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(54) Title: COMPOSITIONS FOR BIOREMEDIATION

(57) Abstract: Water based compositions comprising bacteria and water dispersible or soluble polymers are described for the treatment of contaminated subterranean materials by either ex-situ or in-situ methods. The compositions may additionally contain one or more polyols, one or more monosaccharides or specified ranges for polymer content or a combination of these features.



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COMPOSITIONS FOR BIOREMEDIATION

[0001] The present invention relates to compositions that utilize micro-
microorganisms for the bioremediation of contaminated soils to reduce or
5 remove the contamination. More particularly this invention relates to
compositions for the delivery of microorganisms, especially bacteria, to
contaminated soils and to methods for the delivery of such compositions and is
of particular relevance to the in-situ bioremediation of contaminated soils.

10 **[0002]** It is known that microorganisms such as for example bacteria are
useful in reducing or removing a wide variety of contaminants from a variety of
contaminated environments. It is known that bacteria are capable of degrading
hydrocarbons such as for example oil or fuel oil contamination by the oxidation
or partial oxidation of the hydrocarbon based contaminant to form carbon
15 dioxide and water.

[0003] A particularly challenging environment for bioremediation is
subterranean contamination where it is highly desirable to be able to reduce or
remove the contamination to enable effective and safe re-use of the
20 contaminated land. Often this type of contamination has been treated by ex-
situ methods in which the contaminated soil is removed and treated on or off
site to reduce or remove the contaminants. An alternative method is to treat the
contaminated subterranean regions in-situ.

25 **[0004]** In both the ex-situ and in-situ methods the microorganisms are
often not very effective in reducing or removing the contamination because the
microorganisms themselves and/or enzymes which they produce may be
dispersed too readily from the location of the contaminant or may not be easily
and effectively delivered to the location of the contamination. This is a
30 particularly challenging problem with in-situ bioremediation.

[0005] One approach to delivering microorganisms to a variety of contaminated locations and for protecting locations from contamination is described in United States Patent No. 5,821,113. In this patent a water based composition is described which contains bacteria and a water soluble polymer at a concentration of up to 10% by weight of the composition for in-situ bioremediation. The patent also describes a composition that contains polymer at 0.2 wt% for spraying onto solid surfaces. The compositions may also contain other components such as nutrients for the bacteria such as NPK fertilizer, enzymes, sucrose and other sources of carbon. It is also indicated that surfactants may be utilized as an additional component although no examples or description of their use is provided.

[0006] A further approach is described in United States Patent No. 6,210,955. In this patent a foam based fluid derived from water and surfactants such as Triton X-100 and Tween-80 is utilised to introduce treating agents into contaminated soil. There is no mention of the use of additional polymers in this reference.

[0007] A further approach known in the art is Surfactant Enhanced Aquifer Remediation (SEAR) or surfactant flooding. In this process the contaminants are removed from the contaminated locations under the action of the surfactant and any solvents that are used in the process. In the paper entitled "In-situ Remediation of Coal-Tar Impacted Soil by Polymer-Surfactant Flooding", C.M.Young et.al., a process of surfactant flooding is described which utilises a Xanthan biopolymer in combination with the surfactant. With SEAR the approach is to recover the contamination from the site of contamination. Whilst this approach has some attractions it is also problematic as it requires careful control and assessment to ensure that the contaminant is not merely removed from one subterranean location to another.

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[0008] Whilst the methods and compositions described in the art have to some degree been effective in bioremediation applications there is still a need

for new compositions and new methods of application of such compositions for bioremediation, especially in-situ bioremediation.

