

Sept. 24, 1963

W. M. HARRISON  
BACTERIAL TREATMENT OF MEDIA CONTAINING  
HYDROCARBONS AND SULFIDES

3,105,014

Filed Dec. 7, 1961

3 Sheets-Sheet 1

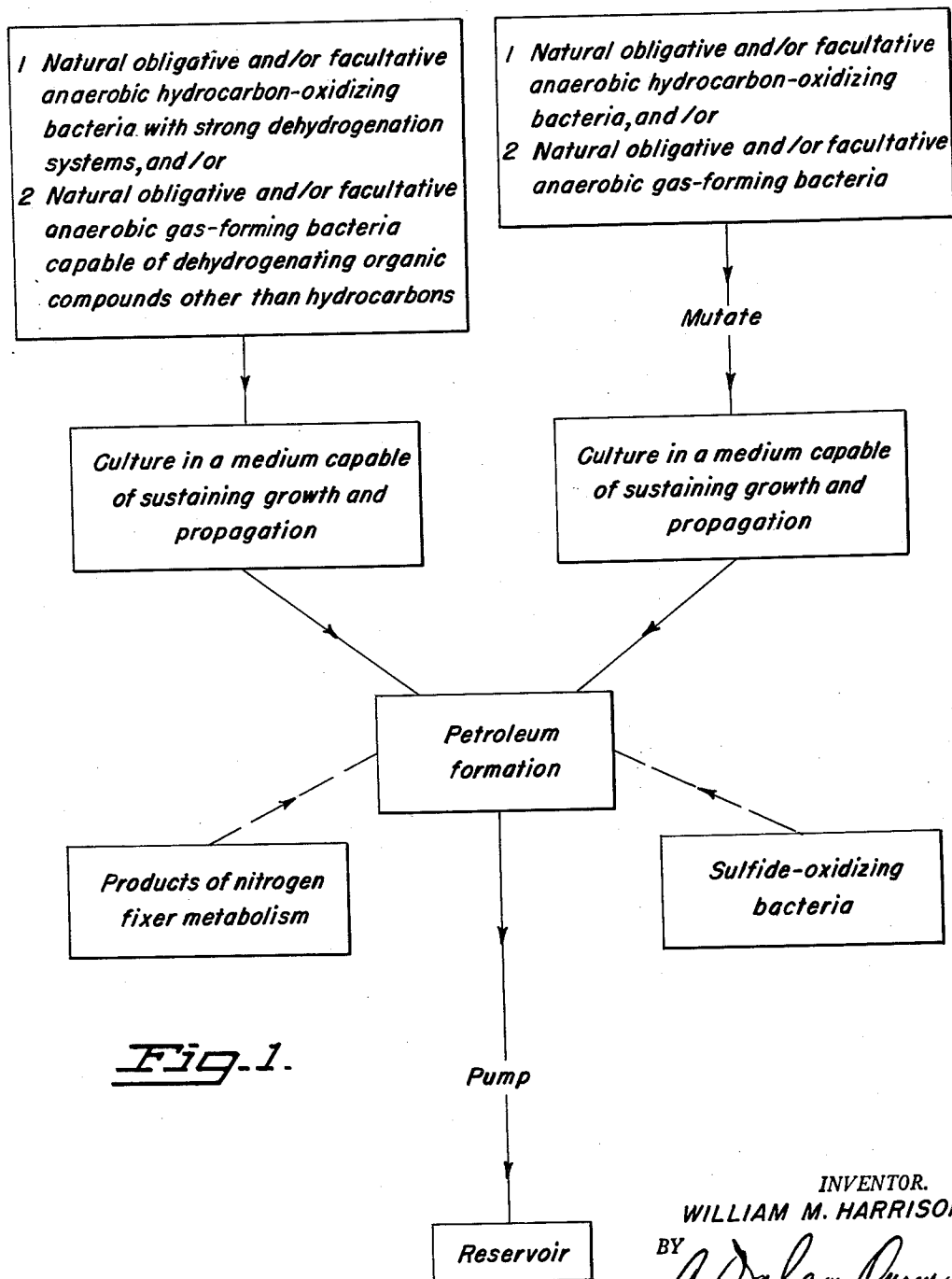


Fig. 1.

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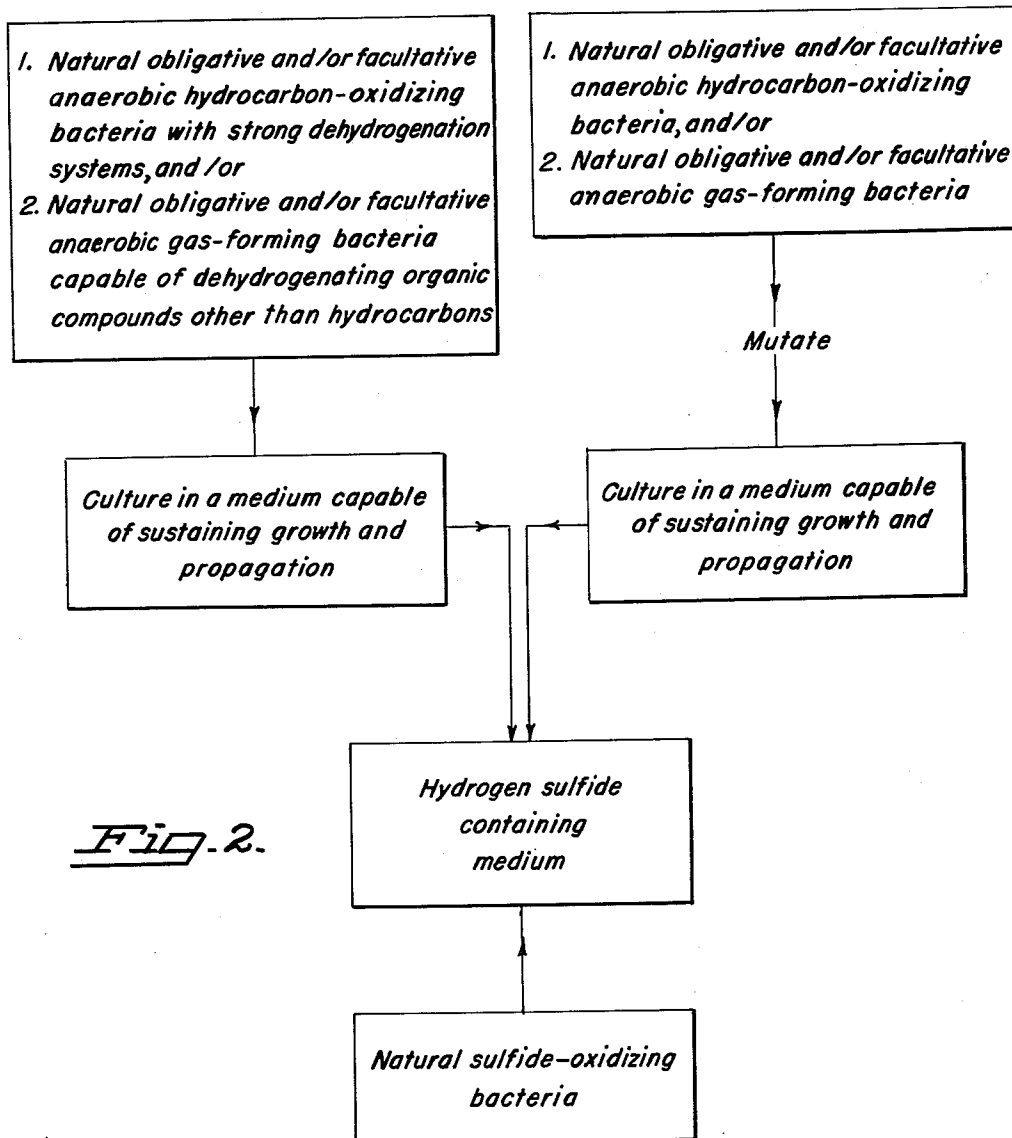
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3 Sheets-Sheet 2



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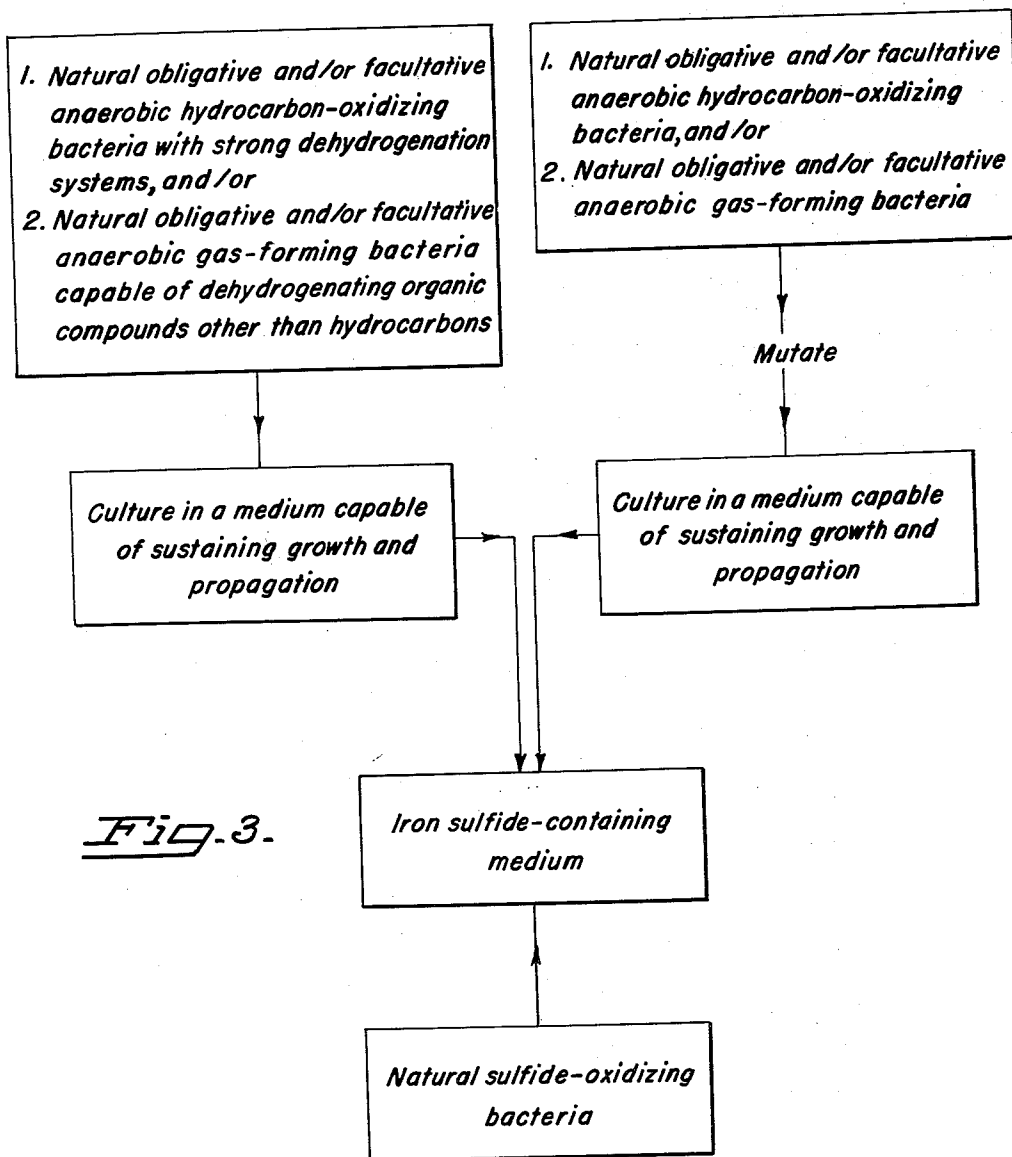
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3 Sheets-Sheet 3



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3,105,014  
**BACTERIAL TREATMENT OF MEDIA CONTAINING HYDROCARBONS AND SULFIDES**  
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Filed Dec. 7, 1961, Ser. No. 153,938  
57 Claims. (Cl. 195—3)

This invention relates to bacterial inoculation and alteration of media containing hydrocarbons and/or sulfides, including, but not restricted to, surface and underground waters, mud, sand, oil formations, and the like. More particularly, this invention relates to the use of a combination of natural and mutant bacteria for (1) recovering oil from surface and underground formations thereof, (2) eliminating undesirable hydrogen sulfide from waste waters, sewage, muds, oil formations, etc., (3) removing precipitated iron sulfide from various deposits thereof, and (4) chemically altering hydrocarbons.

## PROBLEM OF OIL PRODUCTION

The rapid depletion of world oil reserves and the increasing demand for petroleum fuels, lubricants, and petrochemicals, has established the need for a means to increase the known available petroleum deposits. Exploration and development of new deposits, and secondary recovery of crudes, are conventional procedures for increasing the supply of crude oil. Exploration and development are expensive, due to the great depths at which most undiscovered oil deposits now are found. Secondary methods of recovery, such as flooding and pressurizing with gas, increase the yield; however, after all present secondary recovery methods (excluding burning, which consumes as much oil as it reclaims and involves high conversion costs) have been tried, 40%–95% (depending upon the gravity of the oil and type of formation) of the original oil remains underground.

Another problem in recovering crude petroleum from underground deposits is that it has a great tendency to cling to the formation. For example, clays occlude ring-type compounds. Also, where heavy-viscosity crudes are concerned, not only do they cling to the rock strata but their tendency to flow toward a well-bore or tunnel is greatly restricted and easily prevented by plugging conditions and comparatively low temperatures.

