

BIOREMEDIATION OF HYDROCARBON SLUDGE

Technical Field

5 The present invention generally relates to bioremediation of hydrocarbon sludge.

Background

10 Soil that is contaminated with crude oil or oil sludge must be treated to prevent harmful compounds from seeping into the soil, thereby potentially contaminating ground water.

15 Conventional methods for treating such contaminated soil include physical/mechanical treatment, thermal desorption and incineration. Physical/mechanical treatment typically involves separating the chemicals from the contaminated soil using washing techniques or excavating the contaminated soil for burning in a furnace. Both methods, however, require significant inputs of
20 energy and are therefore costly. Furthermore, burning results in the release of toxic gases into the atmosphere and produces ashes with high concentrations of heavy metals.

25 Another commonly used physical method is cementation. Cementation, however, does not reduce the content of pollutants in the soil but merely prevents leaching of the pollutants into groundwater. This method is also costly because it requires extensive sanitary landfill space.

30 Other than the use of physical/mechanical treatment methods, contaminants in the soil may also be treated by biological means. This traditionally involves allowing the bacteria present in the contaminated soil to naturally degrade the contaminants. However, this method typically

requires extended periods, often a number of years, for the soil to be decontaminated to an acceptable level.

This natural biological degradation process may be accelerated by bioremediation. Bioremediation is a biological treatment process whereby microorganisms are used to biologically degrade hydrocarbon-contaminated waste into non-toxic residues. Because bioremediation is based on a natural process whereby the microbial population increases when the contaminant is present, and declines after biodegradation. Desirably, the product residues of bioremediation processes are typically harmless and therefore there is typically no requirement for any post-process treatment, storage or discharge.

Some other systems for treatment of contaminated soil combine both physical and biological treatment methods. Such systems typically have primary and secondary treatment stages. Primary treatment involves chemical precipitation, sedimentation and generation of chemical sludge, while secondary treatment involves biological treatment. Such conventional systems typically require extensive capital inputs.

There is a need to provide a process for the treatment of petroleum sludge and oil waste that overcomes, or at least ameliorates, one or more of the disadvantages described above.

There is a need to provide a simple and cost-effective yet efficient and environmentally friendly process for treatment of petroleum sludge and oil waste.

Summary

The present invention relates to a novel bioremediation process for treatment of hydrocarbon

sludge, such as petroleum sludge, which is a by-product of the petroleum industry.

According to an aspect, there is provided a process for treating a sludge containing hydrocarbons, the process comprising the step of contacting the sludge with a biofilm comprising at least two of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms, said contacting being undertaken under conditions to at least partly reduce the amount of hydrocarbons in said sludge.

The process may be implemented in a bio-treatment facility, which may comprise means for controlling aeration and moisture levels within the hydrocarbon sludge during said contacting. In some embodiments, a specialty blend of microorganism species may be used to promote formation of biofilms within said sludge. Additionally, the sludge may be supplemented with additives and nutrients to further promote formation of the biofilm.

The process parameters and the consortium of microorganism species may be tailored for treatment of different compositions of petroleum sludge and oil waste.

The bioremediation process disclosed herein may enable direct and on-site treatment of hydrocarbon sludge with the microorganism blend without the need for a primary treatment step (ie physical treatments such as washing and chemical treatments such as precipitation, sedimentation).

Advantageously, the process disclosed herein is preferably a substantially "dry system" in that the sludge is substantially solid and the process utilizes a minimal amount of moisture. More advantageously, the use of minimal moisture at least partly, if not completely, avoids leaching of hydrocarbons into surrounding soils.

Furthermore, the low moisture content of the solid phase sludge typically results in less sludge by volume to be removed at the end of the process. Advantageously, no additional equipment or infrastructure is required to collect leachate and remove or dispose of generated sludge. As the bioremediated waste can be land-filled on-site, no additional transportation or treatment costs need to be incurred.

According to another aspect, there is provided a use of at least two of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms, for the formation of a biofilm for treating a sludge containing hydrocarbons.

According to another aspect, there is provided a sludge hydrocarbon bed having a biofilm comprising at least two of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms.

According to another aspect, there is provided a method of making a bioremediation facility for remediating a hydrocarbon sludge comprising the steps of:

(a) placing said hydrocarbon sludge in a treatment zone;

(b) seeding said hydrocarbon sludge with microorganisms comprising at least two of *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms;

(c) forming a biofilm from said seeded microorganisms; and

(d) degrading said hydrocarbons within said hydrocarbon sludge using said biofilm.

