Title: MINERAL PRECIPITATION METHODS

Abstract: The present invention provides methods for mineral precipitation of porous particulate starting materials using isolated urease.
Mineral Precipitation Methods

5 Cross-Reference
This application claims priority to U.S. Provisional Patent Application Serial No. 61/896340 filed October 28, 2013 and 61/916908 filed December 17, 2013, both incorporated by reference herein in their entirety.

10 Statement of U.S. Government Interest
This invention was made with government support under grant number 1233658 awarded by the National Science Foundation and grant number 0856801 by the National Science Foundation. The government has certain rights in the invention.

15 Summary of the Invention
The present invention provides mineral precipitation methods, comprising
(a) combining a porous starting material with
   (i) a source of urease;
   (ii) urea; and
   (iii) a source of divalent cations;
wherein (i), (ii), (and (iii) are provided in amounts effective and the combining is carried out under conditions suitable to cause carbonate mineral precipitation of the starting material to produce a starting material complex; and
(b) contacting the starting material complex with a base under conditions to mitigate formation of ammonium salts in the starting material complex. In one embodiment, the base is introduced into the starting material complex at one or more locations. In another embodiment, contacting the starting material complex with the base results in an increased pH of the starting material complex at the site of the contacting. In various further embodiments, the base is selected from the group consisting of NaOH, KOH, Ca(OH)₂, Mg(OH)₂, and combinations thereof. In one embodiment, the source of urease comprises isolated urease or cells expressing urease.

In various non-limiting embodiments, the method can be used for one or more of improving bearing capacity of foundations; reducing settlement potential of foundations or embankments; stabilizing slopes; reducing the potential for triggering earthquake-induced
liquefaction; reducing the potential for triggering static liquefaction; mitigating the potential for damaging ground displacements subsequent to triggering of liquefaction; increasing lateral resistance of foundations; enhancing stability of slopes or embankments; reducing lateral earth pressures on retaining walls; increasing passive resistance of retaining walls; increasing capacity of ground anchors or soil nails; increasing the side resistance and tip resistance of deep foundations; facilitating tunneling in running or flowing ground; stabilizing excavation bottoms; soil erosion control; and groundwater control.

In various further embodiments, the starting material can be selected from the group consisting of sand, silt, clay, other sediments, sawdust, igneous rocks, metamorphic rocks, gravel, fractured crystalline rocks, cracked concrete and sedimentary rocks including but not limited to conglomerate, breccia, sandstone, siltstone, shale, limestone, gypsum, and dolostone, and combinations thereof. In other embodiments, the source of divalent cations is selected from the group consisting of calcium, magnesium, strontium, and other cations in the divalent state such as iron, cadmium, manganese, lead, or zinc ions, or combinations thereof.

In a further embodiment, the method further comprises introducing a clay slurry into the starting material prior to or concurrent with combining the starting material with the source of urease, the urea, and the source of divalent cations.

**Description of the Figures**

**Figure 1.** LV-Scanning electron microscope images of a) well-grown and cementing calcite crystals; b) cementing calcite crystals at inter-particle contact; c) indentation of quartz surface (arrows) and nucleation of calcite crystals (red arrows); d) calcite crystal growing on quartz surface.

**Figure 2.** P-q plot failure envelopes for 20-30 silica sand: ■Cemented (Dv = 60%); ○ Uncemented (Dv = 60%).

**Figure 3.** P-q plot failure envelopes for F-60 silica sand: ■Cemented (Dv = 35%); ○ Uncemented (Dv = 37%).

**Figure 4.** Results of triaxial compression tests performed using F-60 Ottawa sand (medium sand).

**Figure 5.** Results of triaxial compression tests performed using medium sand w/bentonite slurry.

**Figure 6.** Results of triaxial compression tests performed using F-60 fine sand.
Figure 7. Image from mix & compact columns using silica 20-30. These are silica sand particles variously covered with CaCO₃ and cemented at the inter-particle contacts.

Figure 8. Image from same mix & compact columns using silica 20-30 as shown in Figure 7. Note the concave CaCO₃ cement where a sand particle was bonded.

Figure 9. Image from the same mix & compact columns using silica 20-30 as shown in Figure 7. These are silica sand particles covered with a growing CaCO₃ layer that forms cement at the inter-particle contact. The large particles to the left appear to be well-developed CaCO and possibly evaporates (far left).

Figure 10. Low-resolution image from a 4”x12” PVC column #2 using silica 20-30 with bentonite; note the scale (10 µm). These are growing CaCO₃ crystals covering silica sand particles (not seen). The CaCO₃ is randomly covered or associated with clumps of bentonite, which may be serving as points of nucleation.

Figure 11. Image from the 4”x12” PVC column #1 using silica 20-30. These are growing CaCO₃ crystals covering silica sand particles. The relatively smooth concave feature is an inter-particle cementation contact.

Figure 12. Image from the same 4”x12” PVC column #1 using silica 20-30 as Figure 11, but zoomed-in to the relatively smooth concave inter-particle feature described above; these are CaCO₃ crystals covering silica sand particles.

Figure 13. Image from the same 4”x12” PVC column #1 using silica 20-30 as in Figure 11. These are CaCO₃ crystals covering silica sand particles.

Figure 14. Image from the same 4”x12” PVC column #1 using silica 20-30 as in Figure 11.

Figure 15. Image from the 4”x12” PVC column #3 using silica F-60. These are silica sand particles variously covered with CaCO₃ crystals.

Figure 16. Image from the same 4”x12” PVC column #3 using silica F-60 as in Figure 15, but at higher magnification. Note the inter-particle cementation.

Figure 17. Image from the same 4”x12” PVC column #3 using silica F-60 as in Figure 15, but showing different location. These are silica sand particles covered with CaCO₃ crystals.

Figure 18. Image from the same 4”x12” PVC column #3 using silica F-60 as in Figure 15, but at higher magnification. These are silica sand particles covered with CaCO₃ crystals.
Figure 19. Image from the same 4”x12” PVC column #3 using silica F-60 as in Figure 15, but at lower magnification. These are silica sand particles covered with CaCO₃ crystals.