Attempts have been made to increase the flow of an oil well by inoculating the well with natural bacteria supposedly capable of oxidizing crude petroleum hydrocarbons to lower molecular weight, less viscous hydrocarbons. Complete oxidization of a hydrocarbon by other than bacterial means begins with dehydrogenation and proceeds through various steps to form an organic acid which is subsequently decarboxylated to a hydrocarbon with a shorter chain than the original compound. This shorter hydrocarbon is then oxidized in the same manner, and on and on until the hydrocarbons have been converted into carbon dioxide and water. This complete oxidation is not, however, achieved by anaerobic bacterial processes heretofore used. Bacteria prefer to oxidize long-chain hydrocarbons, for the shorter the chain the more energy is required to split off carbon. As a result, natural anaerobic bacterial oxidation of petroleum stops short of completion, and a large amount of intermediate products, mainly organic acids, builds up. These intermediate products have higher melting points, boiling points, and greater viscosities than the hydrocarbons from which they stem, so that when natural bacteria alone are introduced into an oil formation the flow of crude from the formation is generally restricted, rather than increased.

For instance, in Zo Bell U.S. Patent No. 2,413,278, the use of natural *Desulfovibrio* bacteria to oxidize petroleum hydrocarbons to less viscous, shorter chain-length compounds is disclosed. Most bacteria in this group will de-

hydrogenate oil as shown with methylene blue reduction, but then stop short of complete oxidation. The addition of peroxidase (a general enzyme which catalyzes the breakdown of hydrogen peroxide into water and oxygen) will cause oxidation of the reduced methylene blue, but twenty-four hours later it is again reduced and the reaction again stops. Hydrogen sulfide also stops the reaction by partially poisoning the oxidizing system of the *Desulfovibrio* bacteria. *Pseudomonas* bacteria have been suggested for oxidizing hydrocarbons, providing a trace of oxygen is present. However, they apparently lack the ability to initiate oxidation. The combination of the two natural-occurring bacteria does very little more than the two separately.

Other problems are involved in using natural bacteria to recover oil, for the bacterial oxidation produces hydrogen sulfide and carbon dioxide, both of which corrode the metallic pipes, pumps, etc., used in well-drilling and oil recovery. Also, the intermediate fatty acids saponify with metallic ions in the cognate waters to form emulsifying soaps that further thicken the oil and plug formations.

An important object of this invention is the provision of a bacterial means for efficiently recovering oil from surface and underground deposits thereof.

Another important object of this invention is to practice bacterial means for the primary, secondary, or tertiary recovery of crude petroleum from oil wells.

Another object of this invention is the provision of a novel bacterial means for reducing the viscosity of crude petroleum, thereby enabling the oil to flow to an accumulating area for subsequent removal.

Another object of this invention is to supply gases, such as methane, carbon-dioxide, hydroxyl amine, nitrogen, and others, to dilute the oil and supply a gas-drive to help move the oil to the well bore-hole.

Still another object of this invention is the provision of a process for increasing the available supply of crude oil.

## HYDROGEN SULFIDE PROBLEM

Waste waters, such as sewage, effluents from oil fields, paper mills and other industries, and those resulting from other natural and human activities, as well as mud, are potential sources of hydrogen sulfide generation. Hydrogen sulfide, even in small concentrations, is a nuisance due to its obnoxious odor, taste, and reactivity; an illustration of the former is the foul odor usually surrounding paper mills, sewage plants, oil fields, refineries, etc.; an illustration of the latter is the blackening of silverware and lead-based housepaints. In moderate-to-large concentrations, hydrogen sulfide is a menace to wildlife, fish in particular, and also to humans.

When a waste-water or mud contains inorganic sulfates and organic material, sulfate-reducing bacteria, usually present therein, feed upon the organic material, using the oxygen component of the sulfate as its oxygen source and excreting sulfide. These bacteria belong to the genus *Desulfovibrio*, are anaerobes, and their oxidative metabolism utilizes sulfate as an hydrogen acceptor, just as oxygen or nitrate functions as an hydrogen acceptor for other organisms. Examples of the sulfate-reducing bacteria are *Desulfovibrio aestuarii* and *Desulfovibrio desulfuricans*. If air is absent, the sulfate-reducing bacteria, which are found nearly everywhere, may thrive.

Oil production operations often result in waste-water streams which contain traces of oil. In order to dispose of the waste-water, it is necessary to remove all oil, which might separate and disfigure or discolor streams or beaches. The separation of this oil is carried out conveniently in sumps or separators, where the oil is impounded long enough to allow a complete separation of oil from water to take place. The condition of the water and mud in these sumps generally is ideal for propagation

of sulfate-reducing bacteria; the layer of crude oil on top of the water effectively excludes sunlight and air, and provides food for bacteria; the water and mud usually contain ample sulfates, and thus the source of sulfates is constantly replenished; the water is generally impounded long enough to allow the sulfate-reducing bacteria to generate substantial amounts of hydrogen sulfide. The hydrogen sulfide thus produced is easily released in subsequent disposal systems and creates a nuisance due to odor, taste, paint damage, metallic corrosion, etc.

This undesirable bacterial generation of hydrogen sulfide is by no means confined to oil-field waste-water systems. Sanitary sewers, certain food processing effluents, paper mills, chemical plants, etc., as well as natural sources, are causes of hydrogen sulfide generation, usually in amounts more than sufficient to be a genuine problem to employees, to residents of the surrounding area, and to wildlife inhabitants of the streams, etc., in which the waste-waters are dumped.

Several methods for controlling the bacterial generation of hydrogen sulfide are known, but they either are too expensive, relatively ineffective, or dangerous. For example, aeration of the water has been used. Insofar as aeration destroys the sulfides by oxidation to sulfur, sulfates, and the like, this method is satisfactory; but the slowness of air oxidation results in expulsion of most of the sulfides into the air as hydrogen sulfide, thereby intensifying the nuisance. Also, sulfides are reproduced farther downstream by bacterial reduction of the oxidized sulfur compounds. Ozone has been used, but it is expensive and the oxidized sulfur compounds so produced are likewise reduced to sulfides downstream. Halogens, such as chlorine or bromine, have been used to destroy sulfides in streams and sumps; but the high halogen demand of the sulfides, trace-oil, and other contaminants, makes this type of treatment very expensive, and toxic to wildlife of streams. This process also is temporary, for sulfides are reproduced from the oxidized sulfur compounds downstream when the concentration of halogen is insufficient. Metal salts, such as iron sulfate and iron chloride, have been used to precipitate out the offending sulfides but, in addition to high chemical cost, such methods are objectionable because the black iron sulfide so produced becomes dispersed in the effluent waste-waters and is mistaken by the public for crude oil, and the sulfate and chloride are very corrosive to iron and other metallic objects. Furthermore, iron sulfate provides sulfate ions for further sulfide production. In some sanitary systems, bacterial inhibitors such as chlorinated aromatics have been used successfully where the mud or sludge is very thin, but since they penetrate only about one inch of stationary mud, etc., they are valueless in oil-field sumps containing large amounts of infested muds, crude oil, and sulfates; also, these inhibitors are very expensive.

Another object of my invention, therefore, is to provide a means for the control of bacterial production of hydrogen-sulfide, both above and below the ground.

Another object of my invention is to provide a means for elimination of hydrogen sulfide in underground oil formations and surface oil-containing ponds and sumps.

Another object of my invention is to provide a cheap and efficient means for rendering waste-waters sufficiently pure and safe that they may be dumped into large, natural bodies of water without menacing the wildlife inhabitants thereof.

Still another object of my invention is the provision of a means for eliminating or greatly reducing the hydrogen sulfide odors in the atmosphere around waste-waters and others, oil fields, and refineries.

A still further object of my invention is the provision of a means for eliminating hydrogen sulfide from natural deposits thereof.

### IRON SULFIDE PROBLEM

In the production of crude petroleum, extensive use is made of steel pipes, valves, pumps and other machinery

for conducting a flow of hydrogen sulfide-containing gas, oil, water, mud and the like. When these fluids come into contact with the iron, a chemical reaction takes place and iron sulfide is formed as a precipitate. Underneath this iron sulfide scale corrosion, as pitting, rapidly takes place. If allowed to accumulate, this iron sulfide precipitate eventually will substantially reduce the fluid flow through the system, at points of restriction, and can even plug the system entirely at these points, cutting down production and necessitating extensive cleaning procedures.

This iron sulfide precipitate also accumulates in oil formations and restricts the flow of crude oil to the bore-hole of the oil well. This problem is especially prevalent in water-flood programs, but can occur naturally.

Other sites of iron sulfide deposits are considerable public problems; for instance, along beaches where wastewater containing iron sulfide has been discharged. The black iron sulfide accumulates on the beach, discoloring it and degrading its quality as a recreational area.

Another object of my invention, therefore, is the removal of iron sulfide from oil formations and other sites where its presence would restrict a flow of oil, gases, etc.

A further object of my invention is to remove sulfide ions from oil, gases, etc., that precipitate iron sulfides into piping, valves, and machinery.

A further object of my invention is the elimination of the cause of unsightly iron sulfide contamination of beaches and stream beds.

### PROBLEM OF HYDROCARBON ALTERATION

The metabolism of practically all natural bacteria is somewhat like that of an animal in that it involves the oxidation of organic compounds to obtain the energy that is stored in these complex molecular configurations. Hydrocarbons are one class of organics that natural bacteria oxidize, and this process includes dehydrogenation of the hydrocarbons and reduction of other compounds. Dehydrogenation also involves the formation of new products that, when their concentration becomes great enough, act as anti-metabolites by decreasing the oxidizing activity of the dehydrogenases of the bacteria. In order to keep dehydrogenation and oxidation going at a maximum rate, these antimetabolites must be removed.

Another object of my invention is to provide a new method of chemically altering hydrocarbons and hydrocarbon-containing media.

A further object of my invention is to provide a new method for increasing and maintaining the rate of bacterial oxidation of hydrocarbons and hydrocarbon-containing media.

Yet another object of my invention is to provide a new method of increasing and maintaining the rate of bacterial dehydrogenation of hydrocarbons and hydrocarbon-containing media.

Still another object of my invention is to provide a new method of chemically altering media containing intermediate bacterial hydrocarbon metabolism derivatives.

The aforementioned problems are resolved and the objects accomplished by making use of the metabolic activities of certain bacteria in a new and inventive manner. The metabolisms of both natural bacteria and mutant strains of natural bacteria are combined to provide a result never before achieved. Where increased production of oil is desired, the oil-bearing formation is inoculated with a combination of certain natural bacteria and certain mutant bacteria. Where reduction or elimination of hydrogen sulfide and iron sulfide in oil formations, waste-waters, muds, sands, and the like is desired, these media are inoculated with certain natural bacteria alone, or in combination with certain mutant bacteria. Where prevention of precipitated iron sulfide in piping, valves, and other machinery is the object, the sulfide-producing media are inoculated either with certain natural bacteria or a combination of certain natural bacteria plus certain mutant bacteria, to diminish or eliminate the offending

sulfides. And where chemical alteration of hydrocarbons and/or intermediate hydrocarbon metabolism derivatives is the object, these media are inoculated with a combination of certain natural bacteria and certain mutant bacteria.

The accompanying drawings are flow-sheets of preferred embodiments of the processes of this invention, wherein: FIG. 1 illustrates the invention in producing oil;

FIG. 2 illustrates the invention in eliminating hydrogen sulfide; and

FIG. 3 illustrates the invention in eliminating iron sulfide.

### PRODUCTION OF OIL

For producing oil, the process of my invention involves cooperation in the oil-bearing formation between (1) one or more strains of mutant hydrocarbon-oxidizing bacteria, capable of living under anaerobic conditions, having partially or totally inactivated hydrocarbon-dehydrogenation systems, and which depend therefore on partially oxidized hydrocarbons as an energy source for their metabolism, and (2) one or more strains of natural hydrocarbon-oxidizing bacteria also capable of living under anaerobic conditions. If, as is sometimes the case, oil-bearing formations already contain the (2) natural bacteria in sufficient quantity, then only the (1) mutant bacteria need be added to the oil-bearing formation.

My invention makes use of the hydrocarbon dehydrogenation activity of certain natural bacteria and combines with it the further hydrocarbon oxidation activity of mutants of certain bacteria. In my invention, the natural bacteria are used to initiate hydrocarbon oxidation by dehydrogenation and the mutant bacteria carry on the oxidation by metabolizing the dehydrogenated hydrocarbon "leavings" of the natural bacteria. In this way, the problems of accumulation of higher viscosity hydrocarbons and the plugging of the rock formations are alleviated. Gases in relatively large quantities also are released; these tend to pressurize the formation and reduce the viscosity of the petroleum hydrocarbons, making them easier to recover.

Of the various bacteria usable in the process of my invention, preference is given to those having one or more of the following qualities: (1) bacteria that are non-toxic to both plant and animal life; (2) bacteria, both natural and as mutants, that are able to carry on their metabolism under anaerobic (i.e., in the absence of atmospheric oxygen) conditions, viz. obligate anaerobic and facultative anaerobic bacteria; (3) bacteria that do not require the addition of expensive nutrients to continue hydrocarbon oxidation; (4) bacteria that do not form mats or slimes that would plug up the underground formation; and (5) bacteria that have thermophilic properties or are able to withstand the increased temperature and pressure encountered in oil formations.

The operable natural bacteria are all those capable of dehydrogenating hydrocarbons under anaerobic conditions. It is conceivable that in a water-flood operation oxygen in small quantities could be carried down into a formation and be utilized by some bacteria. In such a situation, those bacteria which can utilize oxygen for hydrocarbon dehydrogenation may also be used.