According to yet another aspect, there is provided a kit for use in treating a sludge containing hydrocarbons, the kit comprising:

(i) a solution containing at least two of *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms, and

5 (ii) instructions for contacting said solution with said sludge under conditions to form a biofilm of said microorganisms and to at least partly reduce the amount of hydrocarbons in said sludge.

Definitions

10 The following words and terms used herein shall have the meaning indicated:

The term "hydrocarbon sludge" refers to any solid or semi-solid material containing hydrocarbons therein. Typically, the hydrocarbon sludge is generated from municipal, industrial or commercial operations which
15 contains hydrocarbons compounds that are at least partially biodegradable, i.e., capable of being broken down by the action of living microorganisms. For example, the hydrocarbon sludge may be "petroleum sludge" generated from the petroleum industry. The hydrocarbon sludge may be
20 generally derived from petroleum crude oil; the composition of the oil present in the sludge may resemble crude oil or derived hydrocarbon products having a boiling range at least as high as kerosene/diesel fuels. The sludge may be in the form of an emulsion, such as a water-
25 in-oil emulsion, an oil-in-water emulsion, or mixtures thereof, wherein the oil phase is typically a hydrocarbon resulting from production well testing, completion, production or refinery operations. This type of sludge may have an initial content of water and sediment of
30 approximately 10.0% volume or greater which severely reduces the commercial value of the hydrocarbon phase. The sludge may typically be produced as a mixture of crude oil, water and organic and inorganic solids, and is created as a waste of the various production processes.

The sludge for treatment may of course have a wide variety of compositions and characteristics. Although a prime example of a hydrocarbon sludge is a petroleum sludge and hence, the examples disclosed herein primarily refer to petroleum hydrocarbon sludge, it should be noted that the term refers to any hydrocarbon sludge derived from any sources.

The term "total petroleum hydrocarbon" or "TPH" refers to the amount of petroleum pollutants in a contaminated material that is to be treated.

The term "treatment", and grammatical variants thereof, when used in connection with hydrocarbon sludge, refers to any method, technique, or process which results in degradation of the hydrocarbon compounds contained within the sludge. For example, the treatment may involve degradation of the hydrocarbons so as to neutralize toxic compounds contained therein, and render such hydrocarbon sludge non-toxic, safer for transport, amenable for recovery, amenable for storage, or amenable for disposal.

The term "toxic" refers to a substance or material which is capable of causing damage to the environment or causing damage to living organisms, severe illness or, in extreme cases, death when ingested, inhaled, or absorbed by the skin. The term "aerate", and grammatical variants thereof, refers to the introduction of air or some other gas into the material to be treated or into the facility in which treatment of said material takes place.

As used herein, the term "mixed culture" refers to a culture composition comprising any combination of microorganisms in a culture medium. The combination may be binary or tertiary, or contain any number of individual species or strains, or contain cells of divergent species.

The word "substantially" does not exclude "completely" e.g. a composition which is "substantially

free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of components of the formulations, typically means $\pm 5\%$ of the stated value, more typically $\pm 4\%$ of the stated value, more typically $\pm 3\%$ of the stated value, more typically, $\pm 2\%$ of the stated value, even more typically $\pm 1\%$ of the stated value, and even more typically $\pm 0.5\%$ of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Brief Description Of Drawings

The accompanying drawings illustrate a disclosed embodiment and serves to explain the principles of the

disclosed embodiment. It is to be understood, however, that the drawings are designed for purposes of illustration only, and not as a definition of the limits of the invention.

5 Fig. 1 is a schematic drawings of one embodiment of a pit used in a biotreatment facility which utilizes the disclosed treatment process; and

 Fig. 2 is a cross-sectional view of the pit of Fig. 1.

10

Disclosure of Optional Embodiments

Exemplary, non-limiting embodiments of a novel process for bioremediation of hydrocarbon sludge will now be disclosed.

15 There is provided a process for treating a hydrocarbon sludge, the process comprising the step of contacting the hydrocarbon sludge with a biofilm comprising at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp.
20 microorganisms and *Brevibacillus* sp. microorganisms, said contacting being undertaken under conditions to at least partly reduce the amount of hydrocarbons in said sludge.