Figure 20. An image from a columnar EICP study using dry sand injected with cementation solution

Figure 21. An image from a columnar EICP study using wet sand injected with cementation solution

Detailed Description of the Invention

As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. “And” as used herein is interchangeably used with “or” unless expressly stated otherwise.

All embodiments of any aspect of the invention can be used in combination, unless the context clearly dictates otherwise.

In a first aspect, the present invention provides mineral precipitation method, comprising

(a) combining a porous starting material with
   (i) a source of urease;
   (ii) urea; and
   (iii) a source of divalent cations;

wherein (i), (ii), (and (iii) are provided in amounts effective and the combining is carried out under conditions suitable to cause carbonate mineral precipitation within the starting material to produce a starting material complex; and

(b) contacting the starting material complex with a base under conditions to mitigate formation of ammonium salts in the starting material complex.

In a soil cementation process based upon carbonate precipitation via hydrolysis of urea, adding a base (such as sodium hydroxide) can mitigate the formation of ammonium salts, inhibit nitrification of ammonia (which can lower the pH and start dissolving CaCO₃), and facilitate carbonate precipitation and cementation. It is applicable to work on enzyme-induced calcite precipitation (EICP) as described herein, as well as microbially induced calcite precipitation (MICP). The use of the base helps drive a shift of ammonium --> ammonia to limit the potential of reaction reversal due to the ammonium, and will temporarily increase pH near the point of introduction, which also has a beneficial effect with
respect to inducing carbonate precipitation. This not only limits the potential for reversal of and enhances carbonate precipitation, but also helps rid the system of toxic ammonium salt by-products. A further effect of adding a strong base to the system is that it may help expose/dissolve some of the soil particle or substances clothing the soil particle and thereby facilitate more and/or stronger bonding of the soil to be improved (e.g., gravel, sand, or silt particles susceptible to degradation/dissolution via base) with the precipitated calcite (calcium carbonate).

Induced carbonate precipitation can enhance the stiffness, strength, and liquefaction resistance of soil. The methods provide an alternative to commonly used soil improvement techniques such as deep soil mixing, stone columns, penetration and compaction grouting, and rammed aggregate piers.

The methods can be used, for example, in stabilizing slopes, improving the bearing capacity of foundations, reducing settlement potential of foundations and embankments, increasing the lateral resistance of foundations, reducing the potential for triggering of earthquake-induced liquefaction; reducing the potential for triggering static liquefaction, mitigating the potential for damaging ground displacements subsequent to triggering of liquefaction; enhancing the stability of slopes and embankments, reducing lateral earth pressures on retaining walls, increasing the passive resistance of retaining walls, increasing the capacity of ground anchors and soil nails, increasing the side resistance and tip resistance of deep foundations, facilitating tunneling in running or flowing ground (dry or saturated cohesionless soil), stabilizing the bottom of excavations, soil erosion control, groundwater control.

Any suitable base may be used that serves to mitigate formation of ammonium salts in the starting material complex. In one non-limiting embodiment, the base is selected from the group consisting of NaOH, KOH, Ca(OH)₂, Mg(OH)₂, and combinations thereof. The amount of base needed is that amount which shifts the acid-base equilibria towards the basic side (i.e., higher pH); this amount will depend on the concentration and the amount of concentrations of urea and divalent cations added to the starting material (or in the starting material complex) used, as will be understood by those of skill in the art. In addition, soil conditions may favor more or less base as certain soils lack buffering capacity and other soils provide some level of buffering, respectively. The goal is to add a sufficient amount of base to shift the acid-base equilibria of the chemical reaction matrix to a high pH (preferably pH>10) which would greatly favor ammonia over ammonium. Thus, as will be understood by those of skill in the art, a lesser amount of stronger base (e.g., higher Molarity or dibasic
base) can be used, or a greater amount of a weaker base (e.g., lower Molarity or monbasic). The exact amount of base added will depend on the concentration of ammonium present in the reacting system, or the amount expected to be formed in the reacting system.

In one embodiment, the base is contacted with the starting material complex after most of the carbonate precipitation reaction has occurred, or after nearly all the urea has been hydrolyzed; these are two different reactions, and even if most to all the urea is depleted, carbonate precipitation may still be occurring and can be further “stimulated” or driven by addition of base—again, by shifting the system to more basic conditions which favor mineral precipitation. The timing of contacting can be determined by those of skill in the art based on the intended use. In one non-limiting example, one could draw a sample and test concentrations of either calcium or urea or both and then estimate the extent of the reaction and add base accordingly. In another embodiment the pH can be checked at appropriate intervals and base added as necessary for the intended use. In various further embodiments, the method may comprise checking the pH of the complex at a location of interest to estimate or determine the ammonia-ammonium concentrations, including but not limited to checking Ca, NH₄ and/or NH₄⁺ levels, which can be used to estimate/determine the ammonia-ammonium ratios, and/or estimate/determine the extent/amount of carbonate precipitation.

The contacting can be carried out for as long as appropriate for an intended use. As will be understood by those of skill in the art, upon contacting of the complex with the base, the reaction and shift from ammonium to ammonia occurs very rapidly, but may be spatially limited to areas near a site of contacting the complex with the base. Thus, in one embodiment, the methods may comprise introducing the base into the complex at more than one location in the complex. Any suitable number of different locations for contacting of the complex with the base can be used, as deemed most appropriate for an intended use.

The base can be contacted with the complex via any suitable technique, including but not limited to injection or percolation of the base into the starting complex, or via any other means of introducing the base into the complex.

“Carbonate mineral precipitation” means mineral precipitates that may include one or more cations that may produce one of several phases of carbonate minerals, including but not limited to calcite. In a preferred embodiment, calcium carbonate precipitates form cementation bonds at inter-particle contacts in the starting material and fill in void spaces in the starting materials (thereby increasing the tendency of the starting material to dilute, or expand in volume, when sheared), and/or cementation of adjacent particles of the starting material.
Several divalent cations, primarily alkaline earth metals and other divalent-state metals (including but not limited to such as calcium, magnesium, strontium, iron, cadmium, manganese, lead, or zinc ions, or combinations thereof), that satisfy the crystalline structure constraints of calcite or calcium mineral carbonates can be used to precipitate carbonate minerals in the present methods. Any suitable source of divalent cations can be used, including but not limited to salts of organic and inorganic compounds such as nitrates, nitrites, chlorides, sulfates, oxides, acetates, silicates, oxalates or mixtures thereof.