[0009] According to a first aspect of the present invention, there is
5 provided an aqueous bioremediation composition comprising one or more
microorganisms capable of degrading organic material and one or more water
dispersible or water soluble polymers, wherein the composition comprises from
0.25 to 5 % by weight of water dispersible or water soluble polymer based on
the total weight of the composition. In a preferred embodiment the composition
10 comprises from 0.3 to 4 % by weight, more preferably 0.3 to 3 % by weight,
more preferably from 0.3 to 2.5 % by weight, more preferably from 0.4 to 2 %
by weight, more preferably from 0.5 to 1.5 % by weight and most preferably
from 0.5 to 1.25 % by weight of water dispersible or water soluble polymer,
based on the total weight of the composition.

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[0010] According to a further aspect the present invention provides for a
method of treating contaminated subterranean material, which method
comprises contacting the contaminated subterranean material with one or more
compositions according to the present invention. The contact may be in-situ or
20 ex-situ. Preferably the contact is in-situ.

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[0011] Thus according to a further aspect of the present invention, there
is provided a method for the in-situ bioremediation of contaminated soil which
method comprises:

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(i) treating contaminated soil by injecting a composition comprising one
or more microorganisms capable of degrading organic material and one
or more water dispersible or water soluble polymers at a concentration of
from 0.25 to 5 % by weight of water dispersible or water soluble polymer
based on the total weight of the composition, into a subterranean region
of contaminated soil via an injection well having an outlet located at a

depth of at least one meter below the subterranean surface and proximate to the subterranean contamination, and in addition

5 (ii) treating contaminated soil by injecting a composition comprising one or more microorganisms capable of degrading organic material and one or more water dispersible or water soluble polymers at a concentration of from 0.1 to 0.5 % by weight of water dispersible or water soluble polymer based on the total weight of the composition, into a subterranean region of contaminated soil via an injection lance which is inserted into the
10 surface of the soil such that the composition is injected at a depth of up to 1 meter depth below the soil surface.

[0012] In preferred embodiments the compositions for lance injection comprise between 0.1 to 0.4, preferably 0.15 to 0.3 and most preferably 0.2 to
15 0.4 wt% of the polymer.

[0013] According to a further aspect of the present invention, there is provided a method for the in-situ bioremediation of contaminated soil which method comprises:
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(i) treating contaminated soil by injecting a composition comprising one or more microorganisms capable of degrading organic material and one or more water dispersible or water soluble polymers at a concentration of from 0.25 to 5 % by weight of water dispersible or water soluble polymer based on the total weight of the composition, into a subterranean region
25 of contaminated soil via an injection well with an outlet located at a depth of at least one meter below the subterranean surface and proximate to the subterranean contamination, and in addition

30 (ii) treating the surface of the contaminated soil region with a composition comprising one or more microorganisms capable of degrading organic material and one or more water dispersible or water

soluble polymers at a concentration of from 0.25 to 5 % by weight of water dispersible or water soluble polymer based on the total weight of the composition.

- 5 **[0014]** In preferred embodiments the compositions for either well injection or for surface soil treatment, comprise from 0.3 to 4 % by weight, more preferably 0.3 to 3 % by weight, more preferably from 0.3 to 2.5 % by weight, more preferably from 0.4 to 2 % by weight, more preferably from 0.5 to 1.5 % by weight and most preferably from 0.5 to 1.25 % by weight of water dispersible
10 or water soluble polymer, based on the total weight of the composition. In a preferred embodiment the composition used for surface treatment is of the same composition as the well injected composition or has a lower % by weight of water dispersible or water soluble polymer.
- 15 **[0015]** According to a further aspect of the present invention, there is provided an aqueous bioremediation composition comprising one or more microorganisms capable of degrading organic material and one or more water dispersible or water soluble polymers, wherein the composition further comprises one or more monosaccharides. In a preferred embodiment the
20 monosaccharides comprise one or more of the following; glucose also known as dextrose monohydrate, fructose and galactose. In a preferred embodiment the one or more monosaccharides are one or more aldose monosaccharides. The most preferred aldose monosaccharide is dextrose, preferably anhydrous dextrose or glucose. Preferred sources of the monosaccharide are corn sugar,
25 corn syrup or grape sugar. In a further embodiment the bioremediation composition may comprise one or more disaccharides, oligosaccharides or polysaccharides in addition to the one or more monosaccharides. The preferred disaccharide is sucrose. The monosaccharides especially aldose monosaccharides are beneficial in supporting the microorganisms present in
30 the aqueous compositions of the present invention. In this aspect of the present invention it is preferred that the compositions comprise from 0.1 to 5% by weight, more preferably from 0.3 to 4 % by weight, more preferably 0.3 to 3

