

TREATMENT OF HYDROCARBON-CONTAMINATED AQUEOUS SOLUTIONS

Technical Field

5 The present invention generally relates to the treatment of aqueous solutions, such as wastewater, contaminated with hydrocarbons, such as petroleum sludge.

Background

10 Aqueous solutions, such as water, are commonly used in various chemical processing industries, such as the petroleum industry. Inevitably in these chemical processing industries, the water becomes contaminated with hydrocarbon containing compounds. For example, process
15 water is used in the petrochemical industry for various applications such as generating steam, cleaning vessels and as a solvent in various unit operations. The water becomes contaminated with oil and other petrochemical compounds such as phenol, benzene, toluene, ethylbenzene,
20 xylene, ketones, cyclohexanes, alcohols and related petrochemical derivatives. This wastewater must be treated to remove the oil contaminants prior to discharge of the wastewater in order to comply with environmental regulatory requirements.

25 Conventional processes for treating such contaminated wastewater typically require an initial physical separation step, such as chemical precipitation, sedimentation and dissolved air flotation (DAF), to remove hydrocarbon contaminants from the wastewater. Such
30 separation systems require significant inputs of energy which results in high treatment costs.

 Biological treatment or "bioremediation" has also been used for treating hydrocarbon contaminated wastewater. Biological treatment may comprise an

exclusively biological treatment process or a hybrid chemical/biological treatment process. Many of the exclusively biological processes rely primarily on the use of enzymes and bacteria that occur naturally in the wastewater to biologically attack the hydrocarbon compounds by breaking them down into non-toxic constituents. However, due to the often harsh environment in which these microorganisms populations must not only survive but flourish in order to be effective, known bioremediation processes have been difficult to sustain and implement. Furthermore, such bioremediation processes may stimulate the growth of pathogenic organisms in addition to the growth of the desired beneficial microorganisms.

Hybrid wastewater treatment processes, on the other hand, have many of the environmental and cost drawbacks of chemical treatment processes. Furthermore, not only do exclusively biological and hybrid treatment processes require extended retention times in the wastewater treatment facility for the wastewater to be decontaminated to an acceptable level for discharge, such systems are also known to be susceptible to failure, known as "shocking", when subjected to high loadings.

There is a need to provide a process for the treatment of aqueous solutions, such as wastewater containing hydrocarbons, 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 wastewater containing hydrocarbons.

Summary

The present invention relates to a novel bioremediation process for treatment of aqueous solutions containing hydrocarbons, such as wastewater generated from petrochemical plants and petrochemical storage facilities.

According to an aspect, there is provided a process for treating an aqueous solution containing hydrocarbons, the process comprising the step of exposing said aqueous solution to a biofilm comprising at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms, in a treatment zone containing said aqueous solution while providing substantially aerobic conditions in said treatment zone during at least part of said exposing step to reduce the amount of said hydrocarbons in said aqueous solution.

According to another aspect, there is provided a method for forming a biofilm comprising at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms, in a treatment zone, said method comprising the steps of:

- (a) providing a plurality of support substrates in a treatment zone;
- (b) seeding said plurality of support substrates with said at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms;
- (c) growing said at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms, on said plurality of support substrates; and
- (d) aerating said treatment zone during at least one of said seeding step (b) and said growing step (c).

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

- 5 (i) a solution containing at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms, capable of forming a biofilm on support substrates and reducing the amount of said hydrocarbons in said aqueous solution, and
- 10 (ii) instructions for seeding said support substrates with said solution under conditions to form said biofilm and reduce the amount of said hydrocarbons in said aqueous solution.

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Definitions

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

The term "chemical oxidation demand" or "COD", when used in connection with an aqueous solution, refers to the
20 total oxidizable material present in the aqueous solution. The oxidizable material may be an organic or an inorganic compound. The COD may be determined by various methods known in the art, for example using American Society for Testing and Materials (ASTM) Test Method D 1252-67.

25 The term "treatment", and grammatical variants thereof, when used in connection with an aqueous solution containing hydrocarbons, refers to at least partial degradation of the hydrocarbon compounds contained therein. For example, the treatment may involve
30 degradation of the hydrocarbons so as to at least partially neutralize toxic compounds contained therein, and render such aqueous solution at least partially 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
5 by the skin.

The term "aerate", and grammatical variants thereof, refers to the introduction of an oxidant, such as air or some other oxygen containing gas, into the aqueous solution to be treated or into the treatment zone within
10 which treatment of said aqueous solution takes place.

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
15 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
20 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
25 inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of components of the formulations, typically means +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the
30 stated value, more typically, +/- 2% of the stated value, even more typically +/- 1% of the stated value, and even more typically +/- 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
5 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,
10 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.

15 **Disclosure of Optional Embodiments**

Exemplary, non-limiting embodiments of a novel process for bioremediation of an aqueous solution, such as wastewater, containing hydrocarbons will now be disclosed.

