjustment of the hydraulic gradient and i_{cr} was identified when the sand column was boiled (liquefied) or cracked by the hydraulic pressure in the test.

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Test arrangement

Nine tests were conducted on bio-gelled sand with three different alginate concentrations and three different degrees of saturation, and one reference test was conducted on fully saturated sand sample, as shown in Table 4. All the sand samples were prepared to have a relative density of 40%.

Table 4. Arrangement of quicksand model test on bio-gelled sand.

Test ID	Relative Density	Degree of saturation	Alginate concentration	Remarks
GQT01	40%	100%	1 g/L	Medium
GQT02		100%	3 g/L	loose
GQT03		100%	5 g/L	sample
GQT04	40%	95.5%	1 g/L	Medium
GQT05		95.9%	3 g/L	loose
GQT06		96.3%	5 g/L	sample
GQT07	40%	91.2%	1 g/L	Medium
GQT08		90.9%	3 g/L	loose
GQT09		91.1%	5 g/L	sample
RE01	40%	100%	-	Reference sample

Results and discussion

FIG. 14 shows the coefficient of permeability measured at different hydraulic gradients applied to the fully saturated bio-gelled medium loose sand samples (Tests GQT01 to GQT03). It can be seen that the coefficient of permeability gradually reduced with the increase in the alginate concentration in the bio-gelled samples. With increasing hydraulic gradient, a quicksand condition eventually occurred in each test and the hydraulic gradient at which is the i_{cr} . The effect of hydrogel on the permeability and critical hydraulic gradient can be seen from FIG. 14. It can be seen that i_{cr} increased with the increase in the alginate concentration. The increase in i_{cr} was more pronounced by increasing the alginate content and the i_{cr} increased from 0.97 for fully saturated medium loose sand (Test RE01) to 1.18 for samples treated with 1 g/L alginate concentration (Test GQT01).

The measured i_{cr} were also plotted against the alginate concentration in FIG. 15 for bio-gelled sand samples. In FIG. 15, the i_{cr} increased with the increase in alginate concentration. The influence of the degree of saturation on i_{cr} became less significant at a higher alginate

concentration. The i_{cr} reached as high as around 1.58 in the bio-gelled medium loose sand treated with 5 g/L alginate concentration.

The existence of hydrogel in the pore space would reduce the permeability of the sand sample as has been demonstrated in FIG. 14. The measured coefficient of permeability at a hydraulic gradient of 0.25 was plotted in FIG. 16 for bio-gelled sands which shows that the reduction in the permeability of sand can be 3 ~ 4 orders of magnitude for medium loose sand after bio-gelation treatment. The higher the alginate concentration, the lower the coefficient of permeability. The overlapping of the three data lines for sand with three different degrees of saturation in FIG. 16 indicates that the effect of degree of saturation on the permeability becomes negligible in the bio-gelled sand samples.

Example 9. Advantages of bio-gelation method

Previous researches have studied the properties of hydrogel formed through direct chemical reaction (I. Chang, J. Im & G.-C. Cho, *Can. Geotech. J.* **2016**, 53, 1658-1670; and K. Wen *et al.*, *Constr. Build Mater.* **2019**, 207, 174-180). However, chemically induced gelation is problematic when applied in-situ because the rapid generation speed makes it impossible for the solution to be injected to a desired distance. The bio-gelation method can overcome this shortcoming by increasing the gelation time by controlling the generation of calcium ions through a microbial process so that the low viscous treatment solution can be distributed evenly in soil before the hydrogel is formed in-situ. As indicated in FIG. 10, the massive formation of hydrogel as indicated by the decreasing of permeability only took place 28 hours after the injection of the bio-gelation solution. Furthermore, the cost involved in the production of hydrogel in soil is much lower than conventional soil improvement methods for liquefaction or seepage control such as deep cement mixing and chemical grouting.

Example 10. Environmental impact

As microbial activities are involved in the bio-gelation method, there is a need to evaluate its environmental influence. The principle behind the bio-gelation is to use the bio-acidification to release the metal ions to produce the hydrogel. Although the bio-acidification process may modify the pH value of the indigenous soil, it can be avoided by careful determination of the substrate concentrations for bio-acidification to match the metal content of the soil. With proper substrate concentrations, the produced acids by bio-acidification can be all consumed by the dissolution of metal compounds. One bio-gelled sample was prepared for daily extraction of the pore water for the measurement of pH value in this study. As can be seen in FIG. 17, the

pH value of the extracted pore fluid remains relatively neutral throughout the whole bio-gelation treatment process. Thus, the microbially induced gelation method does not change the pH value of the soil fluid if properly adopted. Further, the only by-product of the method is carbon dioxide and most of it will be dissolved in water.

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Another concern regarding the impact of bio-acidification is that the dissolution of CaCO_3 minerals may cause unwanted settlements or particle rearrangement. Based on the test data in this study, an extra axial strain within 0.02% was found in the bio-gelled sand samples treated with 3 g/L alginate concentration compared to clean sand samples during the bio-gelation process. However, even if all the extra strain is due to the dissolution of CaCO_3 minerals, it would only cause a less than 2 mm settlement for every 10-meter depth in soil treatment. Hence, the deformation due to dissolution of CaCO_3 minerals by acids may be neglected under the test conditions adopted in this study.