% by weight, more preferably from 0.3 to 2.5 % by weight, more preferably from 0.4 to 2 % by weight, more preferably from 0.5 to 1.5 % by weight and most preferably from 0.5 to 1.25 % by weight of water dispersible or water soluble polymer, based on the total weight of the composition.

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[0016] According to a further aspect of the present invention, there is provided an aqueous composition comprising one or more microorganisms capable of degrading organic material and one or more water dispersible or soluble polymers, wherein the composition further comprises one or more polyols. The polyols are preferably hydrocarbon based polyhydroxyl compounds, which preferably contain between two and four hydroxyl groups per monomeric unit of the polyol. The polyol may be a polymeric polyol; in this case the polyol may contain more than four hydroxyl groups. The preferred polyols are tri-hydroxy alcohols such as tri-hydroxy aliphatic alcohols. The preferred tri-hydroxy aliphatic alcohols are 1,2,3 aliphatic triols. In a particularly preferred embodiment at least one of the polyols is glycerine or glycerol. A preferred polymeric polyol is polyglycerol. Other suitable polyols include the polyalkylenepolyols such as polyethyleneglycol or polypropyleneglycol, and alkyloxy derivatives thereof, such as alkyloxypolyethyleneoxyethanol or nonylphenolpolyethyleneglycolether. The polyols are beneficial in aiding formulation of the aqueous compositions of the present invention and also in supporting the microorganisms present in the aqueous compositions. In this aspect of the present invention it is preferred that the compositions comprise from 0.1 to 5% by weight, more preferably from 0.3 to 4 % by weight, more preferably 0.3 to 3 % by weight, more preferably from 0.3 to 2.5 % by weight, more preferably from 0.4 to 2 % by weight, more preferably from 0.5 to 1.5 % by weight and most preferably from 0.5 to 1.25 % by weight of water dispersible or water soluble polymer, based on the total weight of the composition.

[0017] The present invention also provides for aqueous compositions that comprise in any combination two or more of each of the foregoing aspects of the present invention.

[0018] In all aspects of the present invention the one or more microorganisms may be any microorganism that is capable of effecting the decomposition or conversion of organic contaminants to relatively harmless compounds or intermediary compounds that may be converted by the action of further bioremediation to relatively harmless compounds. Relatively harmless compounds includes but is not limited to compounds such as water and carbon dioxide. Particularly preferred microorganisms are those that are capable of degrading or decomposing hydrocarbon based contaminants in the soil such as crude oil, crude oil derived products such as hydrocarbon fuels such as diesel, lubricant oils, petrochemicals and speciality chemicals, such as for example MTBE. Also included are microorganisms that may degrade or decompose halogen containing organic materials such as chlorinated aliphatic solvents such as trichloroethylene and aromatic compounds. Other contaminants to be treated include for example phthalates and phthalates esters e.g. dimethylphthalate, pesticides such as 2,4-D, phenols, chlorinated phenols, nitroaromatics such as TNT and DNT, styrene, quinones, BTEX, paraffin, gasoline, naphthalene, biphenyls, benzopyrene, brake fluids, xylene, pentane, organic pesticides, kerosene, isoprene, heptane, dichlorobenzene, silicon oils, isobutanol, hexene, ethylene glycol, chloroform, butanol, and acetone.