There is provided a process for treating an aqueous
20 solution containing hydrocarbons, the process comprising the step of exposing said aqueous solution to a biofilm comprising at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms, in a
25 treatment zone containing said aqueous solution while providing substantially aerobic conditions in said treatment zone during at least part of said exposing step to reduce the amount of said hydrocarbons in said aqueous solution.

30 In one embodiment, the aqueous solution is water. For example, the water may be domestic or industrial wastewater containing hydrocarbon compounds.

The process may be performed in a treatment zone. The treatment zone may be located in at least one vessel.

The vessel may be a closed vessel having a substantially impervious base and wall and equipped with means for controlling conditions within the treatment zone during the process.

5 The means for controlling the process conditions, may include a controller for controlling at least one of aeration, pH, temperature and nutrient supply of said aqueous solution within said treatment zone. While the volume of the vessel may depend on the treatment load, it
10 typically ranges from about 100 litres to about 1,000 litres. For example, the treatment load may be about 250 litres, about 320 litres, about 410 litres, about 510 litres, about 630 litres, about 750 litres or about 930 litres.

15 Suitable materials for use in construction of the vessel may include galvanized steel, stainless steel, fiber glass, polyvinyl chloride, plastics, including Teflon or the like.

 The aqueous solution to be treated may be transported
20 from a reservoir and introduced into the vessel via a first inlet pipe, optionally controlled by a first inlet valve. Typically, the hydrocarbons in such aqueous solutions may include combinations of both aliphatics (C₅-C₃₆) and aromatics (C₉-C₂₂). Examples of hydrocarbons found
25 in such aqueous solutions include pentachlorophenols (PCPs); polychlorinated byphenyls (PCBs); polyaromatic hydrocarbons (PAHs) such as naphthalene, anthracene, acenaphthene, acenaphthylene, and pyrene; polynuclear aromatics (PNAs); 2,4,6-trinitrotoluene (TNT);
30 nitrocellulose (NC); benzene, toluene, ethylbenzene, xylene (BTEX); olefins; paraffins; isoparaffins; and other xenobiotics. The initial COD of the aqueous solution typically ranges from about 3,000 ppm to about 30,000 ppm. More typically, the initial COD ranges from about 4,000

ppm to about 15,000 ppm and most typically, from about 5,000 ppm to about 10,000 ppm. For example the initial COD range may be about 3,250 ppm, about 5,250 ppm, about 7,250 ppm, about 9,250 ppm, about 13,250 ppm, about 16,250 ppm, about 18,250 ppm or about 19,250 ppm.

In one embodiment, the aqueous solution may be pre-conditioned prior to being introduced into the vessel. Pre-conditioning typically involves adjustment of pH of the aqueous solution, for example using suitable pH buffers, to an optimal level for promoting growth of the biofilm of microorganisms and enhancing biodegradation of the hydrocarbons. Typical optimal pH levels for promoting biofilm growth are within the range about pH 5 to about pH 9. For example, the optimal pH may be about pH 5.5, about pH 6.0, about pH 6.5, about pH 7.0, about pH 7.5, about pH 8.0, or about pH 8.5.

Once introduced into the vessel, the aqueous solution may be filtered through a headspace that provides space for expansion of the support substrates. Preferably, the headspace may comprise about 5% to about 15% of the total volume of the vessel. More preferably, the headspace may comprise about 7% to about 13% of the total volume of the vessel. Most preferably, the headspace comprises about 9% to about 11% of the total volume of the vessel. For example, the headspace may comprise about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14% or about 15% of the total volume of the vessel.

After filtering through the headspace, the aqueous solution may be permitted to filter onto the biofilm growing on the plurality of support substrates provided within said treatment zone for growing said biofilm.

The support substrates are typically positioned approximately in the central regions of the vessel.

Preferably, the support substrates comprise about 15% to about 55% of the total volume of the vessel. More preferably, the support substrates comprise about 25% to about 45% of the total volume of the vessel. Most preferably, the support substrates comprise about 30% to about 40% of the total volume of the vessel. For example, the support substrates may comprise about 20%, about 25%, about 30%, about 35%, about 40%, about 45% or about 50% of the total volume of the vessel.

10 The support substrates provide support for growth of the microorganisms to form a biofilm. Suitable materials for use as a support substrate for biofilm formation include, but are not limited to, ceramic, plastic, cloth, fiber, metalloid, crystal, polymer, clay, sepiolite, silica, feldspar, sericite, alumina, zeolite, glass, titanium oxide, zirconium oxide, silicone carbide and the like. The support substrate preferably has a surface area that is sufficiently large to provide maximum surface for supporting formation and growth of the biofilm. The

20 particle size of the support substrate typically depends on the material used. For example, where ceramic is used, the particle size may range from about 0.5 cm to about 2 cm, and where plastic spheres are used, the particle size may range from about 3 cm to about 6 cm.