The appropriate amount of ions can be determined by one of skill in the art based on the teachings herein; factors to be considered in determining an appropriate amount of ions include, but are not limited the required amount of carbonate precipitate required for the particular application as determined on a stoichiometric basis. In one non-limiting example, if 100 grams (approximately 1 mole) of calcium carbonate (CaCO₃) is desired, then 1 mole of urea ((NH₂)₂CO) and at least 1 mole of calcium (Ca²⁺) are required (in addition to alkalinity, the urea also provides the necessary 1 mole of carbon to ultimately form CO₃²⁻).

Any suitable starting material, or combinations of starting materials, may be used in the methods of the invention, such as those having a particulate structure or those consisting of discrete soil particles forming a stable framework (or skeleton) or relatively impervious blocks delineated by an interconnected network of fractures or fissures. In a preferred embodiment, the starting material may be unconsolidated or partially consolidated particulate material such as sand, silt, soil, clay, sediments, sawdust or other material that is amenable to in situ cementation, or combinations thereof. As used herein, “particulate” starting materials are starting materials comprising separate particles.

In another embodiment, the starting material may comprise fractured rock masses. Such starting materials may be mixed in situ or mixed and compacted with the urease, the urea, and the source of divalent cations. In a further embodiment, the starting material is saturated.

In further embodiments, the starting material may be gravel, igneous, metamorphic, or sedimentary rocks including but not limited to conglomerate, breccia, sandstone, siltstone, shale, limestone, gypsum, and dolostone, or combinations thereof. In one preferred embodiment, the starting material comprises sand. In another preferred embodiment, the starting material comprises silt. In a further preferred embodiment, the starting material is fractured crystalline rock or cracked concrete.

The starting material may be “porous” in that it enables sufficient passage of the urease, the urea, and/or the source of calcium or other ions and constituents including, but not
limited to, buffers and stabilizers, to enable carbonate precipitation with or without
cementation. In some applications, the method may work simply by filling the void spaces
(e.g. soil pores, rock fractures) with the carbonate precipitate, without any actual cementation
of/bonding to the host material.

The components can be combined in any way suitable in light of the specific starting
material, the amount of starting material, the components to be used, etc. In various
embodiments, the starting material and components are combined by a technique selected
from the group consisting of flushing, injecting, mixing, spraying, dripping or trickling onto
or into the starting material. The starting material may also be immersed in one or more ways
as described above. In addition, secondary non-specific methods may be employed to
facilitate carbonate precipitation including, but not limited to, moisture control measures,
crystal seeding, and initiation of nucleation sites. In one embodiment, the methods comprise
mixing powdered urease or urease in solution with a particulate starting material prior to
percolation of a solution comprising the urea and the divalent ion source or combining the
urease, urea, and divalent ion in solution with the starting material, mixing them with in situ
or mixing them ex situ and then placing or compacting the mixture. In another embodiment,
the combining comprises injection of the urease, urea, and/or divalent cations into the starting
material via one or more central porous injection tubes, such as are described in the examples
that follow. Any number of such injection tubes may be used, depending on the size and
depth of the starting material to be treated, among other factors, and any design or size of
such injection tubes may be used. In one non-limiting embodiment, field injection tubes may
comprise 2 inch or 3 inch diameter perforated pipe that is vibrated or pushed into the soil.
The tubes may be placed at varying depths and orientations in the starting material. In one
embodiment, injection occurs all along the length of the tube at once, or may comprise
injection over small intervals (such as 1-3 foot intervals) working from one end of the tube,
which can be accomplished, for example, by sealing off sections where injection is to occur
with packers, as is known in grouting technology.

The source of urease may comprise isolated urease or cells expressing urease.

As used herein, “isolated urease” is urease that is extracted from cells and cellular
materials. The urease may be synthetically produced or obtained by extraction from any
suitable source, including but not limited to bacteria, plants, invertebrates, and fungi. In one
non-limiting embodiment, a plant derived urease extract can be used. For example, urease
activity is present in various plant leaves and this activity can be realized using crude extracts
of the leaves, or isolated enzyme. Urease enzyme as discussed herein is characterized by the
reaction it catalyzes and identified by EC 3.5.1.5 (i.e. Reaction: \( \text{urea} + \text{H}_2\text{O} = \text{CO}_2 + 2\text{NH}_3 \)). In one embodiment, the urease enzyme is isolated from the jack-bean plant (SEQ ID NO:1). The amino acid sequences of exemplary ureases for use with the present invention are provided below. However, it will be clear to those of skill in the art that any enzyme identified by EC 3.5.1.5 can be used in the methods of the invention, including but not limited to a urease comprising or consisting of any one of SEQ ID NOS: 2-5, where SEQ ID NO:2 is a soybean urease, SEQ ID NO:3 is a *Agaricus bisporus* urease, SEQ ID NO:4 is a *Schizosaccharomyces pombe* (strain 972 / ATCC 24843) urease, SEQ ID NO:5 is a *Sporsarcina pasteurii* urease, and SEQ ID NO:6 is a *Pseudomonas syringae* (strain B728a) urease.

The appropriate amount of urease needed can be determined by one of skill in the art based on the teachings herein; factors to be considered in determining an appropriate amount of urease include, but are not limited to:

(a) urease source type (e.g. Jack bean vs. other source, such as a cell expressing urease)

(b) urease purity, which dictates enzymatic activity (i.e. rate of conversion of urea to products); and

(c) the stability/half-life of the enzyme matrix used, where the “enzyme matrix” refers to the specific form of the enzyme mixture used such as liquid, powder and/or solid when combined with or used apart from stabilizers, buffers, fillers or other media to facilitate its desired use.