[0019] The preferred microorganisms for use in all aspects of the present invention are bacteria in the form of cultures. Examples include:

- Enterobactor sakazakii (strain 1)
- Enterobactor sakazakii (strain 2)
- Klebsiella sp.
- Arthrobacter sp. (strain 1)
- Arthrobacter sp. (strain 2)
- Pseudomonas sp.
- Pseudomonas fluorescens
- Pseudomonas putida

Pseudomonas pseudoalcaligenes JS45

Acinetobacter

Mycobacterium

Burkholderia sp eg *cepacia*

5 *Rhodospirium* sp

Dehalococcoides sp

Dehalococcoides ethenogenes strain 195

Dehalococcoides CBDB1

Bacillus (sp 1), (sp 2), & (sp 3)

10 *Bascillus*, sp, *subtilis*

Pseudomonas (sp 1) & (sp 2)

Pseudomanas aeruginosa

Pseudomanas alcaligenes

Arathrcbcter crystallopoietes

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[0020] Mixtures of micro-organisms may also be used. In one preferred embodiment the micro-organism are introduced to the composition in the form of a dried bacterial cultures (DBC), such as those grown and supplied on substrates such as bran. In a preferred embodiment the micro-organisms are introduced into the composition in the form of a liquid culture. Examples of suitable bacterial cultures that are commercially available are cultures supplied by Biosystems Europe, as EU70 and EU80 being suitable cultures for hydrocarbon degradation, B350, B350/10 and L1800 as supplied by Biosystems Corporation Ltd, The Fox, Ousby, Penrith, Cumbria, CA10 1QA, United Kingdom, PDM-7 HC as supplied by Phase III Inc, 916 E. Baseline Road, Suite 101 Mesa, Arizona USA 85204. In a preferred embodiment the microorganisms are provided in admixture with an organic substrate preferably a sugar and most preferably a monosaccharide. In a preferred embodiment the monosaccharide is glucose or dextrose and most preferably is anhydrous dextrose. The microorganism compositions with sugar substrate comprise up to 80% be weight of sugar, preferably up to 70% by weight of sugar, more preferably up to 60% by weight of sugar and most preferably up to 50% by

weight of sugar. In a further preferred embodiment the microorganism compositions further comprise nutrients. When nutrients are present in the microorganism composition they are preferably present as part of the substrate for the microorganisms and are preferably present at a ratio of 1:1 with the sugar if this is present. Preferred microorganism compositions have nutrients have nitrogen to phosphorous ratio of 5:1. In a further preferred embodiment the microorganism composition comprises a blend of microorganisms. In one preferred embodiment the blend of microorganisms comprises pseudomonas, preferably pseudomonas putida, and bacillus; preferably in a ratio of approximately 1:1, more preferably the amount of pseudomonas present is higher than bacillus

[0021] In preferred embodiments of all aspects of the present invention the one or more water dispersible or water soluble polymers in the compositions of each aspect of the invention are biodegradable. In preferred embodiments the polymers are organic polymers. Typical polymeric materials suitable for use in the present invention include water dispersible or water soluble polysaccharides, polyvinyl alcohols, polyacrylamides, and polyacrylamide copolymers. Particularly desirable polysaccharides include galactomanan gums, derivatives thereof, and cellulose derivatives. Typical polysaccharides include: guar gums, locust bean gum, karagya gum, sodium carboxymethyl guar, hydroxyethyl guar, hydroxypropyl guar, sodium hydroxymethyl cellulose, sodium carboxymethyl-hydroxyethyl cellulose, and hydroxyethyl cellulose. A further preferred polymer is Xanthan gum.