The following Table I contains a partial list of operable natural bacteria that might be used. There are many more such bacteria known, and perhaps as many or more still unknown. No intention is present to limit the invention to this list, for it is representative only and not exhaustive.

Table I.—Some Natural Bacteria Satisfactory for Dehydrogenation

|                                    |                             |
|------------------------------------|-----------------------------|
| <i>Achromobacter sulfureum</i>     | <i>Cellulomonas folia</i>   |
| <i>Bacillus laterosporus</i>       | <i>Cellulomonas flava</i>   |
| <i>Bacillus thermoliquefaciens</i> | <i>Cellulomonas iugis</i>   |
| <i>Bacillus tostus</i>             | <i>Cellulomonas caesia</i>  |
| <i>Bacterium udum</i>              | <i>Cellulomonas gilva</i>   |
| <i>Bacterium bibulum</i>           | <i>Cellulomonas pusilla</i> |
| <i>Cellulomonas surrogens</i>      | <i>Cellulomonas gelida</i>  |
| <i>Cellulomonas galba</i>          | <i>Cellulomonas rossica</i> |

*Clostridium fesceri*  
*Clostridium acetobutylicum*  
*Clostridium sartagoformum*  
*Clostridium roseum*  
*Clostridium felsineum*  
*Corynebacterium helvolum*  
*Desulfovibrio desulfuricans*  
*Desulfovibrio aestuarii*  
*Desulfovibrio rubentschikii*  
*Desulfovibrio halohydrocar-*  
*bonoclasticus*  
*Flavobacterium okeanoikoites*  
*Flavobacterium marinotypi-*  
*cum*  
*Flavobacterium marinoviro-*  
*sum*  
*Flavobacterium suaveolens*  
*Flavobacterium esterocar-*  
*maticum*  
*Pseudomonas viscosa*

*Pseudomonas ureae*  
*Pseudomonas effusa*  
*Pseudomonas myxogenes*  
*Pseudomonas putida*  
*Pseudomonas putrefaciens*  
*Pseudomonas punctata*  
*Pseudomonas minuscula*  
*Pseudomonas mira*  
*Pseudomonas marinogluti-*  
*nosa*  
*Pseudomonas calcis*  
*Pseudomonas calciprecipi-*  
*tans*  
*Pseudomonas fermentans*  
*Pseudomonas trifolii*  
*Pseudomonas caudata*  
*Pseudomonas perlurida*  
*Pseudomonas ochracea*  
*Vibrio leonardii*

The mutant bacteria which are combined with the foregoing natural bacteria according to this invention are prepared from natural bacteria having suitable hydrocarbon oxidizing systems. The following Table II lists some operable species for forming these mutants, but is not all-inclusive thereof. There is no intention to limit the scope of the invention to this list, as other bacteria with hydrocarbon oxidizing systems also will work.

Table II.—Some Natural Bacteria Useful To Form Mutants

|                                      |                                    |
|--------------------------------------|------------------------------------|
| <i>Achromobacter aerophilum</i>      | <i>Corynebacterium fimi</i>        |
| <i>Achromobacter citrophilum</i>     | <i>Corynebacterium tumescens</i>   |
| <i>Achromobacter pastinator</i>      | <i>Corynebacterium simplex</i>     |
| <i>Achromobacter thalassius</i>      | <i>Flavobacterium fuscum</i>       |
| <i>Achromobacter iophagus</i>        | <i>Flavobacterium maris</i>        |
| <i>Achromobacter delicatulus</i>     | <i>Flavobacterium diffusum</i>     |
| <i>Achromobacter aquamarinus</i>     | <i>Flavobacterium rigense</i>      |
| <i>Achromobacter cyclocastes</i>     | <i>Flavobacterium rhenanus</i>     |
| <i>Achromobacter stationis</i>       | <i>Flavobacterium lutescens</i>    |
| <i>Achromobacter delmarvae</i>       | <i>Flavobacterium proteus</i>      |
| <i>Achromobacter agile</i>           | <i>Leuconostoc citrovorum</i>      |
| <i>Achromobacter centropuncta-</i>   | <i>Methanobacterium soehngenii</i> |
| <i>tum</i>                           | <i>Methanobacterium omelian-</i>   |
| <i>Agarobacterium bufo</i>           | <i>skii</i>                        |
| <i>Agarobacterium reducans</i>       | <i>Methanomonas methanica</i>      |
| <i>Agarobacterium viscosum</i>       | <i>Methanomonas carbonato-</i>     |
| <i>Alcaligenes metalcaligenes</i>    | <i>phila</i>                       |
| <i>Alcaligenes recti</i>             | <i>Micrococcus denitrificans</i>   |
| <i>Bacillus subtilis</i>             | <i>Micrococcus paraffinae</i>      |
| <i>Bacillus firmus</i>               | <i>Mycobacterium alium</i>         |
| <i>Bacillus polymyxa</i>             | <i>Mycobacterium rubrum</i>        |
| <i>Bacillus macerans</i>             | <i>Mycobacterium lacticola</i>     |
| <i>Bacillus circulans</i>            | <i>Propionibacterium freuden-</i>  |
| <i>Bacillus laterosporus</i>         | <i>reichii</i>                     |
| <i>Bacillus brevis</i>               | <i>Propionibacterium shermanii</i> |
| <i>Bacillus lactorubefaciens</i>     | <i>Propionibacterium rubrum</i>    |
| <i>Bacillus mycoides corallinus</i>  | <i>Propionibacterium thoenii</i>   |
| <i>Bacillus bruntzii</i>             | <i>Propionibacterium technicum</i> |
| <i>Bacillus thermoamylolyticus</i>   | <i>Proteus vulgaris</i>            |
| <i>Bacillus kaustophilus</i>         | <i>Pseudomonas aeruginosa</i>      |
| <i>Bacillus thermolactatus</i>       | <i>Pseudomonas fluorescens</i>     |
| <i>Bacillus calidolactis</i>         | <i>Pseudomonas boreopolis</i>      |
| <i>Bacillus michaelisii</i>          | <i>Pseudomonas cleovorans</i>      |
| <i>Bacillus thermocellulolyticus</i> | <i>Pseudomonas incognita</i>       |
| <i>Bacillus thermoalimento-</i>      | <i>Pseudomonas scissa</i>          |
| <i>philus</i>                        | <i>Pseudomonas denitrificans</i>   |
| <i>Bacillus viridulus</i>            | <i>Pseudomonas eisenbergii</i>     |
| <i>Bacillus hexacarbovorum</i>       | <i>Pseudomonas mephitica</i>       |
| <i>Bacillus mesentericus</i>         | <i>Pseudomonas multistriata</i>    |
| <i>Bacillus ethanicus</i>            | <i>Pseudomonas hydrophila</i>      |
| <i>Bacillus toluolicum</i>           | <i>Pseudomonas cruciviae</i>       |
| <i>Bacillus naphthalinicus</i>       | <i>Pseudomonas desmolyticum</i>    |
| <i>Bacillus phenanthrenicus</i>      | <i>Pseudomonas rathonis</i>        |
| <i>Bacterium benzoi</i>              | <i>Pseudomonas dacinhae</i>        |
| <i>Bacterium bidium</i>              | <i>Pseudomonas arvilla</i>         |
| <i>Bacterium erythrogenes</i>        | <i>Pseudomonas salopium</i>        |
| <i>Bacterium globiforme</i>          | <i>Pseudomonas tralucida</i>       |
| <i>Bacterium idoneum</i>             | <i>Pseudomonas lindneri</i>        |
| <i>Bacterium lipolyticus</i>         | <i>Pseudomonas gelatica</i>        |
| <i>Bacterium naphthalinicus</i>      | <i>Pseudomonas trifolii</i>        |
| <i>Bacterium phenanthrenicus</i>     | <i>Pseudomonas xanthae</i>         |
| <i>Bacterium stutzeri</i>            | <i>Pseudomonas iridescens</i>      |
| <i>Bacterium rubefaciens</i>         | <i>Pseudomonas cerevisiae</i>      |
| <i>Bacterium latericum</i>           | <i>Pseudomonas pictorum</i>        |
| <i>Bacterium parvulum</i>            | <i>Pseudomonas segnis</i>          |
| <i>Bacterium aliphaticum lique-</i>  | <i>Pseudomonas lemonnierii</i>     |
| <i>faciens</i>                       | <i>Serratia marcescens</i>         |
| <i>Bacterium fluorescens lique-</i>  | <i>Serratia kiliensis</i>          |
| <i>faciens</i>                       | <i>Spirillum itersonii</i>         |
| <i>Bacterium nidium</i>              | <i>Sporovibrio desulfuricans</i>   |
| <i>Cellulomonas blazotea</i>         | <i>Thiobacillus denitrificans</i>  |
| <i>Cellulomonas desidiosa</i>        | <i>Vibrio sp. 11171</i>            |
| <i>Cellulomonas flavigena</i>        | <i>Vibrio neocistes</i>            |
| <i>Chromobacterium amethysti-</i>    | <i>Vibrio cuneatus</i>             |
| <i>nium</i>                          | <i>Vibrio agarliquefaciens</i>     |
| <i>Clostridium visciaciens</i>       | <i>Vibrio cyclosites</i>           |
| <i>Clostridium hastiforme</i>        | <i>Vibrio tyroginus</i>            |
| <i>Clostridium omelianskii</i>       |                                    |
| <i>Corynebacterium pseudodi-</i>     |                                    |
| <i>phtheriticum</i>                  |                                    |

In addition to the list of Table II, all of the bacteria

of Table I with strong hydrocarbon dehydrogenation systems also may be mutated. This type of combination is capable of releasing large quantities of methane and/or carbon dioxide.

The combination of Table I bacteria with Table II mutants often leads to large quantities of carbon dioxide, nitrogen, hydroxylamine, etc., being released.

The mutants may be formed by subjecting the particular natural bacteria to any one of several well-known procedures, including exposure to ultra-violet rays, X-rays, radioactive materials, and to chemicals such as the mustard gases. In carrying out this mutating procedure, the natural bacteria are treated long enough to partially or totally inactivate their dehydrogenation systems, but not long enough to kill them. Thus, the mutant usually cannot initiate hydrocarbon oxidation, but may oxidize partially oxidized hydrocarbons. This means the mutant is dependent upon the "leavings" of the dehydrogenating natural bacteria, i.e., the partially oxidized hydrocarbons. Therefore, the two bacteria, that is, the natural and the mutant, work together, helping one another to release oil.

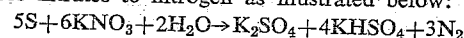
One preferred process of inactivating the dehydrogenation system in the natural bacteria and for forming the mutants of this invention involves the use of ultra-violet radiation. A culture plate containing the natural bacteria is positioned about two inches below an 8-watt, long-wave, ultra-violet lamp and exposed for a time sufficient to partially or totally inactivate the dehydrogenation system and yet not kill the bacteria, such as about five to about twenty-five seconds. About fifteen seconds appears to be an optimum time, under these conditions.

As is readily apparent from the foregoing, both sulfate-reducing and nitrate-reducing bacteria may be used in this invention, since both types of bacteria oxidize hydrocarbons by the removal of hydrogen. Any and all combinations of these bacteria, both single and multiple, natural and mutant, may be made as long as at least one mutant strain is included, and the object of this invention will be achieved.

These combinations of types of natural and mutant bacteria can be tailored for optimum attack on hydrocarbons. For example, if the oil is light, paraffinic, and occluded to clay, and gas is no longer available, a natural sulfate-reducer plus a sulfate-reducer mutant would readily attack the oil and release methane and/or carbon dioxide. If sulfate ion is not present in the cognate waters, nitrate and/or nitrite reducing bacteria can be utilized. If aromatics are present, strong oxidizing bacteria can be utilized to break ring structures. If high heat is expected, mutant thermophilic bacteria can be utilized to allow natural dehydrogenation bacteria to exist at higher temperatures, or to attack the oil themselves. Saline content, temperature, surface conditions, time required for conversion of bacteria, relative availability of hydrocarbon, porosity of formations, types of gases desired, etc., all have to be considered in order to create the best combination of natural bacteria and bacterial mutants.

The initial phase of hydrocarbon oxidation in anaerobic conditions involves dehydrogenation and the formation of hydrogen sulfide. A build-up of hydrogen sulfide is undesirable, for when the concentration reaches a certain point it acts as an antimetabolite by decreasing the oxidizing activity of the hydrogen-carrying enzymes of the natural bacteria. Therefore, in order to enable dehydrogenation to proceed at a maximum rate, oxygen rather than sulfur should be present to combine with the hydrogen, since the hydrogen-carrying enzymes preferentially combine with oxygen. Mutants are forced to live close to natural bacteria in order to receive dehydrogenated organic compounds and, in so doing, intimately supply the required hydrogen-carrying enzymes, thus diminishing or eliminating hydrogen sulfide production. This also can be done by introducing into the

oil-containing medium so-called "sulfide-oxidizing" bacteria which oxidize the hydrogen sulfide, such as natural *Thiobacillus denitrificans*. However, unless they have been mutated, it is not necessary for these bacteria to spread out in the oil formation and oxidize sulfides throughout. *Thiobacillus denitrificans* oxidizes thiosulfates, dithionates, sulfur, and sulfides to sulfates and reduces nitrates to nitrogen as illustrated below:



Where sulfide-oxidizing bacteria which produce sulfate ions are used, the sulfates so formed may be used as food for the sulfate-reducing bacteria. Also, the ammonia formed by some nitrate-reducing bacteria may be utilized by bacteria in their protein metabolism.