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Claims

1. A process of forming an organic barrier layer in a porous material in need thereof, the process comprising the steps of:

(a) providing a porous material in need thereof and a bio-gelation mixture, the bio-gelation mixture comprising:

water;

a polysaccharide suitable for crosslinking;

glucose in an amount of from 1 to 3 g/L; and

a bacterial population suitable for converting glucose to a carboxylic acid which is provided as a bacterial suspension having a bacterial density (OD_{600}) of from 0.3 to 0.8 in an amount of from 5 to 30 mL/L; and

(b) injecting the bio-gelation mixture into; or

spraying the bio-gelation mixture onto a surface of the porous material to form an organic barrier layer comprising the polysaccharide crosslinked by a plurality of calcium ions, wherein the plurality of calcium ions are obtained from reaction of the carboxylic acid with a calcium ion source, where the calcium ion source is present in the porous material and/or is added as an additional component within the bio-gelation mixture.

2. The process according to Claim 1, wherein the bio-gelation mixture comprises:

water;

a polysaccharide in a concentration of from 1 to 10 g/L; and

a calcium ion source provided in an amount of from 0 wt%, or in an amount sufficient to provide from 0.5 to greater than 1 wt% in a volume of the porous material that is to form part of the organic barrier layer.

3. The process according to Claim 2, wherein the bio-gelation mixture comprises:

water;

a polysaccharide in a concentration of from 2 to 4 g/L, such as about 3 g/L;

glucose in an amount of about 2 g/L;

a bacterial population suitable for converting glucose to a carboxylic acid which is provided as a bacterial suspension having a bacterial density (OD_{600}) of about 0.60 in an amount of about 15 mL/L; and

a calcium ion source provided in an amount of from 0 wt% to an amount sufficient to provide from 0.8 to 1.8 wt% in a volume of the porous material that is to form part of the organic barrier layer.

4. The process according to any one of the preceding claims, wherein the porous material in need thereof is one or more of the group consisting of a sand, and a soil, optionally wherein the porous material in need thereof is a sand (e.g. Ottawa sand and/or Coral sand).
5. The process according to any one of the preceding claims, wherein the porous material in need thereof comprises from 0.5 to 1.8 wt%, such as from 0.8 to 1.5 wt%, such as from 0.86 to 1 wt% of a calcium ion source.
6. The process according to any one of the preceding claims, wherein the calcium ion source is CaCO_3 , optionally wherein, when the CaCO_3 forms part of the bio-gelation mixture, it has a diameter of less than or equal to 50 μm .
7. The process according to any one of the preceding claims, wherein the polysaccharide suitable for crosslinking is an alginate, optionally wherein the alginate is sodium alginate.
8. The process according to any one of the preceding claims, wherein the bacterial population suitable for converting glucose to a carboxylic acid is derived from activated sludge.
9. The process according to Claim 8, wherein the activated sludge is incubated in an aqueous medium comprising yeast and KH_2PO_4 at pH 7 for about 24 hours and is then centrifuged to provide a bio-mass pellet for resuspension and formation of the bacterial population.
10. The process according to any one of the preceding claims, wherein the bio-gelation mixture is used immediately after its formation, where the bio-gelation mixture is formed by mixing the polysaccharide suitable for crosslinking, glucose, the bacterial population suitable for converting glucose to a carboxylic acid and, when present, the calcium ion source in water.
11. The process according to any one of Claims 1 to 9, wherein the bio-gelation mixture is used after a period of from 1 to 24 hours after its formation, where the bio-gelation mixture is formed by mixing the polysaccharide suitable for crosslinking, glucose, the bacterial population suitable for converting glucose to a carboxylic acid and, when present, the calcium ion source in water.

12. The process according to any one of the preceding claims, wherein in step (b) of Claim 1, the formation of the organic barrier layer occurs over a period of from 1 hour to 4 days, such as from 2 hours to 2 days, such as from 3 hours to 38 hours.

13. The process according to any one of the preceding claims, wherein in step (b) of Claim 1, the porous material in need thereof has a temperature of from 0 to 60 °C, such as from 5 to 50 °C, such as from 10 to 40 °C, such as from 15 to 37 °C, such as from 20 to 25 °C.

14. The process according to any one of the preceding claims, wherein the permeability of the organic barrier layer is in the order of from 1×10^{-8} to 1×10^{-5} m/s, such as from 1×10^{-7} to 1×10^{-6} m/s.

15. The process according to any one of the preceding claims for mitigating wave erosion of a beach or a shoreline, the process involving:

- (i) spraying the bio-gelation mixture onto a surface of a porous material in need thereof to form the organic barrier layer;
- (ii) placing a porous material on top of the formed organic barrier layer; and
- (iii) subjecting the porous material placed on top of the formed organic barrier layer to microbially-induced calcium carbonate precipitation to form a calcium enriched layer on top of the organic barrier layer.

16. The process according to any one of claims 1 to 14 for mitigating wave erosion of a beach or a shoreline, the process involving:

- (ia) injecting the bio-gelation mixture into a porous material in need thereof at a defined depth to form the organic barrier layer, with a portion of the porous material undisturbed on top of the formed organic barrier layer; and
- (iia) subjecting the undisturbed porous material on top of the formed organic barrier layer to microbially-induced calcium carbonate precipitation to form an inorganic barrier layer on top of the organic barrier layer.

17. The process according to any one of the preceding claims, wherein the process does not involve a step of microbially-induced calcium carbonate precipitation process before or during steps (a) and (b) of Claim 1.