For example, assuming that for practical purposes that transport via diffusion, advection and dispersion is not limiting the availability of urea or calcium to the enzymes—or vice versa—(e.g. we thoroughly mix the soil and cementation constituents or we actively pump the cementation constituents into the soil or do something to assure that the right constituents get to where they need to be), then in a homogenous soil (i.e. without zones of blocked flow or disproportionately high/preferential flow) we could expect an approximately linear relationship between urea conversion and required amount(s) of enzyme needed to convert “x” grams of urea to “y” grams of calcium carbonate over a given time frame. This is also dependent on the on the amount of calcium ions available for precipitation. A sufficiently high concentration of calcium to form calcium carbonate is needed, with hydrolysis of urea just one part of the overall process. If a soil mass requires a total amount “x” grams of urea to be converted into products for calcium carbonate formation, and “y”
grams of enzyme can only convert 50% of “x,” during its functional life time, then theoretically twice as much enzyme is needed to fully convert “x” grams of urea.

Urea is an organic compound of the chemical formula CO(NH₂)₂. Urea is a colorless, odorless, highly water soluble substance with very low toxicity (LD₅₀ = 12g/kg for mouse, Agrium MSDS), and is widely commercially available. Any suitable source of urea can be used, including but not limited to those disclosed herein.

The appropriate amount of urea can be determined by one of skill in the art based on the teachings herein; factors to be considered in determining an appropriate amount of urea include, but are not limited, to the amount of carbonate required for the particular application and the desired increase in pH and alkalinity as determined on a stoichiometric basis. As will be understood by those of skill in the art, the amount of carbonate precipitation required varies from one use to another.

It will be understood by those of skill in the art that the step of "combining" the starting material with effective amounts of urease, urea, and cations covers any process that results in the bringing together of the three constituents in a manner that results in precipitation of carbonate minerals in the starting material. The reactants may be added to the starting material simultaneously or sequentially. For example, there may be applications where one or two of the constituents are already present in the starting material, in which case the step of "combining" will involve the addition of only the missing components. In one embodiment, the urea and cations are admixed and then added to the urease prior to application to the starting material. However, it will be appreciated by those of skill in the art that the constituents may be combined in other ways to carry out the method of the invention.

By manipulating the relative effective amounts of the various components, the methods of the present invention enable the user to control carbonate precipitation by controlling the amount of carbonate formed and the rate at which it is formed. This flexibility means the methods of the present invention can be used in a wide range of applications from those that require a reasonably modest increase in the strength, stiffness, or dilatancy or modest decrease in the permeability in the starting material to those that require larger changes in the relevant property. As used herein, “dilatancy” refers to the tendency of a soil to expand in volume during shear. This is an important property, for example, with respect to reducing liquefaction potential.

The effective amounts of the various reactants combined according to the method of the present invention may vary depending, at least, on the amount of urease used, the characteristics of the starting material and the conditions under which precipitation is to
occur, the desired final strength, stiffness, dilatancy, or permeability of the treated porous material and the amounts of the other reactants in the reaction mix. The present application enables those of skill in the art to determine the relative amounts of the various reactants required for a given application and to apply the method to various starting materials and for a variety of end uses. The method of the present invention may be adapted to allow for the rate of mineral precipitation to be controlled, as required. When rapid or slow formation of the precipitate is desired the amounts and/or relative amounts of the reagents can be selected accordingly to bring about the desired rate of formation. In one non-limiting example, enhancement of the methods may comprise providing stronger nucleation sites on particles of the starting material by high-pH pretreatment of the particles of the starting material to be improved.

Depending on the requirements of a particular application or mode of use of the present invention, rapid formation of the precipitate may be required. Alternatively it may be preferred for the precipitate to be formed slowly. Based on the teachings herein, those of skill in the art will be able to modify the protocol to attain faster or slower formation of the precipitate.

The methods of the invention may be repeated (once, twice, three times, or more) in order to attain the desired amount of mineral precipitate strength, stiffness increase, or permeability reduction. When the method is repeated to gain incremental increases in strength or stiffness or reduction in permeability, not all of the reagents need to be added each time. For example, residual urease activity may still be sufficient for one or more subsequent rounds of the method. A skilled person is readily able to determine the particular amounts of reagents required for use in subsequent rounds of the method of the present invention.

The methods may be applied in situ without disturbing the starting material. This is particularly important for applications where the starting material is delicate or fragile or for other reasons must not be disturbed. Examples include, but are not limited to, when applied in the field where the starting material to be improved (e.g. made resistant to earthquake-induced liquefaction) is underneath or adjacent to an existing structure or facility that is sensitive to ground movement (e.g. settlement or heave).

As will be understood by those of skill in the art, the methods may comprise use of other components as appropriate for a given use. In one embodiment, the methods may further comprise use of a stabilizer (including but not limited to powdered milk) to increase enzyme stability and functional time. The methods can be carried out under any temperature conditions suitable to promote carbonate cementation.
In another embodiment, the method further comprises introducing a clay slurry into
the starting material prior to or concurrent with combining the starting material with the
urease, the urea, and the source of divalent cations. This embodiment is particularly useful
when the starting material comprises a high permeability starting material (e.g. a coarse-
grained soil), as it will help to retain the active components in the starting material where
cementation is desired; the clay particles may also serve as nucleation points for carbonate
precipitation. As used herein, a “clay slurry” is any clay in suspension. In various non-
limiting embodiments, the clay may comprise montmorillonite clay (also known as
bentonite), attapulgite, or combinations thereof. The amount of clay slurry, the specific
amount of clay in the slurry, and the timing/number of times the clay slurry is administered
may vary depending, at least, on the characteristics of the starting material and the conditions
under which precipitation is to occur, the desired final strength, stiffness, dilatancy, or
permeability of the treated porous material and the amounts of the other reactants in the
reaction mix. The present application provides examples that enable those of skill in the art to
determine the relative amounts of the clay slurry appropriate for a given application and to
apply the method to various starting materials and for a variety of end uses.

