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Complete patents

of

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Patented Dec. 24, 1946 2,413,278

UNITED STATES PATENT OFFICE

2,413,278

BACTERIOLOGICAL PROCESS FOR TREATMENT OF FLUID-BEARING EARTH FORMATIONS

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No Drawing. Application March 17, 1944, Serial No. 527,010

4 Claims. (Cl. 195—1)

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This invention relates to an improved process for increasing or facilitating the recovery of valuable fluids from fluid-bearing earth formations. More particularly, the invention relates to introducing bacteria into subterranean formations for the purpose of beneficially influencing the factors which control the quantity of valuable fluids which can be recovered from the formations or which control the facility with which such fluids are recoverable. The invention is primarily concerned with increasing or facilitating the recovery of valuable petroleum products from oil- or gas-bearing formations in the earth, but it is also applicable to the recovery of other fluids such as water or non-hydrocarbon gases from subterranean formations.

There are several factors which are known to influence the amount of valuable fluids which can be recovered from subterranean formations or the facility with which those fluids may be recovered. One of the most important of these factors is the porosity of the formation. Such fluids are customarily recovered through wells extending into the formation from the surface of the earth and it is obvious that the fluid can flow to the wells more readily through a porous formation. Another factor which influences the amount of oil which can be recovered from a formation is the extent to which oil adsorbed in calcareous materials in the formation can be liberated. It has been demonstrated that magnesium carbonate which has been saturated with crude oil may retain as much as fifty gallons of crude oil per ton of magnesium carbonate, which oil cannot be replaced by water. The crude oil adsorbed in magnesium carbonate is held so tenaciously that the equivalent of thirteen gallons of crude oil per ton of magnesium carbonate remain adsorbed after thorough leaching with ether.

The extent to which fluids will flow through a subterranean formation of given porosity is also influenced by the magnitude of surface tension values. If the surface tension values in the formation are high there is greater frictional resistance to flow of fluids through the formation than would be the case if those surface tension values were relatively low. The viscosity of liquids also has considerable bearing on their freedom to flow through porous formations. The pressure differential which exists between a producing well and zones spaced from the well in the formation is perhaps one of the most important factors in determining the rate or extent of flow of fluid to the producing well.

Several procedures have heretofore been resorted to in efforts to favorably influence one or more of the factors above enumerated. Attempts have been made to use explosives in that portion of a well extending into the producing formation in the hope that fractures could be created in the

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formation which would facilitate the flow of fluids to the well. However, the impossibility of controlling the direction or extent of such fractures and the ever-present hazard of rupturing the cap rock have made this procedure impractical. Some success has been achieved from procedures involving the introduction of gas or water under pressure into the formation at a point or points remote from the producing well and causing such gas or water to flow toward the producing well. The field of utility of these so-called gas and water "drives" has been found to be limited and they have not proven economically practical except in certain types of formations. Dissolution of calcareous materials in the formation adjacent a well by means of acid introduced through the well has given advantageous results which justify the procedure but the activity of the acid has been limited to a relatively small zone immediately surrounding the well. These various procedures which have heretofore been proposed or used do not and cannot advantageously influence all of the factors which bear on the extent or facility of recovery of valuable fluids from subterranean deposits.

According to my invention, a subterranean formation is inoculated with bacteria which are capable of beneficially influencing the factors which control the extent or facility with which fluids can be recovered from the formation. The bacteria are responsible for the production of acids or acidic substances from organic matter through a process of reduction of sulfates in the formation, and those acids or acidic substances attack and dissolve calcareous materials of the formation. The dissolution of the calcareous materials increases the porosity of the formation and effects release of fluids which had been adsorbed in the calcareous materials. Carbon dioxide also is formed and to the extent that this gas is not adsorbed in liquids present in the formation it is effective to increase the gas pressure. The bacteria also produce detergents or surface-active substances which effect release of adsorbed oil from sand and other non-calcareous material and reduce surface tension values in the formations to thereby provide what might be termed lubrication for the flow of fluids through the formation. There is basis for the belief that high molecular weight hydrocarbons are converted into hydrocarbons of lower molecular weight with a resulting decrease in viscosity and an increase in ability to flow through the formation.

One of the primary objects of my invention is to inoculate subterranean formations with bacteria for the purpose of increasing or facilitating the recovery of valuable fluids therefrom.

A more specific although important object of my invention is to increase or facilitate the recovery of petroleum oil and gas from oil and

gas horizons by the introduction of bacteria into those horizons.

A further object of the invention is to increase the porosity of subterranean formations by the introduction of bacteria which function to effect dissolution of calcareous materials in the formations.

Still another object of the invention is to increase and facilitate the recovery of valuable fluids from subterranean formations by introducing bacteria which function to effect release of fluids adsorbed in the formation.

Still another object of the invention is to increase or facilitate the recovery of valuable fluids from subterranean formations by the introduction of bacteria which function to produce detergents or surface-active substances thus facilitating the release and flow of such fluids through the formation.

Another object of the invention is to increase the gas pressure within subterranean formations by introducing into the formations bacteria which function to produce carbon dioxide.

A further object of the invention is to increase the ability of petroleum oils to flow through oil horizons by reducing the viscosity of the oils through the action of bacteria which convert high molecular weight hydrocarbons into hydrocarbons of lower molecular weight.