 The process may be performed *in situ* or *ex situ*. Where an *ex situ* process is desired, the hydrocarbon
25 sludge to be treated is typically excavated and placed in the treatment zone of a biotreatment facility.

 The treatment zone may be a reactor, or a plurality of reactors, equipped with means for controlling conditions within the sludge during the process. The
30 means for controlling the process conditions, include but are not limited to, means for controlling aeration, temperature, moisture level and nutrient supply.

Alternatively, the treatment zone may comprise a pit or a plurality of pits.

Figure 1 shows an embodiment of a pit (1) wherein the process may be performed. The pit (1) may be constructed by forming a substantially impervious base (2), on which the hydrocarbon sludge (3) may be placed, and a substantially impervious cover (4) for covering said pit (1). Suitable materials for use as a base (2) include, but are not limited to, polyolefins, polyvinyls, polyacrylics, and polyesters. Exemplary polyvinyls include polyvinyl chloride (PVC), polyvinyl acetate, ethylene vinyl acetate, and combinations thereof. Exemplary polyesters include polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET) and combinations thereof. Preferably, the material forming the base (2) of the pit (1) comprises heavy-duty high-density polyethylene (HDPE) sheets. Such sheets are preferred in the waste containment industry because they possess desirable chemical resistance and strength. Alternatively, the base (2) may be constructed from a material such as epoxy, cement, gravel or the like.

To ensure uniform access of the microorganisms to the hydrocarbon sludge (3) and to ensure uniform process conditions, for example that of temperature, moisture, aeration and nutrients, the layer of the hydrocarbon sludge (3) on the base (2) preferably has a thickness of about 0.25 m to about 1.75 m, more preferably about 0.5 m to about 1.5 m, and most preferably about 0.75 m to about 1.25 m. In one embodiment, the thickness of the layer of hydrocarbon sludge (3) is about 1.0 m.

The hydrocarbon sludge (3) may be bunker fuels, drill cuttings, shipping sludge, oil spills, grease, jet fuel, diesel fuel, gasoline, hydraulic oil, kerosene, sludge cake from biotreatment plants, biodiesel, refined oil and

crude oil. Typically, the hydrocarbons in such sludge include combinations of both aliphatics (C₅-C₃₆) and aromatics (C₉-C₂₂). Examples of hydrocarbons found in such sludge include pentachlorophenols (PCPs); polychlorinated
5 byphenyls (PCBs); polyaromatic hydrocarbons (PAHs) such as naphthalene, anthracene, acenaphthene, acenaphthylene, and pyrene; polynuclear aromatics (PNAs); 2,4,6-trinitrotoluene (TNT); nitrocellulose (NC); benzene, toluene, ethylbenzene, xylene (BTEX); olefins; paraffins;
10 isoparaffins and other xenobiotics. The initial TPH of the hydrocarbon sludge (3) typically ranges from about 1,000 ppm to about 250,000 ppm. More typically, the initial TPH ranges from about 5,000 ppm to about 200,000 ppm and most typically, from about 10,000 ppm to about 100,000 ppm. The
15 hydrocarbon sludge (3) preferably has a particle size that is sufficiently small to enable access of the microorganisms to the compounds to be degraded. Preferably, the particle size of the material to be treated is about 1 mm to about 15 mm. More preferably,
20 the particle size is about 5 mm to about 12.5 mm. Most preferably, the particle size is about 7.5 mm to about 10 mm.

A grid of porous conduits (5) may be placed on the layer of hydrocarbon sludge (3) for supplying air to the
25 layer of hydrocarbon sludge, and microorganisms forming the biofilm therein, when required from an air compressor (6). Suitable materials for constructing the grid of porous conduits (5) include metals such as galvanized steel and stainless steel, and plastics such as Teflon and
30 polyvinyl chloride, or the like. The scale of the grid typically ranges from about 0.3 m to about 3 m depending on such factors as the overall size of the pit, the load of hydrocarbon sludge and the thickness of the layer of hydrocarbon sludge. More typically, the scale of the grid

ranges from about 0.5 m to about 2 m. Most typically, the scale of the grid ranges from about 0.9 m to about 1.5 m. In one embodiment, the scale of the grid is about 1 m. The perforation in the grid of porous conduits (5) through which air is supplied may be located at suitable intervals of about 5 cm to about 30 cm. Preferably, the intervals of perforation are about 5 cm to about 20 cm, more preferably about 5 cm to about 15 cm, and most preferably about 5 cm to about 10 cm. Berms (7) may be built along the periphery of the pit (1) above ground to avoid run-offs. Such berms are preferably about 0.2 m to about 0.7 m in width. More preferably, the berms are about 0.3 m to about 0.6 m in width and most preferably, the berms are about 0.4 m to about 0.5 m in width.