In a preferred embodiment, the starting material comprises a column of starting
material. As used herein, a “column” refers to relatively linear prisms of soil that are to be
stiffened and/or strengthened to reinforce the in situ soil mass and/or transfer load to greater
depths in the soil stratum. As will be understood by those of skill in the art, the prism of soil
extends below a surface of the starting material. In various embodiments, the column is at
least 0.6 meters long and 0.15 meters in diameter. In various further embodiments, the
column is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, or more meters long/depth. In
various further embodiments, the column is at least 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5,
2, or more meters in diameter. In one exemplary embodiment, for the mitigation of
earthquake-induced liquefaction under residential structures, the columns are between about
0.3 meters to 1.5 meters in diameter; more preferably about 0.9 meters in diameter and up to
20 meters long. In this embodiment, the methods comprise combining the starting materials
so that carbonate precipitation of the starting material occurs at a radial pattern around an
injection tube (e.g. perforated pipe) to form the column. In one embodiment, carbonate
precipitation occurs at only one or more specific areas within the column, such as at a
specific location where the starting materials are combined within the column (for example,
where liquefiable soil strata is intersected by the injection tube). In another embodiment,
carbonate precipitation occurs throughout the column.
This embodiment provides further improvements over prior methods, which are focused on improvement of the entire mass of soil in the improvement zone. In this embodiment, the methods permit production of stone-like materials via local cementation. Columns of improved starting materials, such as soils, have broad application in geotechnical practice, including reducing the potential for earthquake-induced liquefaction; mitigating the potential for damaging ground displacements subsequent to earthquake-induced liquefaction, improvement of foundation bearing capacity, support of embankments, slope stabilization, stabilization of the base of excavations, support of underground openings, and a variety of other geotechnical purposes. The limitations associated with bio-plugging can also be mitigated by using this technique to improve columns of soil (rather than the entire soil mass). Such columns of improved starting material possess, for example, increased strength, stiffness, and liquefaction resistance relative to the non-treated starting material. Bio-plugging refers to hindering the mobility of the compounds that were added to the starting material for the purpose of inducing mineral precipitation due the decrease in permeability associated with mineral precipitation. In another embodiment, the methods can be used for fugitive dust control (e.g., wind-blown erosion).

In one non-limiting example, consider a 4.2-meter long, 1-meter diameter column of soil with a porosity of 0.31 is to be improved by precipitating 5% calcium carbonate by weight. This column has a total volume of 3.29 m$^3$ and a pore space of 1.0 m$^3$. Assuming a calcium carbonate density of 2.71 g/cm$^3$ (2710 kg/m$^3$), approximately 136 kg of CaCO$_3$ is used to precipitate in the improvement zone (i.e. the column). To obtain 136 kg of CaCO$_3$ (1,360 moles), 81.6 kg of urea (1,360 moles) and 54.4 kg of calcium (1,360 moles) are used, assuming that all added constituents are available and used in the reaction process to precipitate CaCO$_3$. Since there are 2 amine groups per urea molecule, 2,720 moles of ammonia nitrogen and 1,360 moles of carbon dioxide are released from 81.6 kg of urea. Assuming a minimum urease activity of 15,000 units/gram urease powder (type 3 urease from Jack bean [Sigma Aldrich]), where one-unit is the release of 1.0 mg ammonia nitrogen from urea in 5 minutes at pH 7.0 at 20 °C, then 10 grams of this particular grade of enzyme (150,000 units) will release 150 grams of ammonia nitrogen (∼8.8 moles) in 5 minutes. Therefore, 26 hours (1546 minutes) are required to fully catalyze the release of carbon dioxide and ammonia from 81.6 kg of urea.

Any suitable technique/configuration for introducing the components into the column may be used as deemed appropriate. For introduction of the urease into columns, injection
could be done at one site per column or at multiple sites along the column. If multiple sites, then the concentration may the same or different at the different locations.

In another embodiment, the starting material is saturated. As used herein, “saturated” means that the soil has reached its maximum water content; if any more water is added it will either drain downward, flow upwards, or turn the soil into a suspension wherein there is little to no inter-particle contact. In one example, the saturated starting material is below the water table. When the starting material is below the water table it is usually saturated, unless there is gas in the soil. Saturated zones may also exist above the water table due to capillary rise, with the extent of the saturated zone above the water table depending upon the pore size of the starting material.

In a second aspect, the present invention provides kits comprising

(a) isolated urease;
(b) urea;
(c) a source of divalent cations, preferably calcium ions; and
(d) a base;

wherein (a), (b), (c) and (d) are provided in amounts effective to cause carbonate cementation when combined with a particulate starting material or a starting material complex. In another embodiment, the kit further comprises a clay slurry.

All definitions, embodiments, and combinations thereof of the first aspect apply equally to this second embodiment, unless the context clearly dictates otherwise. Thus, the kits may further contain any of the components or combinations thereof disclosed for use with the methods of the invention, including but not limited to stabilizers, buffers, nucleation aids, etc.

Example 1. **Carbonate Cementation via Plant Derived Urease**

**METHODS**

*Ottawa 20-30 Sand*

Laboratory column tests were conducted using plant derived urease to induce calcium carbonate (CaCO₃) precipitation in Ottawa 20-30 sand. These tests were carried out in 6” x 2” (152 mm x 51 mm) acrylic tubes and membrane-lined 2.8” x 6” (71 mm x 152 mm) split molds for creating specimens for triaxial testing. Three acrylic tubes and two columns for triaxial testing were filled with 20-30 Ottawa silica sand (mean grain size 0.6 mm, coefficient of uniformity 1.1) and treated as follows: tube #1: the sand was dry pluviated via funnel at ≈3” (76 mm) drop height and then received 5 applications of a cementation solution.
containing urea and calcium chloride mixed with 1.4g/L enzyme (total solution volume ≈ 300 ml); tube #2: sand was added in same manner as tube #1 and then received 2 applications (≈ 150 ml total) of the same cementation solution mixed with 1.4g/L enzyme; tube #3: the lower-third of tube was filled with sand and dry enzyme (≈ 3g), the remainder of the tube contained dry pluviated sand without enzyme, and the tube then received 2 applications (≈ 150 ml) of the cementation fluid with no enzyme added.

Approximately 100 mL of a pH=7.8 solution containing 383 mM urea (reagent grade, Sigma-Aldrich), 272 mM CaCl$_2$-2H$_2$O (laboratory grade, Alfa Aesar) was used for the first application in each acrylic tube. Subsequent applications employed approximately 50 mL of a pH=7.6 solution containing 416 mM urea and 289 mM CaCl$_2$-2H$_2$O. Solution concentrations, while variable, were formulated within a reasonably similar range as a matter of convenience. In each application, the cementation fluid was poured into the top of the acrylic tube with the bottom closed off. The cementation fluid was allowed to stand, loosely covered, in the acrylic tube for at least 24 hours and then drained out the bottom of the cylinder. The next application followed immediately after drainage was complete. Drainage was accomplished by puncturing the base of the cylinder with a 20-gauge needle. When drainage was complete, the needle was removed and the puncture was plugged with a dab of silicone. Occasionally, the needle became plugged and an additional needle was inserted through the base. The triaxial columns were filled with sand in the same manner as tube 1 and then received 2 applications (each application ≈ 250 ml) of cementation solution with 1.4g/L enzyme.