When nitrate-reducing bacteria are used in anaerobic conditions, nitrate or nitrite ions must be present for their survival. Nitrates or nitrites or their ions are not common in petroleum deposits, and therefore they must be added. This can be done by a waterflood program. They can be formed by growing nitrogen fixers, such as the nitrifying bacteria *Nitrosomonas*, *Nitrosococcus*, and *Nitrobacter* genera, in surface flood-water ponds. The nitrate-fixing efficiency of these bacteria is not as great as that of certain algae, e.g., *Anabena*, *Calothrix*, *Anabaenopsis*, *Plectonema*, *Tolypothrix*, *Nostoc*, *Schizothrix*, which also may be used for manufacturing nitrate and nitrite ions. This water containing nitrogen fixers is an oxidizing medium and should not be mixed with the floodwater containing the reducing bacteria, which is a reducing medium prior to entering the ground, or the reaction desired underground could occur above ground. By varying the flow of water from the nitrogen-fixer pond, the oxidation-reduction potential of the oil formation may be controlled. So, too, may the viability of the bacteria be controlled. This also gives control over the existence of nitrate reducers in the petroleum formations so that changes can be made if desired in types of nitrate reducers being utilized.

In culturing bacteria for use in this invention, the following media have given satisfactory results. However, other media may be and have been used, and therefore it is not my intention to limit the invention to these two.

Table III.—A Preferred Culture Medium for Sulfate-Reducing Bacteria

| Component:                                                        | Parts by weight |
|-------------------------------------------------------------------|-----------------|
| Sea water                                                         | 750.0           |
| Tap water                                                         | 250.0           |
| K <sub>2</sub> HPO <sub>4</sub>                                   | 0.2             |
| NH <sub>4</sub> Cl                                                | 0.1             |
| MgSO <sub>4</sub>                                                 | 0.2             |
| Na <sub>2</sub> SO <sub>3</sub>                                   | 0.5             |
| Na <sub>2</sub> SO <sub>4</sub>                                   | 1.0             |
| CaCO <sub>3</sub>                                                 | 0.2             |
| (NH <sub>4</sub> ) <sub>2</sub> Fe(SO <sub>4</sub> ) <sub>2</sub> | 0.5             |
| Agar                                                              | 4.0 to 7.0      |

In field use, the sulfate-reducing bacteria may be cultivated in produced water ponds and sumps without need of additional nutrients.

Table IV.—A Preferred Culture Medium for Nitrate-Reducing Bacteria

| Component:                                                        | Parts by weight |
|-------------------------------------------------------------------|-----------------|
| Sea water                                                         | 500.0           |
| Tap water                                                         | 500.0           |
| K <sub>2</sub> HPO <sub>4</sub>                                   | 0.2             |
| NH <sub>4</sub> Cl                                                | 0.1             |
| MgSO <sub>4</sub>                                                 | 0.2             |
| Na <sub>2</sub> SO <sub>3</sub>                                   | 0.5             |
| Na <sub>2</sub> SO <sub>4</sub>                                   | 1.0             |
| CaCO <sub>3</sub>                                                 | 0.2             |
| (NH <sub>4</sub> ) <sub>2</sub> Fe(SO <sub>4</sub> ) <sub>2</sub> | 0.5             |
| NaNO <sub>3</sub>                                                 | 1.0             |
| NaNO <sub>2</sub>                                                 | 0.5             |
| Agar                                                              | 4.0 to 7.0      |

The invention is further illustrated by the following examples, which describe various tests performed in accordance with this invention. These examples are set forth only for purposes of description and no intention is present to limit the invention thereto.

## EXAMPLE 1

A culture of natural *Desulfovibrio aestuarii*, a sulfate-reducing bacterium, was mutated by exposure to an 8-watt ultraviolet lamp held at a distance of 2 inches of fifteen seconds. The mutant bacteria so formed had inactive hydrocarbon dehydrogenation systems.

Absorption oil was placed over an aqueous sulfate culture medium containing methylene blue as an oxidation-reduction indicator. The sample was inoculated with this mutant and natural *Desulfovibrio aestuarii*. The cultures were incubated at 100° F. After 24 hours the methylene blue was colorless and the absorption oil darker. At the end of 48 hours the methylene blue was light blue, showing that it was being oxidized, and thereby also indicating that the absorption oil was being reduced.

As a control comparison, the same absorption oil-methylene blue composition was inoculated only with natural *Desulfovibrio aestuarii* and than incubated at 100° F. In 24 hours the methylene blue had turned colorless; i.e., had been reduced. There was no change afterward, and hydrogen sulfide was formed. However, when aqueous extracts of horseradish (rich in peroxidase enzyme) were added, the blue color of the methylene blue returned. Incubation for another 24 hours again bleached the methylene blue, showing that the natural bacteria have a weak oxidizing enzyme system. When air was bubbled through the media containing the peroxidase enzyme, methylene blue was again reduced temporarily. Infra-red studies showed that the absorption oil which had been attacked by mutants and natural bacteria differed from that which had been attacked only by natural bacteria. The addition of the mutant seemed to speed up the attack on hydrocarbons.

## EXAMPLE 2

Example 1 was repeated, using octadecane (reagent grade). A change in the blue color of the mutant-inoculated media was observed at 24 hours. Infra-red studies showed that oxidation of the octadecane had occurred. Slight changes in the blue color of the medium inoculated only with natural bacteria were observed after 30 days, but the changes were not as significant as shown in 24 hours with the mutant.

## EXAMPLE 3

Example 1 was repeated, using *Pseudomonas fluorescens*, a nitrate-reducing bacterium. With methylene-blue, it was established that natural *Pseudomonas fluorescens* is strongly reducing, but no change in pure hydrocarbons (i.e., oxidation) was observed in infra-red analyses. When its mutant was combined with natural *Desulfovibrio aestuarii*, carbon-dioxide was released and hydrocarbons were oxidized after a period of three days, as shown by subsequent infra-red analysis.

## EXAMPLE 4

A heavy crude was inoculated with a culture of natural *Desulfovibrio aestuarii* and a culture of mutant *Pseudomonas fluorescens*, and incubated at 100° F. for two months. During this time, long fingers formed from the top layer of the oil and after one month approximately one-half the oil dropped to the bottom of the bottle. Gas was then liberated and the oil on the bottom formed long fingers pointing upward. At the end of two months, all oil again was floating on the surface and appeared to be less viscous.

## EXAMPLE 5

A heavy crude from Santa Maria was run as in Example 4, along with a control. Again similar results were observed. The heavy, tacky composition of the oil precluded normal methods of determining viscosity. A slanted hot sheet of aluminum was used to determine times for the oil to run between two lines. The oil treated by bacteria took .41, .42, .79, and .94 minute. The control took .50, .74, and 2.17 minutes, showing that bacteria had lowered the viscosity of the oil.

## EXAMPLE 6

Bottles containing 100 g. of sump mud, rich in sulfides and sulfates, and 100 cc. of nutrient medium were inoculated as follows: (1) control (no bacteria other than already present); (2) natural *Desulfovibrio aestuarii*; (3) mutant *Desulfovibrio aestuarii* (impure culture); (4) natural *Desulfovibrio aestuarii* plus its mutant; (5) natural *Desulfovibrio aestuarii* plus *Pseudomonas fluorescens* mutant; (6) natural *Desulfovibrio aestuarii* plus its mutant plus the *Pseudomonas fluorescens* mutant.

After one month the following was observed: in bottles 1 and 2, hydrogen sulfide was present and little gas or oil was on the surface; Nos. 3 and 4 had produced methane along with carbon dioxide and some oil on the surface; Nos. 5 and 6, the most efficient combinations, released large quantities of carbon dioxide and the oil on the surface disappeared, and reappeared later on. After two months, the bottom of the mud began clearing in Nos. 5 and 6, indicating the removal of oil and sulfides therefrom.

## EXAMPLE 7

50 g. of a core sample from which oil had long ago migrated was ground up and to this was added 50 cc. of a culture medium. The control and a sample inoculated with regular *Desulfovibrio aestuarii* showed no change. The sample inoculated with *Desulfovibrio aestuarii* mutant (impure culture) alone showed the slow evolution of carbon dioxide. The sample inoculated with regular and mutant *Desulfovibrio aestuarii* showed a trace of oil on the surface and evolved considerably more carbon dioxide than the impure mutant. (Since the mutant requires the presence of natural bacteria to exist, only impure cultures are used in these experiments. This accounts for mutative experiments acting similarly to mixtures.)

## EXAMPLE 8

A test tube of mutated *Desulfovibrio aestuarii* was added to a large sump containing hydrogen sulfide. Five months later the sump began to produce oil and gas (methane and carbon dioxide). In the preceding six weeks an excess of 10,000 barrels of oil could not be accounted for except by bacterial action. Oil present is cool at night, and methane and carbon dioxide being released formed a blanket of foam 8-10 inches deep. During warm weather, at night, this foam layer built up to two feet in depth. However, as soon as the hot sun hit the oil, the oil became less viscous and released the gas. The oil appeared thin and had a gravity of 17.

For the next three weeks all oil into and out of the sump was estimated carefully, and over 3,000 barrels of excess oil were produced. Core samples taken at depths of 12 to 20 feet in the mud showed no hydrogen sulfide present. Prior testing of core samples from the same spot gave 50-90 p.p.m. hydrogen sulfide. In the area of inoculation, only traces of oil in the form of droplets the size of pinheads or smaller remained, and 150 to 200 feet away the oil occurred in small droplets about the size of matchheads. Samples acquired at a later date showed the black mud to be turning light brown in color; i.e., the color of the sand and clay, without organic material or iron sulfide. Large bubbles of methane gas came to

the surface as the sampler was lowered into the mud. Often the bubbles were four or so feet in diameter, which became smaller as the oil was depleted. Mud samples released large quantities of methane and carbon dioxide.

EXAMPLE 9

To each of six test bottles were added 50 g. of heavy tar having an A.P.I. gravity of 3 and a density greater than 1, and 100 cc. of the culture medium of Table III. In order to determine whether or not the thermophilic bacteria to be tested were anaerobic, 0.5 g. sodium nitrate, 0.5 g. sodium nitrite, 0.5 g. sodium sulfate and 0.5 g. sodium sulfite were added to each bottle. The bottles were then inoculated as follows:

| Bottle No.     | Inoculated with—                                                                                                                    |
|----------------|-------------------------------------------------------------------------------------------------------------------------------------|
| 1 (control)--- |                                                                                                                                     |
| 2-----         | Natural <i>Bacillus kaustophilus</i> .                                                                                              |
| 3-----         | Natural <i>Desulfovibrio desulfuricans</i> .                                                                                        |
| 4-----         | Natural <i>Desulfovibrio desulfuricans</i> and mutant <i>Bacillus kaustophilus</i> .                                                |
| 5-----         | Natural <i>Desulfovibrio desulfuricans</i> plus mutant <i>Desulfovibrio desulfuricans</i> .                                         |
| 6-----         | Natural <i>Desulfovibrio desulfuricans</i> and mutant <i>Desulfovibrio desulfuricans</i> plus mutant <i>Bacillus kaustophilus</i> . |

The test bottles were then incubated at 150° F., and the following facts were observed:

- (1) In 16 hours, the tar in test bottles 2, 4, and 6 (i.e., those containing either natural or mutant *Bacillus kaustophilus*) had expanded and was floating on top of the culture medium; gas was being released in large quantities.
- (2) At the end of 24 hours, the gas coming from the bottles was collected in test tubes under 110 mm. water pressure. The tubes connected to bottles 2, 4, and 6 were 75 percent filled in 4 hours, as compared with relatively little gas in the tubes connected to bottles 3 and 5, and substantially none in the control tube.
- (3) The gas samples from bottles 2-6 were subjected to infra-red analysis, with the following results:

| Bottle No. | Gas analyzed as—                            |
|------------|---------------------------------------------|
| 2-----     | Carbon dioxide, methane and hydroxyl amine. |
| 3-----     | Carbon dioxide.                             |
| 4-----     | Carbon dioxide, methane and hydroxyl amine. |
| 5-----     | Carbon dioxide.                             |
| 6-----     | Carbon dioxide, methane and hydroxyl amine. |

The structure of the tar in each of the test bottles was not appreciably changed, but the viscosity and the density were substantially modified.

EXAMPLE 10

For determining relative rates of gas production, to each of five test bottles were added 50 g. of the same tar used in Example 9, and 100 cc. of the culture medium of Table IV. The bottles were then inoculated as follows:

| Bottle No.     | Inoculated with—                                                                                                                                                            |
|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 (control)--- |                                                                                                                                                                             |
| 2-----         | Natural <i>Desulfovibrio desulfuricans</i> and mutant <i>Desulfovibrio desulfuricans</i> .                                                                                  |
| 3-----         | Natural <i>Desulfovibrio desulfuricans</i> and mutant <i>Desulfovibrio desulfuricans</i> and mutant <i>Pseudomonas fluorescens</i> .                                        |
| 4-----         | Natural <i>Desulfovibrio desulfuricans</i> , mutant <i>Desulfovibrio desulfuricans</i> , mutant <i>Pseudomonas fluorescens</i> , and natural <i>Bacillus kaustophilus</i> . |
| 5-----         | Natural <i>Desulfovibrio desulfuricans</i> and mutant <i>Pseudomonas fluorescens</i> .                                                                                      |

These test bottles were then incubated at 100° F. for one week, and the following data were obtained:

- (1) The tar in bottle 2 was just beginning to rise to the top of the culture medium;
- (2) The tar in bottle 4 had risen about half-way up, and was still rising;

(3) Most of the tar in bottle 3 had risen to the top of the culture medium, and strings of tar extended from the bottom of the bottle to the top of the medium;

(4) In bottle 5, all the tar had risen completely to the top of the culture medium, establishing this as the most active inoculation group of the series.