The pit (1) may be covered with an impervious cover (4) to maintain controlled conditions within the treatment zone (8). Any suitable material may be used as a cover (4); for example, the cover may comprise the same material used for the base, i.e. heavy-duty HDPE, polyolefins, polyvinyls (e.g. polyvinyl chloride (PVC), polyvinyl acetate, ethylene vinyl acetate), polyacrylics, polyesters (e.g. polyethylene terephthalate (PET)) or combinations thereof. Preferably, the cover is tilted at a suitable angle to avoid retention of water, such as rainwater, on the top surface of the cover. For example, the cover may be tilted at an angle of about 5°, about 6°, about 7°, about 8°, about 9°, about 10°, about 15°, about 20°, about 25°, about 35°, or about 45°.

The size and number of each pit (1) in a biotreatment facility may be varied according to the treatment load. Typically, the treatment load ranges from about 100 metric tons to about 10,000 metric tons. More typically, the treatment load ranges from about 1,000 metric tons to about 5,000 metric tons. Most typically, the treatment

load ranges from about 2,000 metric tons to about 4,000 metric tons. For a treatment load of about 220 metric tons, for example, a pit dimension of about 50 m x about 10 m x about 0.5 m is typically used. Hence, for higher
5 treatment loads, a pit with a larger dimension may be used or a plurality of pits with said dimension may be used. In one embodiment, about eight pits are constructed as described above for the treatment of about 2,000 metric tons of hydrocarbon sludge.

10 The microorganisms useful in the process are those that are capable of degrading hydrocarbon compounds. Preferably, the microorganisms are capable of forming biofilms. Such microorganisms may be isolated and enriched from the hydrocarbon sludge. Other
15 microorganisms that may be useful and enrichment methods used to increase microbial population are well within the ordinary skills of those knowledgeable in the art.

Advantageously, mixed cultures of the microorganisms are used in order to obtain a broad spectrum of
20 biodegradability.

In one embodiment, the process comprises the step of adding a solution comprising at least one of *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms. In a preferred
25 embodiment, said *Bacillus* sp. is selected from the group consisting of *Bacillus lentus*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus sphaericus*, and *Bacillus licheniformis*. In another preferred embodiment, the *Acinetobacter* sp. is
30 selected from the group consisting of *Acinetobacter haemolyticus* and *Acinetobacter baumannii*. In yet another preferred embodiment, the *Brevibacillus* sp. is *Brevibacillus brevis*.

In one embodiment, the microorganisms may form a biofilm. A biofilm is a community of microorganism species that form a surface which enable the microorganisms to anchor thereto. Advantageously, the
5 inventors have found that due to formation of the biofilm, the microorganisms population more effectively treat the sludge. Without being bound by theory, it is speculated that the microorganisms are able to more effectively
10 resistant the inhibitory effects of inherent toxic compounds present in the hydrocarbon sludge relative to non-biofilm microorganisms. This may be due to the biofilm providing a support environment which allows the microorganisms to flourish in toxic environments.

The biofilm may further comprise one or more
15 microorganisms selected from the group consisting of *Pseudomonas* sp., *Listeria* sp., *Arthrobacter* sp., and *Alcaligenes* sp. In one embodiment, said biofilm comprises one or more microorganisms selected from the group consisting of *Pseudomonas aeruginosa*, *Pseudomonas*
20 *stutzeri*, *Listeria seeligeri*, and *Alcaligenes faecalis* type II.

The selected microorganisms may be combined with other additives to form the microorganism solution. The microorganism content of said microorganism solution may
25 comprise about 30% (vol) to about 70% (vol) microorganisms, about 40% (vol) to about 60% (vol) microorganisms or about 45% (vol) to about 55% (vol) microorganisms in a microorganism culture. The microorganism solution may further comprise additives and
30 nutrients for the microorganisms that are useful for promoting growth and stability of the microorganisms and enhancing their attachment to the hydrocarbon sludge thereby enhancing the efficacy and efficiency of the biodegradation process. The additives may include

biological catalysts (such as oxygenases and monooxygenases), buffers (such as phosphate buffer) and surfactants (such as sorbitan, polysorbates, sorbitan esters and polyxamers). Examples of nutrients typically included in the microorganism solution to enhance microbial growth and biodegradation include carbohydrates (such as glucose, fructose, maltose, sucrose, and starch); other carbon sources (such as mannitol, sorbitol and glycerol); nitrogen sources (such as urea, ammonium salts, amino acids or crude proteins, yeast extract, peptone, casein hydrolysates and rice bran extracts); and inorganic compounds (such as magnesium sulfate, sodium phosphate, potassium phosphate, sodium chloride, calcium chloride and ammonium nitrate).