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[0022] The polysaccharides are preferred polymers. Examples of suitable polymers are those based on cellulose such as cellulose ethers e.g. hydroxypropyl methyl cellulose. A suitable polymeric material is that sold by Courtaulds Fibres Limited under the designation Celacol HPM15000DS or by Dow Chemical as Methocell K15 MDGSE. These polymers have a molecular weight distribution such that a 2% aqueous solution at 20°C. has a viscosity in the region of 13,000 to 17,000 cp. The polymeric material may be supplied as a

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powder containing up to 10% water and be mixed with the required amount of water. In some circumstances, a part or all of the hydroxypropylmethyl cellulose may be substituted by carboxymethyl cellulose. Carboxymethyl cellulose provides a more viscous aqueous composition for a given weight of polymer per
5 liter of the composition. However, the composition may be more susceptible to precipitation in consequence of a reduction of the pH value of the composition and also may be more susceptible to the presence of ions in the composition. In some situations, the greater susceptibility of carboxymethyl cellulose to biodegradation, as compared with hydroxypropylmethyl cellulose, renders the
10 carboxymethyl cellulose less suitable as a thickening agent than is hydroxypropyl methyl cellulose.

[0023] In preferred embodiments the compositions of each aspect of the present invention further comprise one or more nutrients for the
15 microorganisms. There is also incorporated in the composition an appropriate nutrient or mixture of nutrients to promote growth and reproduction of the microorganisms. The nutrients for the microorganisms may comprise ammonium nitrate present in an amount from 0.01 g to 10 g per liter of the aqueous composition and a similar weight of a composition comprising
20 nitrogen, phosphorous and potassium in the proportions 30:5:5. Preferably the nutrient comprises NPK fertilizer and most preferably liquid fertilizer.

[0024] Suitable surfactants for use in the present invention include those surfactants as described in US 6,210,955, the contents of which are hereby
25 incorporated by reference. Suitable surfactants are also described in the art in relation to the technology of surfactant flooding for bioremediation. Other examples of suitable surfactants are as described in Environ.Sci.Technol. 2002, 36, 5491-5497, and 2000, 34, 4842-4848, the contents of which are all hereby incorporated by reference. In a preferred embodiment the electrolyte
30 concentration is controlled using sodium chloride or calcium chloride.

[0025] One example of a suitable composition according to the first aspect of the present invention comprises an aqueous solution of hydroxypropyl methyl cellulose. The composition contains from 0.5 to 2% by weight of polymer and up to 10% by weight of selected microorganisms, together with an appropriate proportion of nutrient for the microorganisms. The weight of microorganisms provided in the composition generally corresponds to a mass of the microorganisms, measured on a dry basis, which is within the range 0.1 milligram to one kilogram per liter of the aqueous composition. The polymeric material may be supplied as a powder containing up to 10% water and be mixed with the required amount of water. The microorganisms may be provided in admixture with the nutrient or nutrients in the form of an aqueous paste or dispersion in water and the required weight of this paste or dispersion is added to the aqueous solution of the polymer. Alternatively, the microorganisms and nutrient may be mixed with the water before the polymer is added.

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[0026] In all aspects of the present invention the aqueous bioremediation compositions may be manufactured in bulk on the site to be treated or off-site for transportation to the site in suitable tankers. In a preferred method the aqueous bioremediation compositions are manufactured in the following manner. The mixing plant comprises at least two vessels which are interconnected for circulation between the two vessels one of the vessels may be a mixing vessel and the other may be for example a transportation tanker. The plant is primed with water for the final composition. In a first stage the NPK nutrient is added to the mixing vessel and the contents are circulated between the bulk tanker and the mixing vessel. Once the nutrient has been uniformly mixed into the water then when used the monosaccharide may be added to the mixing vessel with circulation to and between the tanker and the mixing vessel. Once the monosaccharide has been added and the composition is uniformly mixed then any further additives such as for example polyol may be added and mixed via re-circulation between mixing tank and tanker. At this stage the water dispersible or water soluble polymer may be added to the mixture. Finally the

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bacteria, preferably in the form of a liquid inoculum, is added to the mixing vessel and the mixture re-circulated until homogeneous.