In each application of cementation fluid, the fluid was added until it rose to approximately ½-inch (12-mm) above the soil line. After 2 applications, tubes #2 and #3 were allowed to air dry for several days and then analyzed. Experimentation with tube #1 was continued for several more days as three more batches of cementation fluid were applied.

The last 2 applications of cementation fluid were allowed to slowly drain through the needle in the base immediately after application rather than sit for 24 hours (drainage rate ≈10-25ml/hour). The triaxial columns were allowed to stand for at least a week after the second cementation fluid application and then drained.

After drainage was complete, the triaxial columns were moved to a triaxial testing device. After draining the specimens from the acrylic tubes and after the completion of the triaxial tests, all samples were triple washed with de-ionized water. Tubes #2 and #3 were separated in 3 layers, while tube #1 was separated into six layers (for better resolution). Each layer from the specimens in the acrylic tubes and the entire mass of the triaxial specimens were acid washed to determine CaCO$_3$ content by oven drying for 48 hours, weighing,
digesting with warm 1M HCl, washing, drying, and reweighing to determine carbonate mineral content.

Several of the cemented specimens were analyzed for mineral identification using X-Ray Diffraction (XRD). Samples were ground in an agate mortar and pestle and powdered onto a standard glass slide for analysis. Scanning electron microscopy (SEM) imaging was performed on intact cemented chunks of material with an Agilent 8500 Low-Voltage SEM (LV-SEM). A LV-SEM is a field emission scanning electron microscope capable of imaging insulating materials, such as organic and biological substances without the need for a metal coating and without causing radiation damage to samples.

*Ottawa F-60 Sand*

A triaxial column was prepared using Ottawa F-60 silica sand (mean grain size 0.275 mm, coefficient of uniformity 1.74) to investigate enzymatic ureolytic CaCO₃ precipitation in a finer grained material than Ottawa 20-30 sand. The specimen was prepared in the same manner as described for the triaxial columns for the Ottawa 20-30 sand. The cementation fluid for the first of the two applications contained approximately 2.0 g/L enzyme, 400 mM urea (reagent grade, Sigma-Aldrich), 300 mM CaCl₂·2H₂O (laboratory grade, BDH) at pH=7.7. The fluid for the second application contained 1 M urea-CaCl₂·2H₂O solution at pH=7.8 without any enzyme. After the test, the triaxial specimen was washed and subject to acid digestion in the same manner as the Ottawa 20-30 triaxial specimens.

**RESULTS**

*Acrylic Tubes*

Approximately 100 ml of cementation solution was delivered per application for the first application in each acrylic tube. However, the amount of solution the tube would accept was notably reduced in subsequent applications, when less than 75 ml was typically required to fill the tubes to ≈ ½ inch (12 mm) above soil line. At the conclusion of the experiment, precipitation was visible along the entire length of tubes 1 and 2. Internally the cementation was variable, with some highly cemented zones and other zones with little to no cementation.

Tube 1 yielded mostly small, loose chunks of sand with strong effervescence upon digestion. Most of this column appeared un-cemented and exhibited unusually viscous behavior when wet. A fairly large (compared to column diameter) piece of strongly cemented sand (not breakable without tools) formed in the deepest layer of tube 1. Tube 2 had many small chunks of weakly cemented sand with strong effervescence upon digestion. Tube 3 had
little to no precipitation in the top layer (i.e. this layer did not show any indication of carbonate upon acid digestion.) The deepest layer of tube 3 contained many pieces of weakly cemented sand that effervesced strongly upon digestion. The middle layer of tube 3 contained a few pieces of cemented sand that effervesced moderately upon digestion. The results from the acid washing are presented in Table 1.

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<th>Tube #</th>
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<th>Weight Change via Digestion (%)</th>
<th>Amt. of CaCO₃ (g)</th>
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<th>Theor. Max CaCO₃ (g)</th>
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<td>3.57</td>
<td>11.8</td>
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<td>2</td>
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<tr>
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<td>3</td>
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<tr>
<td></td>
<td>4</td>
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Table 1. Results from Experiment Set 1 using 20-30 Ottawa silica sand

The theoretical maximum CaCO₃ content is the stoichiometric maximum balanced on initial concentrations. The primary experimental differences between the tests are (1) the number of applications of cementation fluid and (2) the manner in which the urease was delivered. The results indicate that there is greater carbonate precipitation with increasing number of applications, as expected. The data show more precipitation in (or on) the top layer of tubes 1 and 2 but not in tube 3, as the enzyme was physically confined to the lower-third layer in tube 3 during sample preparation. In the top layer of tube 3, where no urease was mixed with the sand, carbonate precipitation was nearly undetectable. There was no visual evidence of precipitation and practically no measurable change in weight of this layer after acidification (weight change = 0.23%). In the bottom layer of tube 3, where 3 g of dry enzyme was mixed with the soil, there was a weight change of 1.7% following acid washing. The middle layer of this specimen had a minor change in weight (0.58%), possibly due to uneven distribution of the layers during preparation or splitting of the specimen or to upward migration of urease from the bottom layer. XRD analysis confirms that calcite is the
mineral phase present in the cemented soil chunks. LV-SEM images, presented in Figure 1, show silica (quartz) sand particles cemented with calcium carbonate and various morphological features associated with the cementation process on the silica surface.

**Triaxial Columns**

The three triaxial sand columns (2 Ottawa 20-30 sand columns and 1 Ottawa F-60 sand column) were tested in drained triaxial compression prior to acid digestion. All three columns were able to stand upright after removal of the split mold. The results of the triaxial compression tests performed on the 20-30 Ottawa sand are presented in Figure 2 and the results for the F-60 Ottawa sand are presented in Figure 4. The carbonate cement content for one of the 20-30 silica sand columns was 2.0% CaCO₃ (by weight). The carbonate content of the other 20-30 Ottawa sand column could not be quantified due to unintended sample loss. The carbonate cement content for the finer grained F-60 Ottawa sand was 1.6% CaCO₃ (by weight). The results show substantial strength increase for all 3 sand columns tested.