A typical microorganism solution may comprise:

	Relative proportion (%)
Microorganisms	
<i>Bacillus lentus</i> A 5019 ST	2
<i>Bacillus megaterium</i> B 5013	2
<i>Bacillus megaterium</i> C 6019	2
<i>Bacillus pumilus</i> C 5011 ST	2
<i>Bacillus cereus</i> C 5014	2
<i>Bacillus cereus</i> C 603	2
<i>Bacillus cereus</i> C 6011	2
<i>Bacillus cereus</i> Z 508	2
<i>Bacillus subtilis</i> C 601/1	2
<i>Bacillus subtilis</i> C 601/2	2
<i>Bacillus sphaericus</i> C 605	2
<i>Bacillus licheniformis</i> Z 507 ST	2
<i>Brevibacillus brevis</i> Z 6030 PT	2
<i>Pseudomonas aeruginosa</i> A 6017	2
<i>Pseudomonas stutzeri</i> B 5012 ST	2

<i>Listeria seeligeri</i> B 5011	2
<i>Pseudomonas stutzeri</i> B 509 PT	2
<i>Pseudomonas stutzeri</i> B 6013	2
<i>Acinetobacter haemolyticus</i> C 602	4
<i>Acinetobacter baumannii</i> Z 6011	4
<i>Acinetobacter baumannii</i> Z 6013	4
<i>Alcaligenes faecalis</i> type II Z 6020 ST	2
<i>Arthrobacter</i> sp. C 505 PT	2
Additives	
Phosphate buffer (1 M)	1
Nutrients	
Yeast extract	15.7
Sodium chloride	15.7
Glucose	15.7

The microorganism solution is preferably kept cool under refrigeration until just before application to the hydrocarbon sludge (3). In some embodiments, the microorganism solution may be kept at room temperature (ie. at about 25°C) for up to about 4 hours.

In one embodiment, one or more solutions of microorganisms may be provided in a kit. Other than said solutions of microorganisms, the kit may comprise instructions for use as well as additives and nutrients as described above.

Once the hydrocarbon sludge (3) is seeded with the microorganism solution, the conditions within the treatment zone (8) is controlled and monitored such that the conditions are maintained at an optimum required for enhancing microbial growth and biodegradation. The conditions to be monitored include, but are not limited to, moisture, temperature, pH, aeration and nutrient supply. The optimal values for such conditions typically

depend on the selection of microorganisms in the microorganism solution.

In one embodiment, the moisture content of said hydrocarbon sludge is about 5% (wt) to about 20% (wt).
5 Preferably, the moisture content of said hydrocarbon sludge is about 8% (wt) to about 12% (wt). Advantageously, the low moisture content is optimal for promoting formation of the biofilm by the microorganisms. Where the moisture content of the hydrocarbon sludge (3) are not
10 within the preferred ranges, the moisture content of said hydrocarbon sludge (3) may be adjusted so that it falls within the preferred ranges by means that are well known to those skilled in the art. For example, where the moisture content falls below the preferred ranges, the
15 said hydrocarbon sludge (3) may be sprayed with water so that the moisture content is increased to a preferred level.

The hydrocarbon sludge (3) may be aerated with air during the process. Advantageously, the air promotes
20 formation and maintenance of the biofilm. In one embodiment, the air is injected into the sludge by a grid of porous conduits (5) disposed within the treatment zone (8). The source of the air may be air compressors (6).

A suitable atmospheric temperature within the
25 treatment zone typically ranges from about 25°C to about 40°C, from about 30°C to about 40°C or from about 35°C to about 40°C; while a suitable pH typically ranges from about 5 to about 9, from about 6 to about 8 or from about 6.5 to about 7.5.