[0027] In a further aspect of the present invention the compositions may be used in bioremediation especially in-situ bioremediation of contaminated subterranean material. In the case of in-situ treatment the composition may be injected into a mass of particulate material, for example soil, at a number of positions to form a substantially continuous region containing the composition. This acts as a barrier to restrict migration through the mass of material of a contaminant which is degraded by the microorganisms in the composition. For example, in a case where the ground has been contaminated with oil, the composition may be injected in an annular region around the periphery of the contaminated region to prevent spread of the oil beyond that region.

[0028] Compositions embodying the present invention may also include surfactants, additional enzymes, for example lipases, carbohydrases and proteases. A further additive that may be used is ORC™ (Oxygen Release Compound) as supplied by Regenesis, USA, which is an intercalated magnesium peroxide compound; such compounds are well known in the art.

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[0029] The present invention will now be described by way of example with reference to the accompanying drawing in which:

[0030] FIGURE 1 shows a sectional view of a well for in-situ bioremediation using the compositions and methods of the present invention.

[0031] With reference to Figure 1 an injection well (1) is shown which may be used for the subterranean injection of an aqueous bioremediation composition according to the present invention. The well (1) comprises a pipe (2) which comprises a plain section (A) and a slotted section (B). The slotted section (B) is located towards the bottom (3) of the pipe (2), the pipe is held in position at the location for in-situ bioremediation by means of a concrete sleeve

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(4) which extends from the top (5) of the slotted section (B) to the ground level (6). At ground level (6) the concrete section (4) widens to form a cap section (7). Located below the concrete section (4) is a porous medium (8) which is proximate to the slotted section (B) of the pipe (1). At the base of the pipe (1) is a cap (9), which may be in the form of a screw-on cap with preferably a locating spike (10), which may impact the base of the well (11) when the well is assembled. The pipe (1) may also be adapted at the top (12) to accommodate a screw on cap (13) or connection means (not shown) for attaching the feed lines (not shown) for injection of the aqueous bioremediation composition. In a preferred embodiment the pipe (1) is approximately 4 metres in length, with preferably a slotted section (B) of approximately 1 metre, which is located at a depth of approximately 2.5 to 3.5 metres below ground level (6). The slotted section (B) may have any number of slots or holes (14) of any dimension. Preferably, the slots (14) are arranged perpendicular to the bore of the pipe (1) and are narrower than they are long; in a preferred embodiment the slots (14) have a width of from 1 to 15 mm, preferably 1 to 10 mm, most preferably 1 to 5 mm. The concrete section (4) assists in ensuring that the aqueous bioremediation composition does not break out along the side of the pipe (1) to the surface of the well during injection to the location to be bioremediated. The porous medium (8) provides an intermediate region between the exit slots (14) of the pipe (1) and the surrounding subterranean material (15) which may be of variable density and porosity. The porous medium (8) may be particulate material such as gravel and preferably is gravel of 1 to 2 mm particle size.

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[0032] The well (1) may be assembled by first auguring a hole at the desired location of the well (1). The pipe (1) is fitted with the required end cap (9) and this combination is located within the bore of the augured hole. Then the bore surrounding the pipe (1) is backfilled with the porous medium (8) to a depth that reaches the top (5) of the slotted section (B) of the pipe (1). After backfilling with porous medium (8) the remaining bore is filled with concrete to ground level. Once the concrete is set the well is ready for use.

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[0033] Preferably the pipe is a polymer pipe e.g. a polvinylchloride pipe of outside diameter of from 40 to 100 mm, preferably 40 to 80 mm and most preferably from 50 to 70 mm, typically about 60 mm outside diameter.