25 A single type of support substrate or several different types of support substrates may be used. Preferably, several different types of substrates are used to enable adjustment of the size of the inter-particle pores to prevent blockage during passage of the aqueous solution. The different types of support substrates used

30 may be of the same particle size, or they may be of different particle sizes. Preferably, the different types of support substrates are of different particle sizes. The volumes taken up by each of the different types of

support substrates may be equal, or they may vary in ratios that depend on the inter-particle pores required. For example, where two different types of substrates are used, the ratios may be about 1:10, about 1:9, about 1:8, 5 about 1:7, about 1:6, about 1:5, about 1:4, about 1:3, about 1:2 or about 1:1.

The plurality of support substrates in the vessel may be seeded with said at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* 10 sp. microorganisms and *Brevibacillus* sp. microorganisms, which grow on said plurality of support substrates to form a biofilm.

The *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms useful 15 in the process are those that are capable of degrading hydrocarbon compounds. Preferably, the *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms are capable of forming biofilms. Such microorganisms may be isolated and 20 enriched from the aqueous solution to be treated. Other 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 *Bacillus* sp. 25 microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms are used in order to obtain a broad spectrum of biodegradability. More advantageously, the microorganism species may be tailored for treatment of different compositions of aqueous 30 solutions.

In one embodiment, the process comprises the step of adding a solution comprising at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms.

In a preferred embodiment, said *Bacillus* sp. may be selected from the group consisting of *Bacillus lentus*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus sphaericus*, and *Bacillus*
5 *licheniformis*. In another preferred embodiment, the *Acinetobacter* sp. is selected from the group consisting of *Acinetobacter haemolyticus* and *Acinetobacter baumannii*. In yet another preferred embodiment, the *Brevibacillus* sp. is *Brevibacillus brevis*.

10 In one embodiment, the microorganisms may form a biofilm. A biofilm is a community of microorganism species that forms a slimy substance that enable the microorganisms to anchor and adhere to a surface. The advantage of forming a biofilm is that the microorganisms
15 will be more resistant to the inhibitory effect of the toxic compounds in the aqueous solution.

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

The selected microorganisms may be combined with
25 other ingredients to form the microorganism solution. The microorganism content of said microorganism solution may 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)
30 microorganisms in a microorganism culture. The other ingredients in the microorganism solution may comprise additives and nutrients for the microorganisms that are useful for promoting growth and stability of the microorganisms and enhancing their attachment to the

support substrates 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

5 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);

10 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,

15 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

In one embodiment, said microorganism solution may be provided in a kit together with instructions for seeding said support substrates with said microorganism solution under conditions to form said biofilm and reduce the amount of said hydrocarbons in the aqueous solution to be treated. The kit may optionally comprise one or more pH buffers that are capable of maintaining the pH of said aqueous solution within a range of about pH 5 to about pH 9 to enhance formation of said biofilm as described above. For example, the pH may be maintained at about pH 5.5, about pH 6.0, about pH 6.5, about pH 7.0, about pH 7.5, about pH 8.0, or about pH 8.5. Examples of suitable pH buffers include, but are not limited to, sodium carbonate, potassium carbonate, sodium bicarbonate, potassium bicarbonate, sodium or potassium diacetate, sodium or potassium phosphate, sodium or potassium hydrogen phosphate, sodium or potassium dihydrogen phosphate,

sodium borate, sodium or ammonium diacetate, sulfamic acid, and the like.

The microorganism solution is preferably kept cool under refrigeration until just before application to the support substrates in the vessel. The microorganism solution may be retained in the vessel for a period of about 30 minutes to about 90 minutes to seed said support substrates. Preferably, the microorganism solution may be retained in the vessel for about 35 minutes, 40 minutes, about 50 minutes, about 60 minutes, about 70 minutes, about 80 minutes or about 85 minutes.

Once the support substrates are seeded with the microorganism solution, the conditions within the vessel may be 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, aeration, pH, temperature, and nutrient supply. Such conditions typically depend on the selection of microorganisms in the microorganism solution.

The aqueous solution may be aerated, either continuously or periodically, with air during the process. Advantageously, the air promotes formation and maintenance of the biofilm. In one embodiment, the air may be injected into the aqueous solution by an air pump via a pipe which may be controlled by a valve. The source of the air may be air compressors.

A suitable temperature within the vessel typically ranges from about 25°C to about 40°C, about 20°C to about 30°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. The pH may be adjusted by addition of an appropriate pH buffer(s), as described above, to maintain

the pH in a desired range suitable for promoting formation and growth of the biofilm and thereby enhance the degradation of the hydrocarbons.

5 In one embodiment, the process comprises the step of dosing the biofilm, to which the aqueous solution is exposed, with a microorganism solution. Advantageously, the dosing promotes maintenance of the biofilm during the exposing step.

10 In one embodiment, said dosing may be undertaken in a dosing regime comprising applying the microorganism solution containing said at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms, to the support substrates in the vessel every about 7 days
15 to about four months. For example, the dosing may be undertaken every about 2 weeks, about 1 month, about 1.5 months, about 2 months, about 2.5 months, about 3 months or about 3.5 months.