30 Advantageously, the hydrocarbon sludge (3) is unstirred to avoid disruption of the biofilms formed.

In one embodiment, the process comprises the step of dosing the biofilm contacting the hydrocarbon sludge (3) with a microorganism solution. Advantageously, the dosing

promotes maintenance of the biofilm during the contacting step.

In one embodiment, said dosing is undertaken in a dosing regime of applying microorganism solution to said hydrocarbon sludge (3) about every three weeks for a period of about nine to about twelve weeks. The dosing regime undertaken may be determined based on such factors as the treatment load, the type of hydrocarbon sludge to be treated, the concentration of pollutants in said hydrocarbon sludge, the time period within which treatment is to be completed and the concentration of the microorganisms in the solution.

The process may be allowed to proceed for a period of time until the level of the compounds to be biodegraded reaches the target TPH level. The period of time required may depend on factors such as the initial TPH level of the material to be treated, the type and concentration of the microorganisms used, and the treatment conditions applied in the treatment zone.

Typically, the target TPH level is an acceptable level specified by environmental regulations. For example, the acceptable TPH level specified by the U.S. Environmental Protection Agency (USEPA) is about 1,000 ppm. Preferably, the TPH level is reduced to less than about 1,000 ppm. More preferably, the TPH level is reduced to less than about 500 ppm. Most preferably, the TPH level is reduced to less than about 250 ppm.

Alternatively, the efficiency of the process may be expressed based on the percentage of reduction in TPH level. The percentage of reduction is determined by the following formula:

$$\frac{(\text{Initial TPH Level} - \text{Final TPH Level})}{\text{Initial TPH Level}} \times 100\%$$

Preferably, the TPH level is reduced by about 50% to about 75%, more preferably by about 75% to about 85%, and most preferably by about 85% to about 100%.

- 5 After the toxic compounds in the hydrocarbon sludge (3) have been degraded to said acceptable target level, the biotreatment facility may be decommissioned or a fresh batch of untreated hydrocarbon sludge may replace the treated hydrocarbon sludge in the treatment zone (8).

10

Example

- 15 **Bioremediation of soil contaminated with bunker fuel**

Hydrocarbon sludge to be treated

- 20 The hydrocarbon sludge to be treated was soil contaminated with bunker fuel. The volume of contaminated soil was about 2,000 metric tons. The TPH originally present in the contaminated soil was approximately 50,000 ppm (5%). At the hot spot, the TPH was about 100,000 ppm (10%). The targeted result was to reduce the TPH level to less than 1,000 ppm (0.1%).

- 25 The contaminated soil was subjected to primary treatment involving centrifugation and washing to reduce the TPH to approximately 25,000-50,000 ppm. The centrifuged and washed contaminated soil was excavated and placed in a treatment zone. Each batch was treated over a
30 period of 9 weeks. Testing was carried out before and after treatment for 5 sample points per pit per batch.

Pit construction

A pit was used as the treatment zone to treat about 250 metric tons of soil (i.e. contaminated material). The pit had a dimension of 22 m x 10 m x 0.5 m. The bottom of the pit forming the base was a mixture of epoxy and concrete. A grid of galvanized steel pipes (1 m apart) was laid on the base. The pipes were perforated at 10 cm intervals to supply air from compressors. A thin 1 m layer of the contaminated soil was then placed on the base and grid. The layout of a typical pit is shown in Figures 1 and 2.

Excessive moisture loss or gain may result in loss of reduction in TPH levels. 0.5 m berms were built along the periphery of the pit above ground to avoid any run-offs. Heavy-duty HDPE sheets or PVC tarps with galvanized 2" steel scaffolding were placed over the pit to form the cover.

20

Treatment of the contaminated soil

The solutions of microorganisms for treatment of the contaminated soil was kept cool under refrigeration at about 4°C until just before application to the contaminated soil. The first solution comprised *Bacillus lentus*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus sphaericus*, *Bacillus licheniformis*, *Acinetobacter haemolyticus*, *Acinetobacter baumannii* and *Brevibacillus brevis* while the second solution comprises *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Listeria seeligeri*, and *Alcaligenes faecalis* type II.

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Air supply and moisture were controlled at 7 psig and 10%, respectively, throughout the experiment.

Analysis of TPH using gas chromatography (GC)

Soil samples were collected about 0.35 m (1 foot) below the surface. Each 15 g of soil sample was pounded to homogenize the sample. Sodium sulphate was added into the separating funnel with the soil sample. Seventy-five milliliter of methylene chloride was added as solvent and the sample mix was shaken for 1 min. Separation was allowed to occur overnight. The top layer of the separated sample (containing extracted soil sample) was collected and pre-filtered with a 0.45 μ m filter. The 6 μ l of sample was injected into the GC and analyzed against Alphasaz PIANO Calibration Standards (Supelco).