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CLAIMS

1. An aqueous composition comprising one or more microorganisms capable of degrading organic material and one or more water dispersible or
5 soluble polymers, wherein the composition comprises from 0.25 to 5 % by weight of water dispersible or soluble polymer based on the total weight of the composition.
2. A aqueous composition as claimed in claim 1, which comprises from 0.3 to
10 4 % by weight, more preferably 0.3 to 3 % by weight, more preferably from 0.3 to 2.5 % by weight, more preferably from 0.4 to 2 % by weight, more preferably from 0.5 to 1.5 % by weight and most preferably from 0.5 to 1.25 % by weight of water dispersible or water soluble polymer, based on the total weight of the composition.
- 15 3. An aqueous composition as claimed in claim 1 or claim 2 wherein the composition further comprises one or more monosaccharides.
4. An aqueous composition as claimed in any one of the preceding claims
20 wherein the composition further comprises one or more polyols.
5. An aqueous composition comprising one or more microorganisms capable of degrading organic material and one or more water dispersible or
25 soluble polymers, wherein the composition further comprises one or more monosaccharides.
6. An aqueous composition as claimed in claim 5 wherein the composition
30 comprises from 0.25 to 5 % by weight of water dispersible or soluble polymer.
7. An aqueous composition as claimed in claim 5 or claim 6 wherein the composition further comprises one or more polyols.

8. An aqueous composition comprising one or more microorganisms capable of degrading organic material and one or more water dispersible or soluble polymers, wherein the composition further comprises one or more polyols.
- 5
9. An aqueous composition as claimed in claim 8, wherein the composition further comprises one or more monosaccharides.
- 10
10. An aqueous composition as claimed in claim 8 or claim 9 wherein the composition comprises from 0.25 to 5 % by weight of water dispersible or soluble polymer.
11. An aqueous composition as claimed in any one of the preceding claims wherein the composition further comprises one or more nutrients.
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12. An aqueous composition as claimed in any one of the preceding claims wherein the water dispersible or soluble polymer is an organic polymer.
13. An aqueous composition as claimed in any one of the preceding claims wherein the water dispersible or soluble polymer is a non-ionic polymer.
- 20
14. An aqueous composition as claimed in any one of the preceding claims wherein the water dispersible or soluble polymer is a polysaccharide.
- 25
15. An aqueous composition as claimed in claim 14 wherein the polysaccharide is a cellulose ether or Xanthan gum.
16. An aqueous composition as claimed in claim 15 wherein the cellulose ether is polysaccharide is a cellulose ether is a hydroxy methyl cellulose.
- 30

17. A method of treating contaminated subterranean material, which method comprises contacting the contaminated subterranean material with one or more compositions according to any one of claims 1 to 16.
- 5 18. A method of in-situ bioremediation of contaminated soil which method comprises: (i) treating contaminated soil by injecting a composition as claimed in any one of claims 1 to 16 into a subterranean region of contaminated soil via an injection well having an outlet located at a depth of at least one meter below the subterranean surface and proximate to the
10 subterranean contamination, and in addition, (ii) treating contaminated soil by injecting a composition comprising one or more microorganisms capable of degrading organic material and one or more water dispersible or water soluble polymers at a concentration of from 0.1 to 0.5 % by weight of water dispersible or water soluble polymer based on the total
15 weight of the composition, into a subterranean region of contaminated soil via an injection lance which is inserted into the surface of the soil such that the composition is injected at a depth of up to 1 metre depth below the soil surface.
- 20 19. A method of in-situ bioremediation of contaminated soil which method comprises: (i) treating contaminated soil by injecting a composition as claimed in any one of claims 1 to 16, into a subterranean region of contaminated soil via an injection well with an outlet located at a depth of at least one meter below the subterranean surface and proximate to the
25 subterranean contamination, and in addition (ii) treating the surface of the contaminated soil region with a composition as claimed in any one of claims 1 to 16.
- 30 20. A method as claimed in claim 19 wherein the composition used for surface treatment is of the same composition as the well injected composition or has a lower % by weight of water dispersible or water soluble polymer.

21. A method as claimed in claim 18 wherein the compositions for lance injection comprise between 0.1 to 0.4, preferably 0.15 to 0.3 and most preferably 0.2 to 0.4 wt% of the polymer.
- 5 22. A method as claimed in claim 17 wherein the subterranean material is treated ex-situ.
23. A method as claimed in claim 17 wherein the subterranean material is treated in-situ.