20 The dosing regime undertaken may be determined based on such factors as the treatment load, the type of hydrocarbons in the aqueous solution to be treated, the concentration of hydrocarbons in said aqueous solution, the time period within which treatment is to be completed and the concentration of the microorganisms in the
25 solution.

The aqueous solution may be retained within the vessel for a period of time to enable sufficient exposure and contact between the biofilm of microorganisms and the aqueous solution such that the biofilm of microorganisms
30 is able to assimilate the hydrocarbons from the aqueous solution and thus remove at least part of said hydrocarbons from the aqueous solution. The aqueous solution may be retained in the vessel and the process allowed to proceed for a period of time until the level of

the compounds to be biodegraded reaches the target COD level. Preferably, the aqueous solution may be retained within the vessel for about 12 hours to about 168 hours. More preferably, the aqueous solution may be retained
5 within the vessel for about 12 to about 120 hours. Most preferably, the aqueous solution may be retained within the vessel for about 12 to about 48 hours.

Typically, the target COD level is an acceptable level specified by environmental regulations. Preferably,
10 the COD level may be reduced to less than about 1,000 ppm. More preferably, the COD level may be reduced to less than about 700 ppm. Most preferably, the COD level may be reduced to less than about 500 ppm.

Alternatively, the efficiency of the process may be
15 expressed based on the percent of reduction in COD level. The percent of reduction may be determined by the following formula:

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

20

Preferably, the COD level may be reduced by about 50% to about 75%, more preferably by about 75% to about 85%, and most preferably by about 85% to about 100% according
25 to the time that the aqueous solution is exposed to the biofilm.

After the toxic compounds in the aqueous solution have been degraded to said acceptable target level, the treated aqueous solution may be discharged from the vessel
30 via an outlet pipe using an outlet pump. Alternatively, the treated aqueous solution that is not discharged from the vessel may be recycled into the vessel via a second outlet pipe using a second outlet pump and re-introduced into the vessel via a second inlet pipe controlled by a

second inlet valve. The recycled aqueous solution may be combined with the aqueous solution from the first inlet pipe and filtered through the headspace for further exposure and contact with the biofilm of microorganisms on the support substrates to further reduce the amount of hydrocarbons in the aqueous solution. Any suitable type of pump may be used, for example, an M2 type pump may be used.

The size and number of vessels may be varied according to the treatment load. Typically, the treatment load ranges from about 50 litres to about 3,000 litres of aqueous solution. More typically, the treatment load ranges from about 500 litres to about 2,500 litres of aqueous solution. Most typically, the treatment load ranges from about 1,000 litres to about 2,000 litres of aqueous solution. For a treatment load of about 120 litres of aqueous solution, for example, a vessel with a volume of about 220 litres is typically used. Hence, for higher treatment loads, a plurality of vessels with said volumetric dimension may be used. For example, for a treatment load of about 240 litres of aqueous solution, two vessels with a volume of about 220 litres each may be used.

In one embodiment, the treatment zone may be located in a plurality of vessels in series fluid flow with each other. By this it is meant that the aqueous solution to be treated is introduced into one vessel and is passed on to the other subsequent vessels sequentially.

In another embodiment, the treatment zone may be located in a plurality of vessels in parallel fluid flow with each other. When in use, the plurality of vessels in parallel fluid flow permits passage of two or more streams of aqueous solutions, derived from a single stream, through an equal number of separate vessels, with each of

the two or more streams of aqueous solutions passing through a different vessel.

Brief Description Of Drawings

5 The accompanying drawings illustrate a disclosed embodiment and serve 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
10 of the invention.

Fig. 1 is a schematic cross-sectional view of a treatment unit implementing the process for treating an aqueous solution containing hydrocarbons in accordance with one embodiment disclosed herein.

15 Fig. 2 is a process flow diagram showing a first train of five treatment units of Fig. 1 in parallel fluid flow.

Fig. 3 is a process flow diagram showing a pair of trains disclosed in Fig. 2 above, which are in parallel
20 fluid flow.

Fig. 4 is a process flow diagram showing a train of three treatment units of Fig. 1 in series fluid flow.

Fig. 5 shows a graphical representation of the pH, temperature and COD profile of the treatment unit of Fig.
25 1 treating an aqueous solution containing hydrocarbons over a period of about 96 hours (Batch 1).

Fig. 6 shows a graphical representation of the pH, temperature and COD profile of the treatment unit of Fig. 1 treating an aqueous solution containing hydrocarbons
30 over a period of about 135 hours (Batch 2).

Detailed Disclosure of Embodiments

Referring to Fig. 1, there is shown a vessel 101 located within a treatment zone 100 for treatment of an aqueous solution contaminated with hydrocarbons. The vessel 101 is equipped with an aerator (134), pH
5 controller (103), temperature controller (105) and nutrient supply conduit (107) which are utilized during the treatment process.

The total volume of the vessel 101 is about 220 litres with a capacity for a treatment load of about 120
10 litres of aqueous solution.