**CONCLUSION**

Sand column tests have shown that agriculturally-derived urease can be used to induce calcium carbonate precipitation in sand. Sand columns were developed using Ottawa 20-30 and F-60 sand and three different preparation methods: dry pluviation followed by percolation of a calcium-urease-urea cementation solution, pluviation into a calcium-urease-urea cementation solution, and mixing the sand with urease prior to pluviation with a calcium-urea solution. Cementation was observed in all of the columns. XRD and SEM testing confirmed that calcium carbonate (specifically calcite) was the cementing agent. Acid digestion showed that increased applications yielded correspondingly greater carbonate precipitation. The quality of cementation, as determined by the effort needed to break apart cemented chunks of sand, varied depending on the sampling location within the column. Triaxial test results on cemented columns showed substantial strength increase over non-cemented columns at the same relative density.

**Example 2**

**METHODS:**

Three sand columns were constructed in 12” long by 4” (I.D.) clear PVC tubes (schedule 40) and labeled “Column #1,” “Column #2,” and “Column #3.” Column #1 and Column #2 used
Ottawa 20-30 sand, a medium (grain size) sand, while Column #3 used Ottawa F60 sand, a fine sand. Each PVC column was closed-off on one end using a flexible cap (Qwik Cap) and fastened with a hose clamp. The 3 columns were filled with densified sand to depth of approximately 4". Next, an injection tube was made of flexible ¼" (O.D.) Tygon tubing with 6-8 needle size holes (18 gauge) in a radial pattern along the last 1.5” of the tubing. The injection tube was then placed along the axis of the column with the end containing needle holes approximately 0.25” above the 4” densified soil layer. The columns were then loosely filled with the designated sand using a small scoop to a height of 10” (i.e., 6” above the 4” base layer). Each column was then filled through the injection tube with approximately 700 mL of tap water at 35°C in order to fully hydrate the columns—the final water level was just above the top of the soil (i.e. slightly more than 10” from the bottom of the tube). In order to create a uniform soil column, the columns were then densified by firmly tapping the outside of the PVC tube in a radial pattern using a blunt instrument, which reduced the final soil column height from 10” to approximately 9.4” in each column. Approximately 40 mL of bentonite slurry was injected into Column #2 through the Tygon injection tube. Each column then received 155 mL of the reaction medium consisting of tap water with 1.36 M urea and 0.765 M calcium chloride dehydrate (pH=7.3, 35°C). After receiving the reaction medium, the injection tubes were flushed with 2 mL water. Then 15-20 mL of enzyme solution consisting of 0.44g/L urease enzyme (Sigma Aldrich Type-III, Jack Bean Urease, 26,100 U/g activity) and 4 g/L stabilizer (nonfat powdered milk) were introduced to each column through the injection tube. Finally, another 3-4 mL of reaction medium was injected into each column followed by a 2mL water flush. The injection tubes were closed with polypropylene pinch clamps and the columns were capped with plastic clear wrap and placed in dark, warm (30°C) environment for 28 days. On day 7, an additional 115 mL of reaction medium and 10 mL of enzyme solution was delivered to each column in a manner similar to described above. Approximately 3 mL (0.1% v/v) of 1M NaOH was injected into each column 48 hours before the experiment terminated.

**RESULTS:** Upon disassembly, all 3 columns showed strong carbonate cementation in a cylindrical zone around the end of the inject tube. The results varied slightly for each column, as follows:

**Column #1** (20-30 medium sand)- A region of strongly cemented soil began ~2.5” from the column bottom. The cemented zone was ~4.5” in length and displayed a prominent rounding at the top and a flat surface at the bottom. Overall, the cemented region appeared to have a cylindrical bottom and bulb-shaped upper portion (Figure 4). Several chunks of cemented soil
were dislodged to access the most strongly cemented region which had the injection tube firmly embedded. The cemented region could not be dislodged from the PVC column without the use of hand tools.

**Column #2 (20-30 medium sand w/ 40 mL bentonite slurry)** - A cylindrical region of strongly cemented soil began ≈3” from the column bottom, was ≈4” in length and displayed small rounding near the top and a flat surface at the bottom. Overall, the cemented region was mostly cylindrical (Figure 5). Several chunks of cemented soil were dislodged to access the most strongly cemented region which had the injection tube loosely embedded. The cemented region could not be dislodged from the PVC column without the use of hand tools.

**Column #3 (F-60 fine sand)** - A region of strongly cemented soil began ≈3” from the column bottom, was ≈2.5” in length and displayed a clear bell-shaped top and a flat surface at the bottom. Overall, the cemented region appeared bell-shaped (Figure 6). The entire soil column was dislodged upon disassembly and many chunks of cemented soil were dislodged to access the most strongly cemented region which had the injection tube firmly embedded. In addition, the soil overall mass contained many small (1-3 mm) pieces of cemented sand. The column had a strong smell of ammonia and displayed significant amounts of gas bubbles during washing.

Example 2 demonstrates (for example) the ability to create roughly cylindrical columns in a saturated soil (i.e. below the water table); stabilize a fine grained material; and the optional facilitation of cementation by first injecting a bentonite slurry into the column, which provides particular benefit when used with more porous (coarser) starting materials.

**Example 3**

In one experiment, using 4” by 12” PVC columns, a final step was used as follows: the injection of approximately 0.01% by volume (~2 ml/column) of pH=13 NaOH during the last week of the experiment. The purpose of this injection was to help drive a shift of ammonium → ammonia to limit the potential of reaction reversal due to the ammonium (this will temporarily increase pH near the point of injection, which is also has a beneficial effect with respect to inducing carbonate precipitation)--this not only limits the potential for reversal of and enhances carbonate precipitation, but also helps rid the system of toxic ammonium salt by-products. A further effect of adding this strong base (NaOH) to the system is that it may help expose/dissolve some of the soil particle or substances dressing the soil particle and thereby facilitate more and/or stronger bonding of the soil to be improved (e.g.
gravel, sand, or silt particles susceptible to degradation/dissolution via base) with the precipitated calcite (calcium carbonate).