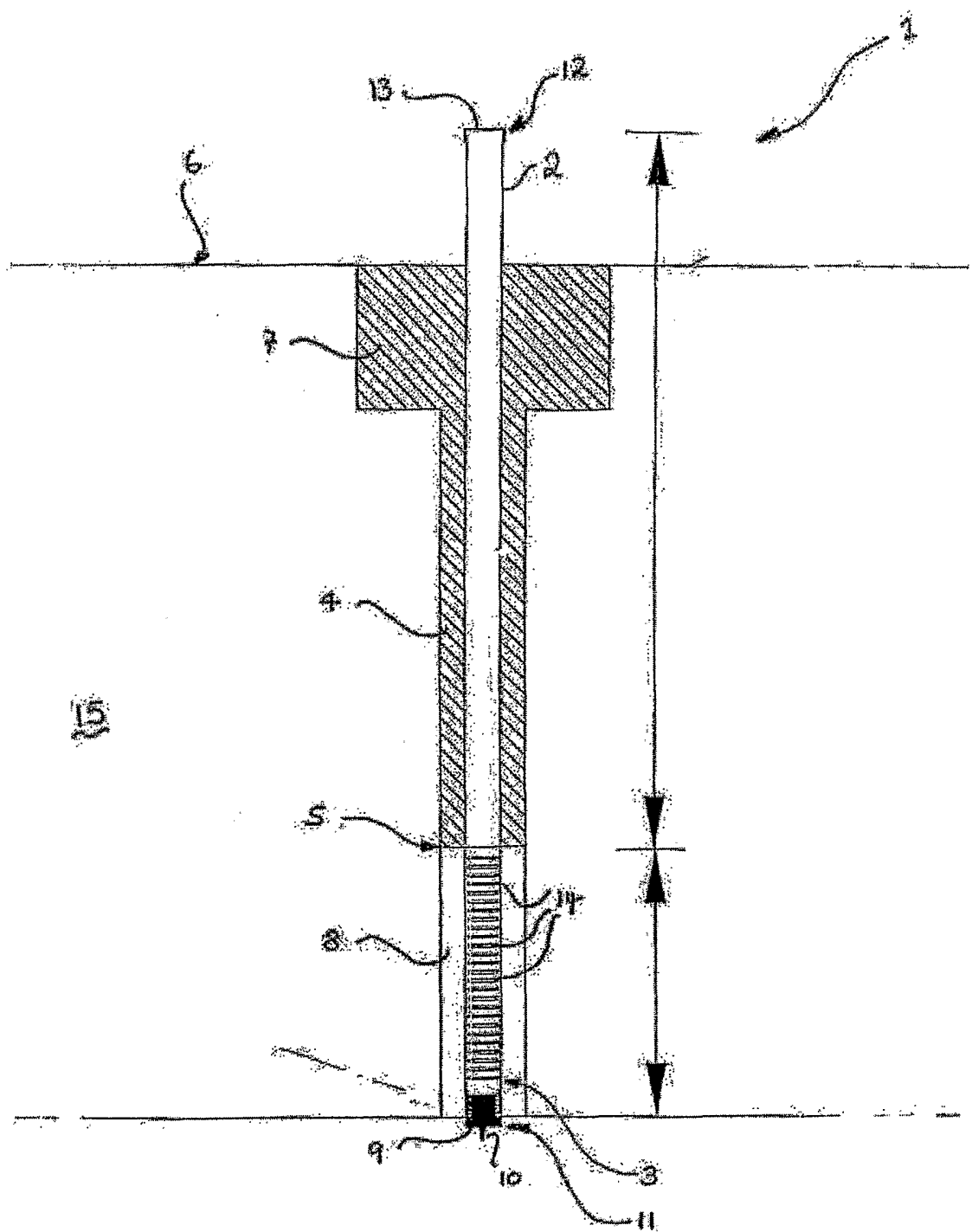


Figure 1