The aqueous solution to be treated is transported from a reservoir and introduced into the vessel 101 via the first inlet pipe 104 controlled by the first inlet valve 102. The initial COD of the aqueous solution
15 introduced into the vessel 101 is about 5,000 ppm.

In one embodiment, the aqueous solution may be pre-conditioned prior to being introduced into the vessel 101. As discussed above, pre-conditioning typically involves adjustment of pH of the aqueous solution to the optimal
20 level of about pH 7 for promoting growth of the biofilm of microorganisms and enhancing biodegradation of the hydrocarbons.

Once introduced into the vessel 101, the aqueous solution is filtered through a headspace 106 that provides
25 space for expansion of the support substrates (128, 130). Preferably, the headspace 106 comprises about 10% of the total volume of the vessel 101.

After filtering through the headspace 106, the aqueous solution is permitted to filter onto the biofilm
30 growing on the plurality of support substrates (128, 130) provided within said vessel 101 for growing said biofilm.

The support substrates (128, 130) are positioned approximately in the central regions (108, 110) of the vessel 101. The support substrates (128, 130) comprise

about 40% of the total volume of the vessel 101. The support substrates are ceramic 128 and plastic 130 particles. The ceramic 128 and plastic 130 particles used are of different particle sizes to provide a suitable inter-particle pore size and thereby prevent blockage during passage of the aqueous solution undergoing treatment. The volumes taken up by the ceramic 128 and plastic 130 are in a ratio of about 1:3.

The ceramic 128 and plastic 130 particles in the vessel 101 are seeded with *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms, *Brevibacillus* sp. microorganisms and one or more other microorganisms which grow on the ceramic 128 and plastic 130 particles to form a biofilm. As discussed above, the *Bacillus* sp. microorganisms useful in the process include *Bacillus lentus*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus sphaericus*, and *Bacillus licheniformis*; the *Acinetobacter* sp. microorganisms include *Acinetobacter haemolyticus* and *Acinetobacter baumannii*; and the *Brevibacillus* sp. microorganisms include *Brevibacillus brevis*. The one or more other microorganisms include *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Listeria seeligeri*, and *Alcaligenes faecalis* type II.

The microorganism solution is retained in the vessel 101 for a period of about 60 minutes to seed the ceramic 128 and plastic 130 particles. Once the ceramic 128 and plastic 130 particles are seeded with the microorganism solution, the conditions within the vessel 101 are controlled and monitored such that the conditions are maintained at an optimum pH of about 7 and at an optimum temperature of about 30°C required for enhancing microbial growth and biodegradation. The aqueous solution is aerated to promote formation and maintenance of the

biofilm. In one embodiment, air from an air compressor is injected into the aqueous solution by the aeration pump 132 via the pipe 136 which is controlled by the valve 134.

5 The aqueous solution is retained within the vessel 101 for a period of about 72 hours to enable sufficient exposure and contact between the biofilm of microorganisms and the aqueous solution such that at least part of said hydrocarbons is removed from the aqueous solution and the target COD level of less than about 1,000 ppm is achieved.

10 Where the toxic compounds in the aqueous solution have been degraded to a predetermined acceptable target level, the aqueous solution is discharged from the vessel 101 via the outlet pipe 118 using the outlet pump 116. Where the toxic compounds in the aqueous solution have not
15 been degraded to said acceptable target level, the aqueous solution is recycled into the vessel 101 via the second outlet pipe 120 using the second outlet pump 122 and re-introduced into the vessel 101 via the second inlet pipe 126 controlled by the second inlet valve 124. The
20 recycled aqueous solution is combined with the aqueous solution from the first inlet pipe 104 and filtered through the headspace 106 for further exposure and contact with the biofilm of microorganisms on the ceramic 128 and plastic 130 particles to further reduce the amount of
25 hydrocarbons in the aqueous solution.

There is shown in Fig. 2, a treatment zone 200 comprising five vessels (201, 201a, 201b, 201c and 201d). The treatment zone 200 and vessels (201, 201a, 201b, 201c and 201d) are similar to that in Fig. 1 except that the
30 reference numerals for the same features are denoted by numbers by an additional 100, 100', a, b, c, or d. The treatment zone 200 is used to treat about 600 litres of aqueous solution containing hydrocarbons in parallel fluid flow. The vessels (201, 201a, 201b, 201c and 201d) are

equipped with means for controlling aeration (234), pH (203, 203a, 203b, 203c and 203d), temperature (205, 205a, 205b, 205c and 205d) and nutrient supply (207, 207a, 207b, 207c and 207d) within the treatment zone 200 during the
5 treatment process. The volume of each of the vessel (201, 201a, 201b, 201c and 201d) is about 220 litres with a capacity for a treatment load of about 120 litres of aqueous solution per vessel.