The foregoing disclosure makes it apparent that I have invented a new method of extracting valuable hydrocarbons from their subterranean formations, a method is not succeeds where all others have failed. The method is not limited to the certain bacteria listed herein, for all bacteria having a strong hydrocarbon dehydrogenation system, a strong hydrocarbon oxidizing system, or both, and which are capable of carrying on metabolic processes under anaerobic conditions, are operable. Furthermore, other methods of creating the mutant strains of bacteria, and other culture media, also are within the scope of this invention, so long as they operate to produce the desired bacteria.

ELIMINATION OF HYDROGEN SULFIDE

For eliminating hydrogen sulfide from oil formations, waste-waters, muds, sands, and the like, both above and below the ground, my invention involves growing special bacteria in ponds, sumps, and other reservoirs of waste-waters, in muds, sands, and other media, which special bacteria digest the sulfides present and oxidize (i.e., remove hydrogen from) them to harmless sulfur or, in some cases, thiosulfate or sulfate. These special bacteria thereby reverse the harmful action of the sulfate-reducing bacteria, which convert sulfates or sulfate ions into sulfides and hydrogen sulfide. This establishes, in a manner safe to wildlife inhabitants thereof, waters and muds, etc., from which no hydrogen sulfide odors emanate.

In principle, any bacteria which will oxidize sulfides and which will convert them to harmless sulfur or other non-odorous forms of sulfur may be used. However, in view of the fact that some bacteria convert sulfides into sulfates, thereby supplying more sulfate for the use of the harmful sulfate-reducing bacteria, these sulfate-producing bacteria are less desirable than other bacteria which do not produce sulfates. Furthermore, the sulfates so produced may form corrosive sulfuric acid, depending on whether or not oxygen is present, which is another undesirable material in the waste-waters. Therefore, the preferred bacteria are those which oxidize sulfide to sulfur and store the sulfur internally. Moreover, no acid is generated by the sulfur-producing bacteria. All bacteria which oxidize sulfides to sulfur or further, however, are operable and within the scope of this invention.

Among the operable sulfide-oxidizing bacteria, preference is given to bacteria having one or more of the following qualities: (1) bacteria that only partially oxidize the sulfide, so as to leave it in a form the sulfate-reducing bacteria cannot utilize; (2) bacteria that do not excrete the sulfur compound; (3) bacterial that are single and do not form mats or slimes that could possibly plug formations and piping; (4) bacteria that are non-toxic to plant and animal life; (5) bacteria that are motile so as to spread through the waste-water; (6) bacteria that are able to adapt themselves to high and low salinities; (7) bacteria that have one weakness that would enable them to be eliminated easily, if desired; and (8) bacteria that are anaerobic, so as to live underground and/or under the conditions prevalent in surface and subterranean mud and waste-waters, with no special chemicals necessary for their diet.

After extensive investigation into the properties and life-cycles of a great number of possible bacteria for purposes of this invention, the following Table V contains a list of some of the satisfactory natural sulfide-oxidizing bacteria. This list is not to be construed as limiting upon the invention, for there may be many other

species of natural sulfide-oxidizing bacteria just as suitable as the following:

Table V.—Some Natural Sulfide-Oxidizing Bacteria

|                                |                                   |
|--------------------------------|-----------------------------------|
| <i>Achromatium osaliferum</i>  | <i>Chromatium minutissimum</i>    |
| <i>Achromatium volutans</i>    | <i>Clathrochloris sulphurica</i>  |
| <i>Amoebobacter bacillosus</i> | <i>Macromonas mobilis</i>         |
| <i>Chromatium strain A</i>     | <i>Macromonas bipunctata</i>      |
| <i>Chromatium 919</i>          | <i>Rhabdomonas rosea</i>          |
| <i>Chromatium gobii</i>        | <i>Rhabdomonas gracilis</i>       |
| <i>Chromatium warmingii</i>    | <i>Rhabdomonas linsbaueri</i>     |
| <i>Chromatium linsbaueri</i>   | <i>Rhodopseudomonas palustris</i> |
| <i>Chromatium okenii</i>       | <i>Thiobacillus denitrificans</i> |
| <i>Chromatium weissel</i>      | <i>Thiosarcina rosea</i>          |
| <i>Chromatium cuculliferum</i> | <i>Thiospirillum jenense</i>      |
| <i>Chromatium minus</i>        | <i>Thiospirillum sanguineum</i>   |
| <i>Chromatium vinosum</i>      | <i>Thiospirillum violaceum</i>    |
| <i>Chromatium violaceum</i>    | <i>Thiospirillum rosenbergii</i>  |
| <i>Chromatium molischii</i>    | <i>Thiospirillum rufum</i>        |
| <i>Chromatium gracile</i>      | <i>Thiovulum majus</i>            |

In addition to the natural sulfide-oxidizing bacteria, mutants of certain natural bacteria also are operable to alleviate the sulfide problem. Among the natural bacteria suitable for mutation are all those capable of oxidizing hydrocarbons, such as the sulfate-reducing and nitrate-reducing bacteria.

Mutation of these bacteria can be accomplished by exposing them to the effects of X-rays, ultra-violet radiation, mustard gas, and other treatments, in the same way the mutant bacteria used for producing oil are formed, so that the mutants' dehydrogenation systems are partially or totally inactivated.

Although it has not been completely verified, and therefore there is no intention to be bound thereby, the theory underlying the action of the mutant bacteria, in eliminating hydrogen sulfide from waste-waters, muds, sewage, etc., is as follows. In the process of bacterial oxidation of a hydrogen-containing compound, dehydrogenation is the first step, followed by the further oxidation steps, which vary according to the material being oxidized. When the bacterial dehydrogenation system is inactivated, in order for the mutant to carry on its metabolism it must obtain a supply of dehydrogenated material which it can further oxidize. Since the oxidizing enzymes of this mutant are partially excreted, the area surrounding the bacteria is rich in these enzymes. When the mutant is introduced into a medium containing natural sulfate-reducing bacteria (i.e., where hydrogen sulfide is being produced), the mutant migrates to the natural sulfate-reducer which has an active, strong dehydrogenation system and which can partially supply oxidized "food" to the mutant. Since these mutants are excreting oxidizing enzymes but are not dehydrogenating, there is an abnormal amount of oxidizing enzymes around the natural sulfate-reducing bacteria and the hydrogen sulfide produced by these natural bacteria is immediately oxidized to sulfur, thiosulfate, or sulfate.

Hydrogen sulfide partially poisons the oxidizing systems of the natural bacteria by precipitating the iron required by the oxidizing enzymes catalase, cytochrome C, peroxidase, etc. However, since the use of these mutants according to this invention results in prevention of formation of hydrogen sulfide, or in oxidation and thus elimination of the hydrogen sulfide as soon as it is formed, such poisoning does not occur and the oxidation potential of the area is increased many fold.

In support of this theory, tests were run on natural sulfate reducing bacteria and mutants, using a culture containing methylene blue as an oxidation-reduction indicator. In every instance, the natural bacteria (with active dehydrogenation systems) reduced the methylene blue, and the mutants did not. Since reduction of methylene blue requires concurrent dehydrogenation, this showed that the mutants had inactive dehydrogenation systems. Whether or not this theory is accurate, the fact is that the addition of these mutant bacteria to muds and waste-waters containing dehydrogenating bacteria results in control and ultimate elimination of the hydrogen sulfide originally present therein.

Where the dehydrogenating bacteria are present na-

turally, as *Desulfovibrio desulfuricans* in oil-field waste-waters and muds, only the mutants need be added. But in rare instances, where there are no dehydrogenating bacteria naturally present, as in oil refinery operations where hydrogen sulfide may be a contaminant in cooling towers, they must be added to provide "food" for the mutants.

The following Table VI contains a list of some of the many operable species of bacteria which can be mutated, as above, and then used individually or in combination with each other to eliminate hydrogen sulfide according to this invention. This list is representative only and, since there are other operable species of bacteria not included therein, no intention is present to limit the invention thereto.

Table VI.—Some Natural Bacteria Useful To Form Mutants

|    |                                           |                                                |
|----|-------------------------------------------|------------------------------------------------|
| 20 | <i>Achromobacter aerophilum</i>           | <i>Corynebacterium pseudodiphtheriticum</i>    |
|    | <i>Achromobacter citrophilum</i>          | <i>Corynebacterium simplex</i>                 |
|    | <i>Achromobacter pastinator</i>           | <i>Desulfovibrio desulfuricans</i>             |
|    | <i>Achromobacter sulfureum</i>            | <i>Desulfovibrio aestuarii</i>                 |
|    | <i>Achromobacter thalassius</i>           | <i>Desulfovibrio rubenschkii</i>               |
|    | <i>Achromobacter iophagus</i>             | <i>Desulfovibrio halohydrocarbonoclasticus</i> |
|    | <i>Achromobacter delicatulus</i>          | <i>Flavobacterium okeanoikoites</i>            |
| 25 | <i>Achromobacter aquamarinus</i>          | <i>Flavobacterium marinotypicum</i>            |
|    | <i>Achromobacter cycloclastes</i>         | <i>Flavobacterium marinovirusum</i>            |
|    | <i>Achromobacter stationis</i>            | <i>Flavobacterium suaveolens</i>               |
|    | <i>Achromobacter delmarvae</i>            | <i>Flavobacterium esteroaromaticum</i>         |
|    | <i>Achromobacter agile</i>                | <i>Flavobacterium fuscum</i>                   |
|    | <i>Achromobacter centropunctatum</i>      | <i>Flavobacterium maris</i>                    |
| 30 | <i>Agarobacterium bufo</i>                | <i>Flavobacterium diffusum</i>                 |
|    | <i>Agarobacterium reducans</i>            | <i>Flavobacterium rigense</i>                  |
|    | <i>Agarobacterium viscosum</i>            | <i>Flavobacterium rhenanus</i>                 |
|    | <i>Alcaligenes metalcaligenes</i>         | <i>Flavobacterium lutescens</i>                |
|    | <i>Alcaligenes recti</i>                  | <i>Flavobacterium proteus</i>                  |
|    | <i>Bacillus thermoamylolyticus</i>        | <i>Leuconostoc citrovorum</i>                  |
|    | <i>Bacillus laterosporus</i>              | <i>Methanobacterium soehngenii</i>             |
|    | <i>Bacillus brevis</i>                    | <i>Methanobacterium ometian-skii</i>           |
| 35 | <i>Bacillus thermoliquefaciens</i>        | <i>Methanomonas methanica</i>                  |
|    | <i>Bacillus tostus</i>                    | <i>Methanomonas carbonatophila</i>             |
|    | <i>Bacillus hexacarbovorum</i>            | <i>Micrococcus paraffinae</i>                  |
|    | <i>Bacillus lactorubefaciens</i>          | <i>Micrococcus denitrificans</i>               |
|    | <i>Bacillus mycoides corallinus</i>       | <i>Mycobacterium alium</i>                     |
|    | <i>Bacillus druntzil</i>                  | <i>Mycobacterium rubrum</i>                    |
|    | <i>Bacillus toluolicum</i>                | <i>Mycobacterium lacticola</i>                 |
| 40 | <i>Bacillus naphthalenicus</i>            | <i>Propionibacterium freudenreichii</i>        |
|    | <i>Bacillus phenanthrenicus</i>           | <i>Propionibacterium shermanii</i>             |
|    | <i>Bacillus subtilis</i>                  | <i>Propionibacterium rubrum</i>                |
|    | <i>Bacillus firmus</i>                    | <i>Propionibacterium thoenii</i>               |
|    | <i>Bacillus polymyxa</i>                  | <i>Proteus vulgaris</i>                        |
|    | <i>Bacillus maderans</i>                  | <i>Pseudomonas fluorescens</i>                 |
|    | <i>Bacillus circulans</i>                 | <i>Pseudomonas putida</i>                      |
|    | <i>Bacillus ethanicus</i>                 | <i>Pseudomonas putrefaciens</i>                |
|    | <i>Bacillus kaustophilus</i>              | <i>Pseudomonas punctata</i>                    |
| 45 | <i>Bacillus thermodiastaticus</i>         | <i>Pseudomonas minuscula</i>                   |
|    | <i>Bacillus calidolactis</i>              | <i>Pseudomonas mira</i>                        |
|    | <i>Bacillus michaelisii</i>               | <i>Pseudomonas marinoglutinososa</i>           |
|    | <i>Bacillus thermocellulolyticus</i>      | <i>Pseudomonas calcis</i>                      |
|    | <i>Bacillus thermoalimento-philus</i>     | <i>Pseudomonas claciprecipitans</i>            |
|    | <i>Bacillus viridulus</i>                 | <i>Pseudomonas fermentans</i>                  |
|    | <i>Bacillus mesentericus</i>              | <i>Pseudomonas trifolii</i>                    |
| 50 | <i>Bacterium naphthalenicus</i>           | <i>Pseudomonas caudata</i>                     |
|    | <i>Bacterium phenanthrenicus</i>          | <i>Pseudomonas perlurida</i>                   |
|    | <i>Bacterium stutzeri</i>                 | <i>Pseudomonas ochracea</i>                    |
|    | <i>Bacterium fluorescens liquefaciens</i> | <i>Pseudomonas arvilla</i>                     |
|    | <i>Bacterium nidium</i>                   | <i>Pseudomonas tralucida</i>                   |
|    | <i>Bacterium globiforme</i>               | <i>Pseudomonas gelatica</i>                    |
| 55 | <i>Bacterium rubefaciens</i>              | <i>Pseudomonas segnis</i>                      |
|    | <i>Bacterium latericium</i>               | <i>Pseudomonas lemonnieri</i>                  |
|    | <i>Bacterium parvulum</i>                 | <i>Pseudomonas viscosa</i>                     |
|    | <i>Bacterium idoneum</i>                  | <i>Pseudomonas ureae</i>                       |
|    | <i>Bacterium benzoi</i>                   | <i>Pseudomonas effusa</i>                      |
|    | <i>Bacterium bidium</i>                   | <i>Pseudomonas myxogenes</i>                   |
|    | <i>Bacterium aliphaticum liquefaciens</i> | <i>Pseudomonas boreopolis</i>                  |
| 60 | <i>Bacterium lipolyticum</i>              | <i>Pseudomonas cleovorans</i>                  |
|    | <i>Cellulomonas diazotica</i>             | <i>Pseudomonas incognita</i>                   |
|    | <i>Cellulomonas desidiosa</i>             | <i>Pseudomonas aeruginosa</i>                  |
|    | <i>Cellulomonas flavigena</i>             | <i>Pseudomonas scissa</i>                      |
|    | <i>Cellulomonas surogenes</i>             | <i>Pseudomonas denitrificans</i>               |
|    | <i>Cellulomonas galba</i>                 | <i>Pseudomonas eisenbergii</i>                 |
|    | <i>Cellulomonas folia</i>                 | <i>Pseudomonas mephitica</i>                   |
| 65 | <i>Cellulomonas flava</i>                 | <i>Pseudomonas multistriata</i>                |
|    | <i>Cellulomonas inais</i>                 | <i>Pseudomonas hydrophila</i>                  |
|    | <i>Cellulomonas caesia</i>                | <i>Pseudomonas cruciata</i>                    |
|    | <i>Cellulomonas gilva</i>                 | <i>Pseudomonas desmolyticum</i>                |
|    | <i>Cellulomonas pusilla</i>               | <i>Pseudomonas rathonis</i>                    |
|    | <i>Cellulomonas rossica</i>               | <i>Pseudomonas dacunhae</i>                    |
|    | <i>Chromobacterium amethystinum</i>       | <i>Pseudomonas salopium</i>                    |
| 70 | <i>Clostridium fieseri</i>                | <i>Pseudomonas lindneri</i>                    |
|    | <i>Clostridium acetobutylicum</i>         | <i>Pseudomonas vanthe</i>                      |
|    | <i>Clostridium sartagoformum</i>          | <i>Pseudomonas tridescens</i>                  |
|    | <i>Clostridium roseum</i>                 | <i>Pseudomonas cerevisiae</i>                  |
|    | <i>Clostridium felsineum</i>              |                                                |
|    | <i>Clostridium viscofaciens</i>           |                                                |
|    | <i>Clostridium hastiforme</i>             |                                                |
| 75 | <i>Clostridium ometianskii</i>            |                                                |
|    | <i>Corynebacterium helvolum</i>           |                                                |