RESULTS

Batch 1

Table 1 shows the data from a 250 metric ton Biotreatment Pit 1. The mean reduction after 9 weeks was 97.6%. The final TPH at 9 weeks was less than 700 ppm. The maximum TPH at 57,215 ppm for Sample Site B was reduced to only 17 ppm (i.e. a 99.9% reduction). Advantageously, treatment of contaminated soil having high TPH levels with the microorganism solutions can result in almost complete (99.9%) degradation of the hydrocarbons.

Table 1. Results for Batch 1

Sample	A	B	C	D	E	Mean
0 week	3,317	57,215	53,433	38,016	7,088	31,825
9 weeks	430	17	508	2,658	238	770
Reduction at 9 weeks (%)	87.1	99.9	99.1	93	96.6	97.6

Batch 2

Table 2 shows the data from a 250 metric ton Biotreatment Pit 2. The TPH level for Sample B at 0 week was in excess of 200,000 ppm, which was reduced by 91.3% after 9 weeks of treatment. The mean TPH reduction for Batch 2 was 99.3% after 12 weeks. The mean TPH for five sample points at week 12 was less than 400 ppm. Like Batch 1, treatment of contaminated soil having high TPH levels with the microorganism solutions resulted in almost complete degradation of the TPH.

Table 2. Results for Batch 2

Sample	A	B	C	D	E	Mean
0 week	26,707	200,442	14,150	2,526	25,274	53,820
9 weeks	865	17,391	93	132	725	3,841
Reduction at 9 weeks (%)	96.8	91.3	99.3	94.5	97.1	92.9
12 weeks	NA	166	NA	NA	NA	396
Reduction at 12 weeks (%)		99.9				99.3

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Applications

Advantageously, the disclosed process reduces the TPH in hydrocarbon sludge to an acceptable level via biodegradation within a short period of time. Accordingly, embodiments of the disclosed process overcome the disadvantages of the prior art. For example, the process is capable of reducing the TPH by more than about 95% in about 9 weeks of treatment. Advantageously, in some cases, the TPH is almost completely degraded.

Furthermore, and in contrast to conventional chemical treatment processes, the process disclosed herein does not require excessive amounts of water nor does it use any toxic chemicals. Hence, the product residue of the
5 process is substantially dry and harmless and therefore there is no requirement for any post-process treatment, storage or discharge.

It will be apparent that various other modifications and adaptations of the invention will be apparent to the
10 person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.

15

Claims

- 5 1. A process for treating a sludge containing hydrocarbons, the process comprising the step of contacting the sludge with a biofilm comprising at least two of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and
10 *Brevibacillus* sp. microorganisms, said contacting being undertaken under conditions to at least partly reduce the amount of hydrocarbons in said sludge.
2. A process as claimed in claim 1, wherein the moisture
15 content of said sludge is about 5% (wt) to about 20% (wt).
3. A process as claimed in claim 2, wherein the moisture content of said sludge is about 5% (wt) to about 10% (wt).
- 20 4. A process as claimed in claim 1, comprising the step of adding a solution comprising at least one of said *Bacillus* microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms.
- 25 5. A process as claimed in claim 1, wherein said biofilm comprises one or more microorganisms selected from the group consisting of *Pseudomonas* sp., *Listeria* sp., *Arthrobacter* sp. and *Alcaligenes* sp.
- 30 6. A process as claimed in claim 5, wherein said biofilm comprises one or more microorganisms selected from the group consisting of *Pseudomonas aeruginosa*, *Pseudomonas*

stutzeri, *Listeria seeligeri*, and *Alcaligenes faecalis* type II.

5 7. A process as claimed in claim 1, wherein said *Bacillus* sp. is selected from the group consisting of *Bacillus lentus*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus sphaericus*, and *Bacillus licheniformis*.

10 8. A process as claimed in claim 1, wherein said *Acinetobacter* sp. is selected from the group consisting of *Acinetobacter haemolyticus* and *Acinetobacter baumannii*.

15 9. A process as claimed in claim 1, wherein said *Brevibacillus* sp. is *Brevibacillus brevis*.