The aqueous solution to be treated is transported
10 from a reservoir and introduced into the vessels (201, 201a, 201b, 201c and 201d) via the first inlet pipe 204 which separates into five first inlet pipes (204', 204'a, 204'b, 204'c and 204'd) controlled by the first inlet valve 202. Once introduced into the vessels (201, 201a,
15 201b, 201c and 201d), the aqueous solution is filtered through the headspaces (206, 206a, 206b, 206c and 206d) that provide space for expansion of the support substrates (228, 228a, 228b, 228c, 228d, 230, 230a, 230b, 230c and 230d). Each of the headspaces (206, 206a, 206b, 206c and
20 206d) comprises about 10% of the volume of each of the vessels (201, 201a, 201b, 201c and 201d).

The aqueous solution then filters onto the biofilm growing on support substrates (228, 228a, 228b, 228c, 228d, 230, 230a, 230b, 230c and 230d) that are positioned
25 approximately in the central regions (208, 208a, 208b, 208c, 208d, 210, 210a, 210b, 210c and 210d) of the vessels (201, 201a, 201b, 201c and 201d). During the treatment process, air from an air compressor may be injected into the aqueous solution within the treatment zone 200 by the
30 aeration pump 232 via the pipe 236 which is controlled by the valve 234.

After retention of the aqueous solution within the vessels (201, 201a, 201b, 201c and 201d) for 72 hours, the toxic compounds in the aqueous solution have been degraded

to a required acceptable target level, the aqueous solution is discharged from the vessels (201, 201a, 201b, 201c and 201d) via the outlet pipes (218', 218'a, 218'b, 218'c and 218'd), which are combined into a single outlet
5 pipe 218, using the outlet pump (216). Where the toxic compounds in the aqueous solution have not been degraded to said acceptable target level, the aqueous solution is recycled into the vessels (201, 201a, 201b, 201c and 201d) via the second outlet pipes (220', 220'a, 220'b, 220'c and
10 220'd) which are combined into a single second outlet pipe 220 using the second outlet pump 222 and re-introduced into the vessels (201, 201a, 201b, 201c and 201d) via the second inlet pipe 226 which separates into five second inlet pipes (226', 226'a, 226'b, 226'c and 226'd)
15 controlled by the second inlet valve 224 for further treatment so that the amount of hydrocarbons in the aqueous solution is reduced to the required acceptable target level.

There is shown in Fig. 3, a treatment zone 500
20 comprising two units (300, 400) of the treatment zone of Fig. 2. The treatment zone 500 is similar to that in Fig. 2 except that the reference numerals for the same features are numbers by an additional 100, 100', a, b, c, or d (for treatment zone 300) and an additional 200, 200', a, b, c, or d (for treatment zone 400). The treatment zone 500 is
25 used to treat about 1,200 litres of aqueous solution containing hydrocarbons in parallel fluid flow.

Referring to Fig. 4, there is shown a treatment zone 900 located in three vessels (601, 701, 801) operating in
30 series fluid flow. The treatment zone 900 is similar to that in Fig. 1 except that the reference numerals for the same features are numbers by an additional 500, 500', a, b, c, or d (for vessel 601), an additional 600, 600', a, b, c, or d (for vessel 701) and an additional 700, 700',

a, b, c, or d (for vessel 801) and that the aqueous solution exiting the first vessel 601 via the outlet pipe 618, after being retained in said first vessel for a predetermined period of time, is not discharged but is
5 pumped using pump 616 into the second vessel 701 via the inlet valve 702. Similarly, the aqueous solution exiting the second vessel 701 via the outlet pipe 718, after being retained in said second vessel for a predetermined period of time, is not discharged but is pumped using pump 716
10 into the third vessel 801 via inlet valve 802. The aqueous solution is retained in the first vessel 601 for about 150 hours, in the second vessel 701 for about 150 hours and in the third vessel 801 for about 150 hours.

15

Example

Treatment of Industrial Wastewater in Parallel Fluid Flow

Aqueous solution to be treated

20 The aqueous solution to be treated in Batch 1 was industrial wastewater contaminated with aromatic compounds, esters, ketones, alcohols, and benzene, toluene, ethylbenzene and xylene (BTEX) and having a pH of about 6.5 to 7.1. The original COD value of the
25 contaminated wastewater was approximately 17,600 ppm. The targeted result was to reduce the COD level to less than 1,000 ppm in less than 100 hours.

The aqueous solution to be treated in Batch 2 was industrial wastewater contaminated with cyclohexane and
30 related compounds having a pH of about 5 to 8. The original COD value of the contaminated wastewater was approximately 34,400 ppm. The targeted result was to reduce the COD level to less than 1,000 ppm in less than 140 hours.

The contaminated wastewater was pre-conditioned by adjusting the pH to between pH 5 to pH 8 and circulated through the treatment zone (200) of Fig. 2. Testing was carried out on each batch before and after treatment.