*Pseudomonas pictorum*  
*Serratia marcescens*  
*Serratia kiliensis*  
*Spirillum itersonii*  
*Sporovibrio desulfuricans*  
*Vibrio species 11171*

*Vibrio neocistes*  
*Vibrio agartiquefaciens*  
*Vibrio leonardii*  
*Vibrio tyrogenus*  
*Vibrio cuneatus*  
*Vibrio cyclostyles*

Some of these bacteria require special nutrients (e.g., nitrate ion) which can be supplied by the utilization of certain nitrogen fixers (e.g., algae bacteria, as illustrated in regard to oil production). Nitrates and nitrites usually are present in sewage. In oil-field operations, nitrogen fixers could be used, for instance, in the ponds for water-flooding. They require sunlight, and therefore could not survive underground; hence, only the nitrate and/or nitrite ion that they fix is pumped underground when subterranean formations are treated.

Although bacteria which oxidize sulfides to sulfates may be used in the process of this invention, the preferred bacteria are those which feed on sulfides to produce sulfur, and then store the sulfur within their bodies, thereby isolating the sulfur from other bacterial attack. Examples of such preferred bacteria are the *Thiospirillum*, *Rhabdomonas*, and *Chromatium* genera. When bacteria which oxidize sulfides to sulfates or thiosulfates are used, the sulfates produced act as more food for the sulfate-reducing bacteria to produce hydrogen sulfide. Also, when sulfates are produced, sulfuric acid may form, if oxygen is present, and corrosion problems arise. Therefore, if bacteria are used that retain the sulfur they produce, the sulfate-sulfide-sulfate cycle is broken, and the action of the sulfate-reducers is soon overcome.

In carrying out my invention, the following is one method which may be employed. A culture of sulfide-oxidizing bacteria is obtained and placed in a container, together with a suitable culture medium to nourish and propagate the bacteria into a stockpile. As an example, a culture of *Chromatium* strain A bacteria (obtained from the Hopkins Marine Station at Pacific Grove, California) was placed in an old storage tank at Ventura, California, together with 130 barrels of oil-field mud and water. After 40 days, the water had a slight pinkish cast, due to the extensive propagation of the purple sulfur bacteria.

Waste-water, mud, etc., containing sulfate-reducing bacteria, is then inoculated with a supply of the cultured sulfide-oxidizing bacterial. Where mutant bacteria are used and no dehydrogenating bacteria are present, the medium to be treated also must be inoculated with such dehydrogenating bacteria. In an ideal operation, field conditions are set up to assure maximum efficiency of the sulfide-oxidizing bacterial action. That is, provisions are made to: (1) avoid overwhelming the sulfide-oxidizing bacteria with oil, mud, sulfates, and other material containing sulfate reducing bacteria; (2) provide sufficient time between heavy additions or withdrawals of waste-water for the sulfide-oxidizing bacteria to propagate; and (3) avoid excessive withdrawal of the inoculated waste-water or, if this is necessary, periodically re-inoculate with more sulfide-oxidizing bacteria. Even under conditions other than ideal, such as normal oil-field procedures where daily 11,000 to 13,000 barrels of water are added to and removed from a 50,000-barrel sump, the method of this invention produces highly satisfactory results, as the following examples indicate. In all operations, analyses for sulfur content are made periodically to test the effectiveness of the sulfide-oxidizing bacteria, as well as to determine the need of re-inoculation.

The method just outlined relates in particular to treatment of stationary bodies of water and mud. The direct treatment of an open, flowing stream also is possible, as well as underground deposits of water, mud, crude oil, etc. Closed systems, such as pipelines, can be treated by sulfide-oxidizing bacteria which carry on their metab-

olism in the absence of light, such as natural *Thiobacillus denitrificans*, or mutants of suitable bacteria.

To further illustrate the invention, but not to limit it, the following examples describing actual tests and their results are set forth.

#### EXAMPLE 11

Effluent waste-water containing active sulfate-reducing bacteria was placed in two pint jars. One jar was inoculated with *Chromatium* strain A bacteria, and the other with *Chromatium* strain 919 bacteria. Initial hydrogen sulfide content was 15 parts per million, by weight, and 2.5 milligrams of sodium sulfide were added, bringing the total sulfide content to 17.2 parts per million (as hydrogen sulfide). Incubation in sunlight at 95° F. in the absence of air diminished the hydrogen sulfide content in both jars by over one-half in three days, and completely in eleven days. The solution in both jars became clear, with the bacteria in a sludge at the bottom. No slime developed in either jar, nor did the bacteria deposit on the vessel walls. The sludge was easily suspended in the water and was similar to that found in a blank run wherein no sulfide-oxidizing bacteria were added. The solution pH of each jar (a measure of the solution acidity) decreased to 7.5 after three weeks, as compared with 9.0 in a blank; pH 7.5 is approximately that of normal waste-water.

#### EXAMPLE 12

An old oil-storage tank was filled with 150 barrels of oil-field mud and water containing sulfate-reducing bacteria. The mixture was allowed to stand for two weeks, in order for any chemically active ion to react with the sulfides. With the hydrogen sulfide content at the high level of 50 parts per million, the tank was inoculated with *Chromatium* strain A bacteria. In seven days the hydrogen sulfide content had dropped from 50 to 12 parts per million. The weather then turned hot, which accelerates activity of the sulfate-reducing bacteria. However, on the eleventh day the hydrogen sulfide content was only 11 parts per million; the value dropped to 6 parts per million on the 21st day, and zero on the 27th day. At this time, the water had a slight pinkish cast, due to the very large build-up of purple *Chromatium* bacteria.

#### EXAMPLE 13

A stagnating pond, filled with rain-water and large quantities of mud from oil-field sumps, had developed as a bacterial hydrogen sulfide generator. It was inoculated with *Chromatium* strain A bacteria. After four days of warm weather, during which time the sulfide-oxidizing bacteria were incubating, the hydrogen sulfide in the pond water rose from 17 to 24 parts per million. After 14 days, water along the pond shoreline showed zero parts of hydrogen sulfide per million parts of water, and after 20 days ½-part per million, despite shrinkage of the shoreline. Five feet out from the shoreline, the hydrogen sulfide content was 20 parts per million after 14 days; 2 parts per million after 20 days. Further out from the shoreline, the values dropped from 28 to 3 parts per million during this period; and from 35 to zero parts per million, still further out. From these data it can be seen that, once the *Chromatium* strain A bacteria became established and started propagating, the sulfide generation was overcome.

#### EXAMPLE 14

A very large, open catch-basin oil-skimming unit, containing waste drilling muds, oil-field brine, and other oil-field waste-waters, was inoculated with *Chromatium* strain A bacteria.

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The following data were obtained:

| Days since inoculation | Parts of hydrogen sulfide per million parts of waste-water | Days since inoculation | Parts of hydrogen sulfide per million parts of waste-water |
|------------------------|------------------------------------------------------------|------------------------|------------------------------------------------------------|
| 0.....                 | 45                                                         | 35.....                | 20                                                         |
| 11.....                | 12                                                         | 42.....                | 6                                                          |
| 13.....                | 4                                                          | 56.....                | 5                                                          |
| 18.....                | 24                                                         | 63.....                | 1                                                          |
| 21.....                | 25                                                         | 70.....                | 0                                                          |
| 28.....                | 5                                                          |                        |                                                            |

Note a. Hot weather started on the 8th day, causing very high activity of sulfide-producing bacteria.  
 Note b. Hot spell ended on the 15th day and resumed on the 32nd day.  
 Note c. Hot spell broke on the 39th day.

From these data it can be seen that the sulfide-oxidizing bacteria gained control rapidly over the sulfate-reducing bacteria. Hot spells have the effect of temporarily increasing the activity of sulfate-reducing bacteria, but never were able to produce sulfide to the high original level of 45 parts per million.

After each cycle of hot and cold weather, the sulfide-oxidizing bacteria seemed to have established ever-firmer control.

## EXAMPLE 15

Another catch-basin oil-skimming unit of the type of Example 14 was inoculated with Chromatium strain A bacteria. The following data were obtained from this test:

| Days since inoculation | Parts of hydrogen sulfide per million parts of waste-water | Days since inoculation | Parts of hydrogen sulfide per million parts of waste-water |
|------------------------|------------------------------------------------------------|------------------------|------------------------------------------------------------|
| 0.....                 | 40                                                         | 77 <sup>f</sup> .....  | 43                                                         |
| 11 <sup>a</sup> .....  | 24                                                         | 84.....                | 35                                                         |
| 18.....                | 23                                                         | 88.....                | 24                                                         |
| 21.....                | 15                                                         | 89 <sup>e</sup> .....  | 44                                                         |
| 28 <sup>b</sup> .....  | 12                                                         | 90.....                | 36                                                         |
| 35.....                | 13                                                         | 93.....                | 23                                                         |
| 42.....                | 6                                                          | 96 <sup>b</sup> .....  | 24                                                         |
| 56 <sup>c</sup> .....  | 32                                                         | 98.....                | 20                                                         |
| 57.....                | 24                                                         | 106 <sup>f</sup> ..... | 32                                                         |
| 60 <sup>d</sup> .....  | 26                                                         | 112 <sup>j</sup> ..... | 18                                                         |
| 63.....                | 50                                                         | 118.....               | 15                                                         |
| 70 <sup>e</sup> .....  | 43                                                         | 124.....               | 11                                                         |

<sup>a</sup> Hot weather started on the 8th day.

<sup>b</sup> By the 28th day, despite intermittent periods of hot weather, the sulfide-oxidizing bacteria had established firm control.

<sup>c</sup> On the 56th day, excessive pumping of waste-water out of the catch basin sucked bottom-mud infested with sulfate-reducing bacteria into separation tanks. The mud then was dumped back into the catch basin, reinfesting the basin after the harmful bacteria had propagated in the tanks. Also, excessive alum-laden new silt was dumped into the basin.

<sup>d</sup> On the 60th day, large quantities of mud containing iron-sulfide were dumped into the catch basin. This material reacted analytically as hydrogen sulfide.

<sup>e</sup> On the 70th day, excessive and unusual mud dumping into the catch basin was made and the sump waterlevel allowed to rise to avoid pumping mud.

<sup>f</sup> On the 77th day the system was reinoculated with Chromatium bacteria, in case the older culture might be losing activity.