20 10. A process as claimed in claim 1, comprising during said contacting step, the step of aerating said sludge with air.

25 11. A process as claimed in claim 1, comprising during said contacting step, the step of dosing said biofilm with a microorganism solution to increase the microorganism population of said biofilm.

30 12. A process as claimed in claim 11, wherein the microorganism content of said microorganism solution comprises 30% to 70% (vol) microorganisms in a microorganism culture.

13. A process as claimed in claim 11, wherein the microorganisms of said microorganism solution are one or more microorganisms selected from the group consisting of

Bacillus lentus, *Bacillus megaterium*, *Bacillus pumilus*,
Bacillus cereus, *Bacillus subtilis*, *Bacillus sphaericus*,
Bacillus licheniformis, *Brevibacillus brevis*, *Pseudomonas*
aeruginosa, *Pseudomonas stutzeri*, *Listeria seeligeri*,
5 *Acinetobacter haemolyticus*, *Acinetobacter baumannii*, and
Alcaligenes faecalis type II.

14. A process as claimed in claim 11, wherein said dosing
is undertaken in a dosing regime of applying microorganism
10 solution to said sludge about every three weeks for a
period of about nine to about twelve weeks.

15. Use of at least two of the following microorganisms:
Bacillus sp. microorganisms, *Acinetobacter* sp.
15 microorganisms and *Brevibacillus* sp. microorganisms, for
the formation of a biofilm for treating a sludge
containing hydrocarbons.

16. The use of claim 15, wherein the biofilm further
20 comprises microorganisms selected from the group
consisting of *Pseudomonas* sp., *Listeria* sp., *Arthrobacter*
sp., *Alcaligenes* sp., and combinations thereof.

17. The use of claim 15, wherein said *Bacillus* sp.
25 microorganisms are selected from the group consisting of
Bacillus lentus, *Bacillus megaterium*, *Bacillus pumilus*,
Bacillus cereus, *Bacillus subtilis*, *Bacillus sphaericus*,
Bacillus licheniformis and combinations thereof.

30 18. The use of claim 15, wherein said *Acinetobacter* sp.
microorganisms are selected from the group consisting of
Acinetobacter haemolyticus and *Acinetobacter baumannii*.

19. The use of claim 15, wherein said *Brevibacillus* sp. microorganism is *Brevibacillus brevis*.

20. A sludge hydrocarbon bed having a biofilm comprising
5 at least two of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms.

21. The sludge hydrocarbon bed of claim 20, wherein the
10 biofilm further comprises microorganisms selected from the group consisting of *Pseudomonas* sp., *Listeria* sp., *Arthrobacter* sp., *Alcaligenes* sp., and combinations thereof.

15 22. A method of making a bioremediation facility for remediating a hydrocarbon sludge comprising the steps of:

- (a) placing said sludge in a treatment zone;
- (b) seeding said sludge with microorganisms comprising
20 at least two of *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms;
- (c) forming a biofilm from said seeded microorganisms;
and
- (d) degrading said hydrocarbons within said sludge
25 using said biofilm.

23. A method as claimed in claim 22, wherein the
treatment zone is a pit having a substantially impervious
base.

30 24. A method as claimed in claim 22, wherein the treatment zone comprises a substantially impervious cover for covering said sludge.

25. A method as claimed in claim 22, comprising the step of:

(e) aerating said sludge during at least one of said forming step (c) and said degrading step (d).

5

26. A method as claimed in claim 22, wherein the biofilm further comprises microorganisms selected from the group consisting of *Pseudomonas* sp., *Listeria* sp., *Arthrobacter* sp., *Alcaligenes* sp., and combinations thereof.

10

27. A kit for use in treating a sludge containing hydrocarbons, the kit comprising:

(i) a solution containing at least two of *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and
15 *Brevibacillus* sp. microorganisms, and

(ii) instructions for contacting said solution with said sludge under conditions to form a biofilm of said microorganisms and to at least partly reduce the amount of hydrocarbons in said sludge.

20

28. A kit as claimed in claim 27, further comprising a solution containing microorganisms selected from the group consisting of *Pseudomonas* sp., *Listeria* sp., *Arthrobacter* sp., *Alcaligenes* sp., and combinations thereof.

25

29. A kit as claimed in claim 27 or 28, further comprising one or more additives or nutrients, wherein said one or more additives or nutrients are capable of enhancing the growth and biofilm formation of said microorganisms in
30 said solutions.