5

Construction of modular vessel

The treatment zone 200 described above was used to treat the industrial wastewater of Batch 1 and Batch 2. Five drums, each having a volume of about 220 litres, were
10 used as the vessels in which the treatment zone 200 was located, with each vessel built to contain about 150 litres of wastewater. The wastewater from a reservoir storage tank was pumped into the first vessel via an inlet at the top of the vessel. The wastewater was allowed to
15 filter through about 20 litres of headspace onto about 20 litres of bioceramics followed by about 60 litres of plastic bioballs. The wastewater at the outlet located at the bottom of each vessel was recycled to the inlet at a rate of 1 litre/hour for 96 hours (in Batch 1) and 135
20 hours (in Batch 2) before being channeled to a post-treatment holding tank where the wastewater was further treated prior to discharge.

The bioceramics and plastic bioballs were prepared by placing dry ceramic and plastic particles in each of said
25 five vessels. A microorganism solution comprising the *Bacillus* sp. microorganisms *Bacillus lentus* A 5019 ST (2% vol), *Bacillus megaterium* B 5013 (2% vol), *Bacillus megaterium* C 6019 (2% vol), *Bacillus pumilus* C 5011 ST (2% vol), *Bacillus cereus* C 5014 (2% vol), *Bacillus cereus* C
30 603 (2% vol), *Bacillus cereus* C 6011 (2% vol), *Bacillus cereus* Z 508 (2% vol), *Bacillus subtilis* C 601/1 (2% vol), *Bacillus subtilis* C 601/2 (2% vol), *Bacillus sphaericus* C 605 (2% vol), and *Bacillus licheniformis* Z 507 ST (2% vol), and *Brevibacillus brevis* Z 6030 PT (2% vol),

Pseudomonas aeruginosa A 6017 (2% vol), *Pseudomonas stutzeri* B 5012 ST (2% vol), *Listeria seeligeri* B 5011 (2% vol), *Pseudomonas stutzeri* B 509 PT (2% vol), *Pseudomonas stutzeri* B 6013 (2% vol), *Acinetobacter haemolyticus* C 602
5 (4% vol), *Acinetobacter baumannii* Z 6011 (4% vol), *Acinetobacter baumannii* Z 6013 (4% vol), *Alcaligenes faecalis type II* Z 6020 ST (2% vol) and *Arthrobacter* sp. C 505 PT (2% vol) was applied to the dry ceramic and plastic particles and allowed to seed said particles for about 30
10 minutes to about 60 minutes before filtering the wastewater through.

Bio-treatment of the wastewater

Air supply, pH and temperature were controlled throughout
15 the experiment. Air was supplied by a single M2 pump while pH was adjusted by the addition of 0.1 M potassium phosphate buffer. The temperature were controlled between 28.5°C to 29.5°C in Batch 1 and between 26°C to 29.5°C in Batch 2.

20

Analysis of COD by colorimetry

COD analysis was carried out using the closed-reflux micro method of and commercial COD reagents from HACH.
25 The reagents were supplied in 16-mm vials that fit directly into the Hach Colorimeter. 2 mL of a sample was added to a vial, after which the vial was capped and placed in a reactor for digestion at 150°C for two hours. After cooling the vial, the COD was read directly on the
30 colorimeter.

If the colorimetric reading exceeded 1,500 mg/L for a sample being pumped into the first vessel or exceeded 150 mg/L for a sample being pumped out of the fifth vessel, the mixture in the vial turned green. In such instances,

appropriate dilutions were made or higher strength reagents were used, and the analysis was repeated.

RESULTS

5 Fig. 5 shows the treatment results for Batch 1. Under the pH condition of 6.5 to 7.1 and at temperatures of between 28.5°C and 29.5°C, the COD level was reduced by about 96% from a starting value of about 17,600 ppm to about 640 ppm after 96 hours of treatment.

10 Fig. 6 shows the treatment results for Batch 2. Under the pH condition of 5 to 8 and at temperatures of between 26°C and 29.5°C, the COD level was reduced by about 98% from a starting value of about 34,400 ppm to about 780 ppm after 135 hours of treatment.

15 Advantageously, as can be seen in Fig. 5 and Fig. 6 for the period from 0 hour to about 40 hours, COD reduction was exponential at a pH value of about 7. Although the initial COD values of the industrial wastewater in both Batch 1 and Batch 2 were high (above
20 15,000 ppm), the final COD levels after treatment were less than the targeted 1,000 ppm.

25

Applications

It will be appreciated that the process disclosed herein is a simple and cost-effective yet efficient and environmentally friendly process for treatment of aqueous solutions containing hydrocarbons, such as wastewater.

30 Advantageously, the process is capable of reducing high levels of COD by more than about 95% in about 4 to 6 days of treatment depending on the starting COD levels.

Advantageously, and in contrast to conventional chemical treatment processes, the process disclosed herein does not use any toxic chemicals.

Advantageously, the process disclosed herein does not
5 require any pre-dilution steps to prevent failure of the treatment system due to high loadings.

It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing
10 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.