<sup>g</sup> On the 89th day, water containing up to 70 parts per million sulfide plus heavy sulfate-reducing bacteria counts was dumped into the catch basin.

<sup>h</sup> On the 96th day the air temperature rose to 106° F.  
<sup>i</sup> An extraordinary week of 107° F. weather immediately preceded the 106th day.

<sup>j</sup> Two weeks of 107° F. weather began on the 110th day.

Before inoculation with sulfide-oxidizing bacteria, and under conditions when the air temperature was between 70° F. and 80° F., tests of the water from this same basin showed 150 to 200 parts of hydrogen sulfide per million parts of water.

## EXAMPLE 16

To determine what effect this process would have on underground formations, the following test was carried out.

Mutant *Desulfovibrio aestuarii* bacteria were prepared by exposing the natural bacteria to an 8-watt ultraviolet light held at a distance of two inches for fifteen seconds. A basin containing oil-field waste-water, alum floc, and sulfate-reducing bacteria producing substantial hydrogen sulfide was inoculated with these mutants. Five months

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after inoculation, samples of mud were taken at depths of 12 to 15 feet, at points 300 to 400 feet from the place of inoculation. Analyses of all samples showed no sulfides present, in contrast to findings of 50 to 90 parts per million sulfide at these same locations before inoculation. This is a clear indication that these mutants oxidize the sulfides underground, and can be used to eliminate hydrogen sulfides in subterranean formations.

## EXAMPLE 17

Test tubes were filled with samples of mud from an oilfield catch basin containing waste-water, sulfates, and other materials conducive to the production of hydrogen sulfide. Each tube was inoculated with natural *Desulfovibrio aestuarii*. One tube was used as the control. The other tubes were further inoculated with (1) mutant *Desulfovibrio aestuarii*, (2) mutant *Pseudomonas fluorescens*, and (3) a combination of mutant *Desulfovibrio aestuarii* and mutant *Pseudomonas fluorescens*, respectively. The mutants were formed by the process of Example 16. The following data were gathered:

| Test tubes containing—                                                                    | Time since inoculation | Amount of H <sub>2</sub> S present |
|-------------------------------------------------------------------------------------------|------------------------|------------------------------------|
| (1) Mutant <i>Desulfovibrio aestuarii</i> .....                                           | 6 weeks.....           | None.                              |
|                                                                                           | 8 weeks.....           | Some. <sup>1</sup>                 |
| (2) Mutant <i>Pseudomonas fluorescens</i> .....                                           | 6 weeks.....           | None.                              |
|                                                                                           | 8 weeks.....           | Do.                                |
| (3) Mutant <i>Desulfovibrio aestuarii</i> and mutant <i>Pseudomonas fluorescens</i> ..... | 6 weeks.....           | Do.                                |
|                                                                                           | 8 weeks.....           | Do.                                |
| (4) Control.....                                                                          | 2 days.....            | Large.                             |

<sup>1</sup> Due to exhaustion of sulfate ions.

This test, like that of Example 16, shows the efficacy of using these bacteria to eliminate hydrogen sulfide in mud and other underground media.

## EXAMPLE 18

Samples of mud containing 70 p.p.m. hydrogen sulfide and natural *Desulfovibrio aestuarii* were taken from an oil-field sump and introduced into test containers. One container was used as a control, and the remaining container inoculated with (1) mutant *Desulfovibrio aestuarii*, (2) mutant *Pseudomonas fluorescens*, and (3) a combination of mutant *Desulfovibrio aestuarii* and mutant *Pseudomonas fluorescens*, respectively. The mutants were formed by the process of Example 16. All containers were incubated at 100° F. The following results were recorded:

| Test tubes containing—                                                                    | Time since inoculation | Amount of H <sub>2</sub> S present |
|-------------------------------------------------------------------------------------------|------------------------|------------------------------------|
| (1) Mutant <i>Desulfovibrio aestuarii</i> .....                                           | 11 weeks.....          | None.                              |
| (2) Mutant <i>Pseudomonas fluorescens</i> .....                                           | do.....                | Do.                                |
| (3) Mutant <i>Desulfovibrio aestuarii</i> and mutant <i>Pseudomonas fluorescens</i> ..... | do.....                | Do.                                |
| (4) Control.....                                                                          | do.....                | Abundant.                          |

The control released H<sub>2</sub>S within 3 days in large quantities. Inspection of the containers inoculated with cultures 2 and 3 showed ¼" to ⅜" of clear sand had formed in the bottom. This indicates that precipitated iron sulfide may be re-absorbed (oxidized) from mud by these mutants.

As is readily apparent from the foregoing examples, 11 through 18, once the sulfide-oxidizing bacteria gained a foothold in the inoculated medium it quite rapidly multiplied and, ultimately, overcame the activity of the sulfate-reducing bacteria, to virtually eliminate the hydrogen sulfide. Apparently an increase in atmospheric temperature aids in the production of hydrogen sulfide by the sulfate-reducing bacteria to the extent that, before the sulfide-oxidizing bacteria gain this foothold, hydrogen sulfide is produced at a faster rate than the sulfide-oxidizing bacteria can oxidize. When appreciable quantities of sulfate-(alum) rich mud, water, and other media containing sulfate-reducing bacteria are added to the waste-

waters containing the sulfide-oxidizing bacteria, a sudden increase in the hydrogen sulfide content occurs and prevails until the sulfide-oxidizing bacteria again are able to take control of the situation. However, such control is regained in a few days. When mutants are used, large quantities of alum-rich muds, etc., can be added with only a slight increase in sulfides.

From many experiments carried out in different areas, it has been established that about 30 parts per million of hydrogen sulfide in waste-waters is enough to cause atmospheric contamination only under extreme conditions and, even then the actual atmospheric concentration of hydrogen sulfide is below 1 part per million. In practice, sumps that ran in excess of 160 p.p.m. hydrogen sulfide have been reduced to 0 to 3 p.p.m. in hot weather. This more than solves the atmospheric hydrogen sulfide contamination problem.

Since hot weather greatly stimulates hydrogen sulfide production, the fact that the hydrogen sulfide concentration in the waste-waters of, for instance, Example 15, which rose only to 32 parts per million during the 107° F. weather, clearly illustrates the unexpected results obtained with my invention. Subsequent readings on the catch basin of Example 15 showed 14 to 8 p.p.m. hydrogen sulfide in 105° F. weather. Under much cooler conditions, values of 150 to 200 parts per million hydrogen sulfide have been recorded from tests carried out on the same body of waste-water before inoculation with sulfide-oxidizing bacteria.

Although Examples 11 through 18 refer mainly to waste-waters and muds present at the sites of oil-drilling operations, and have been prepared from tests carried out upon such waste-waters, it is readily apparent that other types of waste-waters, muds, sands, etc., containing hydrogen sulfide and sulfate-reducing bacteria are susceptible to treatment with sulfide-oxidizing bacteria to eliminate the hydrogen sulfide problem. For instance, since hydrogen sulfide is removed from densely compacted clay (as illustrated in Examples 16-18) which greatly restricts the movement of bacteria, the hydrogen sulfide problem in porous oil sands, which permit much greater bacterial migration, may easily be remedied. In fact, any medium which contains hydrogen sulfide and which is conducive to the propagation of sulfide-oxidizing bacteria may be so treated, to eliminate the hydrogen sulfide undesirable. No restrictions upon the particular types of media are intended and, in fact, none exist except for the obvious limitation as to the media being able to support sulfur bacterial life.

As previously stated, it is desirable but not necessary that the sulfide-oxidizing bacteria have one weakness by which their elimination from the waters can be secured, if so desired. For instance, since *Chromatium* bacteria need light for efficient survival, they can be controlled easily by covering over in some manner the body of waste-waters. Mutants can be eliminated by destroying the dehydrogenating bacteria. Naturally, there are other easy means, e.g., bacteriophages, amoebas, paramecia, etc., to eliminate the sulfide-oxidizing bacteria, which will become readily apparent to the skilled practitioner.

#### ELIMINATION OF IRON SULFIDE

Where the elimination of iron sulfide from oil formations, mud, sand, water, and the like, is desired, these media are inoculated with bacteria in the same manner as described above in relation to the elimination of hydrogen sulfide. The same bacteria are used for both hydrogen sulfide and iron sulfide elimination, i.e., those set forth in Tables V and VI. Iron sulfide which accumulates in machinery, such as oil-well pipes, pumps, etc., can be eliminated by bacterial removal of sulfide ions contained in the gases and liquids transported by such equipment. In this way, restricting the iron sulfide plugs and corrosion can be prevented.

To illustrate this facet of the invention, e.g., to re-

move precipitated iron sulfide from surface and underground deposits thereof, the following test was performed.

#### EXAMPLE 19

Cultures of natural *Desulfovibrio aestuarii* and *Desulfovibrio desulfuricans* were grown in separate Petri dishes of medium containing sulfates and iron. When the dishes were covered with black iron sulfide, one-half of each dish was covered and the uncovered half exposed to an 8-watt ultraviolet light for from 5 to 25 seconds. In 3 to 7 days, the black iron sulfide had disappeared from the exposed area of the *Desulfovibrio aestuarii* dish, showing that oxidation of the sulfide had occurred. The *Desulfovibrio desulfuricans* mutant "bleaching" extended throughout the dish, showing extensive mobility of the mutant. The controls (viz. Petri dishes with media containing sodium sulfide and iron salts to form iron sulfide, but containing no bacteria) were exposed to ultraviolet radiation in the same manner, but showed no change over the same period.

Further illustrations of the removal of iron sulfide precipitate are shown by the experiments embodied in Examples 6, 8, and 18, which describe, inter alia, how black iron-sulfide-containing muds and sand are restored to their natural color after bacterial treatment.

These tests show that iron sulfide can be removed, effectively and efficiently, from plugged oil formations and other media, by the bacterial treatment of this invention.

#### HYDROCARBON ALTERATION

For altering hydrocarbons and media containing hydrocarbons, including media containing intermediate hydrocarbon metabolism derivatives, the method of my invention involves inoculation with a combination of natural and mutant hydrocarbon-oxidizing bacteria. As disclosed supra, particularly under the heading "Production of Oil," inoculating any hydrocarbon-containing media that will support bacterial metabolism with the combination of natural and mutant bacteria will result in an unexpectedly greater rate of hydrocarbon oxidation, and an unexpectedly greater volume of product, than when natural bacteria alone are used. That these results are indeed unexpected is evident when the nature of the mutant bacteria, and of their metabolism, is observed. Since the dehydrogenation ability of these mutants is partially or totally destroyed, it would be expected that they would not be able to increase, at least measurably, the hydrocarbon oxidation rate of the natural bacteria. But just the reverse occurs, not only in underground oil deposits but in surface containers of hydrocarbons as well.

Illustrations of this method are contained in some of the foregoing examples. These examples vividly point out the difference in results obtained when hydrocarbons and hydrocarbon-containing media are inoculated only with natural bacteria, and when the same media are inoculated with the same natural bacteria in combination with mutant bacteria. For instance, as illustrated in Example 1, when a mixture of methylene blue and absorption oil, the latter containing a multitude of various hydrocarbons, was inoculated with natural bacteria in one container, and a combination of natural and mutant bacteria in another container, significantly greater, more rapid oxidation was observed, and materially different products were produced, in the container with the mutants. Examples 2 and 3 set forth the striking difference in results when a mixture of octadecane and methylene blue is inoculated with natural bacteria, and when the same mixture is inoculated with a combination of natural and mutant bacteria.

The results of inoculating samples of sump mud, rich in hydrocarbons, with natural bacteria alone, and a combination of natural and mutant bacteria, are indicated in Example 6. As set forth in Example 7, core samples from which oil had long migrated were inoculated in

part with natural bacteria alone, and in part with a combination of natural and mutant bacteria; the results clearly show that natural bacteria alone produced no change in the sample, whereas the combination of natural and mutant bacteria produced oil and carbon dioxide, positive evidence of chemical alteration of the hydrocarbons in the medium. Even heavy tars, which contain an abundance of hydrocarbons, were oxidized to an unexpectedly greater extent when inoculated with a combination of natural and mutant bacteria than when inoculated with natural bacteria alone, as set forth in Examples 9 and 10. Thus by my invention hydrocarbons, both above as well as below ground, can now be altered at a much faster rate, and the process carried on for a much longer period, producing unexpectedly greater results, than heretofore possible.

This application is a continuation-in-part of my co-pending application Serial Number 585,991, filed December 11, 1959, now abandoned.

To those skilled in the art, various changes and modifications of the invention may suggest themselves without departing from the spirit and scope of the invention. The disclosures and the examples and descriptions herein are purely illustrative and are not intended to be in any sense limiting.

I claim:

1. A method for the recovery of oil from surface and underground formations thereof, comprising: subjecting the formation to the metabolic activities of (1) mutant hydrocarbon-oxidizing bacteria having partially-to-totally inactivated dehydrogenation systems, and (2) natural hydrocarbon-oxidizing bacteria.

2. A process for reactivating "dry" oil wells and other deposits of crude petroleum, comprising: flooding the oil-bearing formation with water containing a combination of (a) natural hydrocarbon-oxidizing bacteria having strong dehydrogenation systems and (b) natural hydrocarbon-oxidizing bacteria mutated by partial-to-total inactivation of their dehydrogenation systems.

3. The process of claim 2, wherein nitrogen fixers and the products of their metabolism are introduced into the oil formation.