Example 4. Columnar EICP Stabilization Tests

The details of chemical and enzyme preparation are the same as those described in the previous examples, as is the application to different soil types. Test were set-up in 5-gallon buckets using coarse sand. Perforated PVC tubes were placed in center of 5-gal buckets. Buckets then filled w/ coarse silica sand; half were wet studies (the soil was essentially saturated, i.e. there was water to almost the top of the soil layer at the start of the experiment) and half were dry studies (i.e.: dry soil). The wet tests simulate improving soil below the water table while the dry test simulates soil above the water table.

Cementation solution (same conditions as disclosed above) was poured into the perforated PVC tubes. Approximately 3.5 weeks later, 0.1% NaOH (1M) added and the mix was allowed to sit for approximately 2 weeks. The buckets were then rinsed & drained and allowed to sit for approximately 1 week. Cemented soil specimens were then removed from the buckets for analysis. See Figures 20-22. The soil stuck to the tubes in all experiments (wet or dry), while the strength of attachment varied from firmly stuck to weakly stuck based on the extent of cementation near the tube. In general, the larger and more uniform the cemented specimen, the more likely to be stuck to the injection tube; similarly, cementation in wet soil generally resulted in some of the cemented specimen being stuck to the injection tube. Penetration of the cementation solution into the dry soil was somewhat more uniform than in the wet soil. These data demonstrate effective cementation both wet and dry soil. These data demonstrated that the addition of base (1) resulted in a shift towards NH\textsubscript{3} and away from NH\textsubscript{4}\textsuperscript{+}, (2) inhibited nitrification, and (3) shifted the CaCO\textsubscript{3} reaction towards

further precipitation.
We claim

1. A mineral precipitation method, comprising
   (a) combining a porous starting material with
       (i) a source of urease;
       (ii) urea; and
       (iii) a source of divalent cations;
   wherein (i), (ii), and (iii) are provided in amounts effective and the combining is
carried out under conditions suitable to cause carbonate mineral precipitation of the starting
material to produce a starting material complex; and
   (b) contacting the starting material complex with a base under conditions to
mitigate formation of ammonium salts in the starting material complex.

2. The method of claim 1, wherein the base is introduced into the starting material
   complex at one or more locations.

3. The method of claim 1 or 2, wherein contacting the starting material complex with the
   base results in an increased pH of the starting material complex at the site of the contacting.

4. The method of any one of claims 1-3, wherein the base is selected from the group
   consisting of NaOH, KOH, Ca(OH)₂, Mg(OH)₂, and combinations thereof.

5. The method of any one of claims 1-4, wherein the source of urease comprises isolated
   urease or cells expressing urease.

6. The method of any one of claims 1-5, wherein the method is used for one or more of
   improving bearing capacity of foundations; reducing settlement potential of foundations or
   embankments; stabilizing slopes; reducing the potential for triggering earthquake-induced
   liquefaction; reducing the potential for triggering static liquefaction; mitigating the potential
   for damaging ground displacements subsequent to triggering of liquefaction; increasing
   lateral resistance of foundations; enhancing stability of slopes or embankments; reducing
   lateral earth pressures on retaining walls; increasing passive resistance of retaining walls;
   increasing capacity of ground anchors or soil nails; increasing the side resistance and tip
   resistance of deep foundations; facilitating tunneling in running or flowing ground;
   stabilizing excavation bottoms; soil erosion control; and groundwater control.
7. The method of any one of claims 1-6 wherein the starting material is selected from the group consisting of sand, silt, clay, other sediments, sawdust, igneous rocks, metamorphic rocks, gravel, fractured crystalline rocks, cracked concrete and sedimentary rocks including but not limited to conglomerate, breccia, sandstone, siltstone, shale, limestone, gypsum, and dolostone, and combinations thereof.

8. The method of any one of claims 1-7, wherein the source of divalent cations is selected from the group consisting of a source of divalent calcium, magnesium, strontium, iron, cadmium, manganese, lead, or zinc ions, or combinations thereof.

9. The method of any one of claims 1-8, wherein the combining step and step (b) are carried out more than once.

10. The method of any one of claims 1-9, wherein the combining comprises
   (i) mixing the source of urease with the starting material; and
   (ii) introducing a solution comprising the urea and the source of divalent cations into the mixture of starting material and urease source.

11. The method of any one of claims 1-10, wherein the method further comprises introducing a clay slurry into the starting material prior to or concurrent with combining the starting material with the source of urease, the urea, and the source of divalent cations.

12. The method of any one of claims 1-11, wherein the starting material comprises a column of the starting material.

13. The method of any one of claims 1-12, wherein the starting material comprises a saturated starting material.
Figure 1
Figure 3
Figure 7
Figure 9
Figure 16
A. CLASSIFICATION OF SUBJECT MATTER
C12N 9/78(2006.01)i, A01G 7/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
C12N 9/78; E02D 3/12; C02F 1/62; C04B 41/52; E21B 43/22; C04B 41/50; A01G 7/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of database and, where practicable, search terms used)
eKOMPASS (KIPO internal) & Keywords: mineral precipitation, carbonate, urea, urease, divalent cations, cementation

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<td>US 7841804 B2 (OSTVOLD, TERJE et al.) 30 November 2010 See claims 1-2.</td>
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<td>US 5143155 A (FERRIS, FREDERICK G. et al.) 01 September 1992 See claims 1, 4 and 11-12.</td>
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<td>A</td>
<td>US 5730873 A (HAPKA, ALISON MARIE et al.) 24 March 1998 See claims 1, 3 and 7.</td>
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☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier application or patent but published on or after the international filing date
  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed
  "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  "&" document member of the same patent family

Date of the actual completion of the international search
11 February 2015 (11.02.2015)

Date of mailing of the international search report
12 February 2015 (12.02.2015)

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongna-ro, Seo-gu, Daegu Metropolitan City, 302-701, Republic of Korea
Facsimile No. +82-42 472 3473

Authorized officer
HEO, Joo Hyung

Telephone No. +82-42-481-8150

Form PCT/ISA/210 (second sheet) (January 2015)
### Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   - because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   - because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-13
   - because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2))  (January 2015)
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