Claims

1. A process for treating an aqueous solution containing hydrocarbons, the process comprising the step of exposing said aqueous solution to a
5 biofilm comprising at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms, in a treatment zone containing said aqueous solution while providing
10 substantially aerobic conditions in said treatment zone during at least part of said exposing step to reduce the amount of said hydrocarbons in said aqueous solution.
- 15 2. A process as claimed in claim 1, wherein said aqueous solution is water.
3. A process as claimed in claim 1, wherein said
20 *Bacillus* sp. is selected from the group consisting of *Bacillus lentus*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus sphaericus*, and *Bacillus licheniformis*.
4. A process as claimed in claim 1, wherein said
25 *Acinetobacter* sp. is selected from the group consisting of *Acinetobacter haemolyticus* and *Acinetobacter baumannii*.
5. A process as claimed in claim 1, wherein said
30 *Brevibacillus* sp. is *Brevibacillus brevis*.
6. A process as claimed in claim 1, wherein said biofilm further comprises one or more microorganisms selected from the group consisting

of *Pseudomonas* sp., *Listeria* sp., *Arthrobacter* sp., and *Alcaligenes* sp.

- 5 7. A process as claimed in claim 6, 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.
- 10 8. A process as claimed in claim 1, comprising, before said exposing step, the step of providing a plurality of support substrates within said treatment zone for growing said biofilm thereon.
- 15 9. A process as claimed in claim 8, wherein said support substrates are selected from the group consisting of ceramic, plastic, cloth, fiber, metalloid, crystal, polymer, clay, sepiolite, silica, feldspar, sericite, alumina, zeolite, 20 glass, titanium oxide, zirconium oxide, silicone carbide and combinations thereof.
- 25 10. A process as claimed in claim 8, wherein said support substrates comprise 15% to 55% volume of said treatment zone.
- 30 11. A process as claimed in claim 1, wherein said exposing step is performed for a period of 12 hours to 168 hours.
12. A process as claimed in claim 1, wherein said treatment zone is located in at least one vessel.

13. A process as claimed in claim 1, wherein said treatment zone is located in a plurality of vessels in series fluid flow.
- 5 14. A process as claimed in claim 1, wherein said treatment zone is located in a plurality of vessels in parallel fluid flow.
- 10 15. A process as claimed in claim 1, comprising, after said exposing step, the step of recycling said aqueous solution into said treatment zone for further exposing said aqueous solution to said biofilm to thereby further reduce the amount of said hydrocarbons in said aqueous solution.
- 15 16. A method for forming a biofilm comprising at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms, in a treatment zone, said method comprising the steps of:
- 20 (a) providing a plurality of support substrates in a treatment zone;
- 25 (b) seeding said plurality of support substrates with said at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms;
- 30 (c) growing said at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms, on said plurality of support substrates; and

(d) aerating said treatment zone during at least one of said seeding step (b) and said growing step (c).

5

17. A method as claimed in claim 16, wherein said seeding step (b) comprises exposing said plurality of support substrates to a solution containing said at least one of the following microorganisms:

10

Bacillus sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms.

18. A method as claimed in claim 16, wherein said growing step (c) is performed for at least 30 minutes to 90 minutes.

15

19. A method as claimed in claim 16, further comprising the step of:

20

(e) adjusting the pH of said treatment zone during at least one of said seeding step (b) and said growing step (c) to enhance formation of said biofilm, wherein said pH is in the range of pH 3 to pH 10.

25

20. A method as claimed in claim 16, further comprising the step of:

30

(f) dosing said biofilm with a solution containing said at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms, to increase the microorganism population of said biofilm.

21. A method as claimed in claim 20, wherein said dosing is undertaken in a dosing regime comprising applying said solution containing said at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms, to said support substrates in the range of every 7 days to four months.
22. A method as claimed in claim 16, wherein said *Bacillus* sp. 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.
23. A method as claimed in claim 16, wherein said *Acinetobacter* sp. is selected from the group consisting of *Acinetobacter haemolyticus* and *Acinetobacter baumannii*.
24. A method as claimed in claim 16, wherein said *Brevibacillus* sp. is *Brevibacillus brevis*.
25. A method as claimed in claim 16, wherein said biofilm further comprises microorganisms selected from the group consisting of *Pseudomonas* sp., *Listeria* sp., *Arthrobacter* sp., *Alcaligenes* sp., and combinations thereof.
26. A method as claimed in claim 25, 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 27. A kit for use in treating an aqueous solution containing hydrocarbons, the kit comprising:

10 (i) a solution containing at least one of the following microorganisms: *Bacillus* sp. microorganisms, *Acinetobacter* sp. microorganisms and *Brevibacillus* sp. microorganisms, capable of forming a biofilm on support substrates and reducing the amount of said hydrocarbons in said aqueous solution, and

15 (ii) instructions for seeding said support substrates with said solution under conditions to form said biofilm and reduce the amount of said hydrocarbons in said aqueous solution.

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, 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 said solutions.

30

30. A kit as claimed in claim 27, further comprising one or more pH buffers to enhance formation of said biofilm.

5 31. A kit as claimed in claim 30, wherein said one or more pH buffers are capable of maintaining the pH of said aqueous solution within a range of pH 5 to pH 9.