4. The process of claim 3 wherein sulfide-oxidizing bacteria also are introduced into the oil formation.

5. A method for the recovery of crude petroleum from formations thereof, comprising: inoculating the formation with (a) a culture of natural bacteria selected from the group consisting of obligative anaerobic and facultative anaerobic hydrocarbon-oxidizing bacteria having strong hydrocarbon dehydrogenation systems, and (b) a culture of mutated natural bacteria selected from the group consisting of obligative anaerobic and facultative anaerobic hydrocarbon-oxidizing bacteria having partially-to-totally inactivated hydrocarbon-dehydrogenation systems.

6. The method of claim 5, wherein the formation also is inoculated with the products of nitrogen fixer metabolism.

7. The method of claim 6 wherein the formation also is inoculated with a culture of bacteria selected from the group consisting of obligative anaerobic and facultative anaerobic sulfide-oxidizing bacteria.

8. A method for the recovery of crude petroleum from reservoirs thereof, comprising: inoculating the reservoir with (a) a culture of natural bacteria selected from the group consisting of obligative anaerobic and facultative anaerobic sulfate-reducing, hydrocarbon-oxidizing bacteria having strong hydrocarbon-dehydrogenation systems, and (b) a culture of mutated natural bacteria selected from the group consisting of obligative anaerobic and facultative anaerobic sulfate-reducing hydrocarbon-oxidizing bacteria having partially-to-totally inactivated hydrocarbon-dehydrogenation systems.

9. The method of claim 8 wherein the reservoir also is inoculated with the products of nitrogen fixer metabolism.

10. The method of claim 9, wherein the reservoir also is inoculated with a culture of bacteria selected from the group consisting of obligative anaerobic and facultative anaerobic sulfide-oxidizing bacteria.

11. A method for the recovery of crude petroleum from reservoirs thereof, comprising: inoculating the reservoir with (a) a culture of natural bacteria selected from the group consisting of obligative anaerobic and facultative anaerobic nitrate-reducing hydrocarbon-oxidizing bacteria having strong hydrocarbon-dehydrogenation systems, and (b) a culture of mutated natural bacteria selected from the group consisting of obligative anaerobic and facultative anaerobic nitrate-reducing hydrocarbon-oxidizing bacteria having partially-to-totally inactivated hydrocarbon-dehydrogenation systems.

12. The method of claim 11 wherein the reservoir is also inoculated with the products of nitrogen fixer metabolism.

13. The method of claim 12 wherein the reservoir also is inoculated with a culture of bacteria selected from the group consisting of obligative anaerobic and facultative anaerobic sulfide-oxidizing bacteria.

14. A method for the recovery of crude petroleum from reservoirs thereof, comprising: inoculating the reservoir with (a) a culture of natural bacteria selected from the group consisting of obligative anaerobic and facultative anaerobic sulfate-reducing hydrocarbon-oxidizing bacteria having strong hydrocarbon-dehydrogenation systems, and (b) a culture of mutated natural bacteria selected from the group consisting of obligative anaerobic and facultative anaerobic nitrate-reducing hydrocarbon-oxidizing bacteria having partially-to-totally inactivated hydrocarbon-dehydrogenation systems.

15. The method of claim 14 wherein the reservoir also is inoculated with the products of nitrogen fixer metabolism.

16. The method of claim 15 wherein the reservoir also is inoculated with a culture of bacteria selected from the group consisting of obligative anaerobic and facultative anaerobic sulfide-oxidizing bacteria.

17. A method for the recovery of crude petroleum from reservoirs thereof, comprising: inoculating the reservoir with (a) a culture of natural bacteria selected from the group consisting of obligative anaerobic and facultative anaerobic nitrate-reducing hydrocarbon-oxidizing bacteria having strong hydrocarbon-dehydrogenation systems, and (b) a culture of mutated natural bacteria selected from the group consisting of obligative anaerobic and facultative anaerobic sulfate-reducing hydrocarbon-oxidizing bacteria having partially-to-totally inactivated hydrocarbon-dehydrogenation systems.

18. The method of claim 17 wherein the reservoir also is inoculated with the products of nitrogen fixer metabolism.

19. The method of claim 18 wherein the reservoir also is inoculated with a culture of bacteria selected from the group consisting of obligative anaerobic and facultative anaerobic sulfide-oxidizing bacteria.

20. A process for obtaining crude petroleum from a depository thereof, comprising: introducing into the depository (1) a volume of water containing natural hydrocarbon-oxidizing bacteria having strong hydrocarbon-dehydrogenation systems and capable of carrying on metabolism under anaerobic conditions, to partially oxidize the hydrocarbons with which they come into contact, and (2) a volume of water containing mutant hydrocarbon-oxidizing bacteria having partially-to-totally inactive hydrocarbon-oxidizing dehydrogenation systems and capable of carrying on metabolism under anaerobic conditions, to carry on oxidation of the partially oxidized hydrocarbons resulting from the activity of the natural bacteria, so that the viscosity of the crude petroleum is reduced to the extent that it flows from its depository into a collecting area for removal.

21. A process for pressurizing an underground oil formation and reducing the viscosity of oil therein, comprising: subjecting the formation to the gaseous metabolic by-products of a combination of natural and mutant bacteria living in the said formation under anaerobic conditions.

22. A process for pressurizing an underground oil formation, comprising: inoculating the formation with a combination of natural and mutant bacteria capable of producing, under anaerobic conditions, a gas selected from the group consisting of methane, carbon dioxide, hydroxyl amine, nitrogen, ammonia, oxides of nitrogen, and mixtures thereof, by oxidation of the hydrocarbon material present in the zone.

23. The process of claim 22, wherein the gas produced is methane, and the bacteria have weak hydrocarbon-oxidizing systems.

24. The process of claim 22 wherein the gas produced is carbon dioxide, and the bacteria have strong oxidizing systems.

25. The process of supplying nitrates and nitrites to oil-containing formations, comprising: cultivating the growth and propagation of nitrogen fixers in a fluid culture medium, and then introducing this medium into the oil-containing formation.

26. The process of culturing mutant hydrocarbon-oxidizing bacteria in subsurface crude petroleum formations containing natural hydrocarbon-oxidizing bacteria, and then removing the petroleum from its formation.

27. A method for eliminating obnoxious odors from oil formations and surface and underground waste-waters and muds, comprising: inoculating with a combination of natural and mutant bacteria capable of oxidizing sulfides.

28. A method for reducing the hydrogen sulfide content of oil formations, and surface and underground waste-waters and muds, comprising: inoculating the medium with a combination of natural and mutant bacteria capable of oxidizing hydrogen sulfide in the absence of atmospheric oxygen.

29. The method of claim 28, wherein the mutant bacteria are mutant nitrate-reducing bacteria.

30. The method of claim 28, wherein the mutant bacteria are mutant sulfide-oxidizing bacteria.

31. The method of claim 28, wherein the mutant bacteria are mutant sulfate-reducing bacteria.

32. The method of claim 28, wherein the bacteria comprise a combination of (1) natural nitrate-reducing bacteria having strong dehydrogenation systems, and (2) bacteria selected from the group consisting of natural sulfide-oxidizing bacteria, mutant sulfate-reducing bacteria, and mutant sulfide-oxidizing bacteria, and combinations thereof.

33. The method of claim 28, wherein the medium is periodically reinoculated to maintain it free of hydrogen sulfide.

34. A method for reducing and controlling the hydrogen sulfide content of oil formations, and surface and subterranean waste-waters and muds, comprising: inoculating with a combination of (1) natural and mutant hydrocarbon oxidizing bacteria and (2) natural sulfide-oxidizing bacteria selected from the group consisting of the *Achromatium*, *Amoebobacter*, *Chromatium*, *Clathrochloris*, *Macromonas*, *Rhabdomonas*, *Rhodopseudomonas*, *Thiobacillus*, *Thiosarcina*, *Thiospirillum*, and *Thiovulum* genera.

35. The method of claim 34, wherein the (2) natural bacteria are of the *Chromatium* genus.

36. A method of eliminating obnoxious hydrogen-sulfide odors from oil formations, and surface and subterranean waste-waters and muds, comprising: inoculating with natural and mutant bacteria which oxidize hydrogen sulfide to sulfur and retain the sulfur so produced within their body structure.

37. A method of reducing and controlling the hydro-

gen sulfide content of oil formations, and surface and subterranean waste-waters and muds, comprising: inoculating with a culture of mutant sulfate-reducing bacteria selected from the group consisting of *Achromobacter*, *Agarobacterium*, *Alcaligenes*, *Bacillus*, *Bacterium*, *Cellulomonas*, *Chromobacterium*, *Clostridium*, *Corynebacterium*, *Desulfovibrio*, *Flavobacterium*, *Leuconostoc*, *Methanobacterium*, *Methanomonas*, *Micrococcus*, *Mycobacterium*, *Propionibacterium*, *Proteus*, *Pseudomonas*, *Serratia*, *Spirillum*, *Sporovibrio*, and *Vibrio* genera and combinations thereof, said bacteria having partially to totally de-activated dehydrogenation systems and active oxidizing systems.

38. The method of claim 37, wherein the bacteria are mutant *Desulfovibrio*.

39. The method of claim 37, wherein the bacteria are mutant *Pseudomonas*.

40. The method of claim 37, wherein a combination of mutant *Desulfovibrio* and mutant *Pseudomonas* bacteria are used as the inoculating culture.

41. A method for controlling hydrogen-sulfide in the air around bodies of waste-waters, comprising: inoculating the waste-waters with a combination of natural and mutant bacteria which oxidize hydrogen-sulfide to harmless forms of sulfur.

42. A method of eliminating undesirable iron sulfide from oil formations, surface and underground waste-waters, sands, muds, and other media, comprising: inoculating the iron sulfide-containing media with a combination of natural and mutant bacteria capable of oxidizing iron sulfide.

43. The method of claim 42, wherein the mutant bacteria are mutant *Desulfovibrio aestuarii*.

44. The method of claim 42, wherein the mutant bacteria are mutant *Desulfovibrio desulfuricans*.

45. A method of diminishing iron sulfide formation in fluid conducting machinery, comprising: inoculating the sulfide-producing media conducted by the machinery with a combination of natural and mutant bacteria capable of oxidizing sulfide ions.

46. The method of claim 45, wherein the mutant bacteria are mutant *Desulfovibrio aestuarii*.

47. The method of claim 45, wherein the mutant bacteria are mutant *Desulfovibrio desulfuricans*.

48. A method of preventing the formation of iron sulfide in an oil formation, comprising: inoculating the sulfide-producing zone with a combination of natural and mutant bacteria capable of oxidizing both hydrogen-sulfide and iron-sulfide.

49. Inoculating bacterial growth-supporting media, containing natural sulfate-reducing bacteria, with mutant bacteria having partially-to-totally inactive hydrocarbon dehydrogenation systems.

50. Inoculating bacterial growth-supporting media containing sulfides with a combination of natural and mutant sulfide-oxidizing bacteria, to eliminate the said sulfides from said media.

51. Inoculating oil-containing, bacterial growth-supporting media with mutant hydrocarbon-oxidizing bacteria having partially-to-totally inactive hydrocarbon dehydrogenation systems.

52. A method of chemically altering hydrocarbon media, comprising: inoculating the media with a combination of (1) mutant hydrocarbon-oxidizing bacteria having partially-to-totally inactivated dehydrogenation systems, and (2) natural hydrocarbon-oxidizing bacteria.

53. A method of chemically altering media containing intermediate hydrocarbon metabolism derivatives, comprising: inoculating the media with a combination of (1) mutant hydrocarbon-oxidizing bacteria having partially-to-totally inactivated dehydrogenation systems, and (2) natural hydrocarbon-oxidizing bacteria.

54. A method of preventing iron sulfide formation in fluid-conducting machinery, comprising: inoculating the sulfide-producing media conducted by the machinery with

a combination of natural and mutant bacteria capable of oxidizing sulfide ions.

55. The method of claim 54 wherein the mutant bacteria are mutant *Desulfovibrio aestuarii*.

56. The method of claim 54 wherein the mutant bacteria are mutant *Desulfovibrio desulfuricans*.

57. A method of dissolving iron sulfide present in an oil formation, comprising: inoculating the iron-sulfide-containing zone with a combination of natural and mutant bacteria capable of oxidizing both hydrogen sulfide and iron sulfide.

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UNITED STATES PATENT OFFICE  
CERTIFICATE OF CORRECTION

Patent No. 3,105,014

September 24, 1963

William M. Harrison

It is hereby certified that error appears in the above numbered patent requiring correction and that the said Letters Patent should read as corrected below.

Column 1, line 44, for "comporatively" read -- comparatively --; column 4, lines 40 and 43, for "anti-metabolities", each occurrence, read -- anti-metabolites --; line 74, after "of" insert -- the --; column 5, line 23, after "case," insert -- the --; column 9, line 12, for "fiften" read -- fifteen --; column 12, line 9, for "is not" read -- which --; line 57, for "bacterial" read -- bacteria --; column 14, Table VI, second column, between "*Propionibacterium thoenii*", in italics, and "*Proteus vulgaris*", in italics, insert -- *Propionibacterium technicum* --, in italics; column 15, line 47, for "bacterial" read -- bacteria --; column 18, line 16, for "sulfovivrio" in italics, read -- *sulfovibrio* --, in italics; line 42, for "*Pseudomonasfluorescens*", in italics, read -- *Pseudomonas fluorescens* --, in italics; column 21, line 18, for "585,991" read -- 858,991 --.

Signed and sealed this 15th day of September 1964.

(SEAL)  
Attest:

ERNEST W. SWIDER  
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