Further objects and advantages of the invention will be apparent from the following detailed description.

The bacteria which are suitable for carrying out my invention are not yet described in the literature. They belong to the Desulfovibrio genus. Their position within this genus has not been definitely ascertained, and it appears probable that several closely related members of the genus are involved. The bacteria more nearly resemble *Desulfovibrio aestuarii* than any other organisms described in the literature. They require water for their growth and activity. Certain types of the bacteria require saline conditions for their growth. These types grow and are active in aqueous salt solutions and although most of my observations have been made in solutions ranging between 25,000 and 125,000 parts per million of salt, they have been found to tolerate salinities as high as 300,000 parts per million. The bacteria resemble *Vibrio thermodesulfuricans* (*Sporovibrio desulfuricans*) in their ability to tolerate high temperatures.

The bacteria are strict anaerobes. They should be cultured in absence of light. Sunlight and other ultraviolet radiations are inimical to their growth. They are capable of growing in the presence of crude oil, utilizing sulfates as a hydrogen acceptor. Presumably impurities in the crude oil provide for the mineral and nitrogen requirements of the bacteria. They preferentially attack certain nitrogenous and waxy constituents of crude oil although it has been demonstrated that they can assimilate pure hydrocarbons.

The bacteria having salinity requirements have been isolated from cores of limestone-sulfur-anhydrite formations taken from wells of the Freeport Sulfur Company at Grand Ecaille, Louisiana. The limestone-sulfur-anhydrite formation from which the cores were taken lies at a depth of approximately 1530 feet. Cultures of the bacteria having salinity requirements have also been obtained from marine muds taken from the floors of the Pacific Ocean and the Gulf of California, in some instances from water

depths as great as 6,000 feet. All deep sea samples so far taken from the Pacific Ocean were taken between 117° and 122° west longitude and between 31° and 35° north latitude. The Gulf of California samples were collected between Guaymas and La Paz, Mexico, at about 110° west longitude and 25° north latitude. Mud from Mission Bay and Sorrento Slough (both in the vicinity of San Diego, California) have also yielded good cultures. Types of the bacteria which are not salt tolerant have been isolated from mud taken from the Chicago Drainage Canal at Chicago, Illinois.

The name *Desulfovibrio hydrocarbonoclasticus* has been assigned to these bacteria. That name is used in this specification and in the claims. The name *Desulfovibrio halohydrocarbonoclasticus* is used to designate those types of the *Desulfovibrio hydrocarbonoclasticus* which have salinity requirements and this name is also used in this specification and in the claims.

Cultures of *Desulfovibrio hydrocarbonoclasticus* may be prepared in various ways.

Example 1

One method which I have found to be convenient is to prepare an aqueous brine solution containing from 3% to 30% of sodium chloride. The salinity of the brine should roughly correspond to the salinity of the subterranean formation which is to be inocculated with the bacteria culture. To the brine I add 1.0% of calcium sulfate ($CaSo_4$), 1.0% of calcium carbonate ($CaCO_3$), 1.0% of calcium lactate ($Ca(C_3H_5O_3)_2$), and 0.1% ferrous ammonium sulfate

$((NH_4)_2SO_4.FeSO_4.6H_2O)$

This solution is then boiled to exclude atmospheric oxygen. After cooling the solution, I add to it any of the source materials for bacteria having salinity requirements which I have mentioned above. After introduction of the bacteria source material the solution is maintained in any air-tight container. A glass bottle provided with a glass stopper is suitable or any other container may be used by providing an air-excluding layer of paraffin wax to cover the upper surface of the liquid. The incubation should be carried on in darkness for several days at a temperature between 70° F. and 180° F. The incubation temperature should correspond at least approximately to the previously ascertained temperature of the subterranean formation which is to be inoculated with the culture.

In some instances, I have added 0.1% of either ascorbic acid ($C_5H_8O_6$) or sodium formaldehyde sulfoxylate ($NaHSO_2.HCHO.2H_2O$) to the above culture medium to lower the oxidation-reduction potential.

Example 2

Instead of the solution of Example 1, I may use a medium composed of the following constituents:

Sea water	ml	1000.0
Ammonium phosphate $((NH_4)_2HPO_4)$	gm	0.1
Ferrous sulfate ($FeSO_4.7H_2O$)	gm	0.1
Potassium phosphate (KH_2PO_4)	gm	0.2
Calcium sulfate ($CaSO_4.2H_2O$)	gm	50.0
Calcium carbonate ($CaCO_3$)	gm	40.0
Calcium lactate ($Ca(C_3H_5O_3)_2$)	gm	5.0
Sodium chloride ($NaCl$)	gm	60.0

This medium is used in the manner described in Example 1.

Example 3

Another procedure which I have found suitable for isolation of *Desulfovibrio halohydrocarbonoclasticus* and the preparation of a culture is to make a water paste of plaster of Paris

$((CaSO_4)_2.H_2O)$

and calcium carbonate ($CaCO_3$) and to impregnate this paste with mineral oil and brine. The paste is also impregnated with iron-by-hydrogen which serves the dual purpose of reducing the oxidation-reduction potential and acting as an indicator for hydrogen sulfide formation. Any of the source materials for bacteria having salinity requirements mentioned above may then be added to the paste and a layer of molten paraffin wax added to exclude atmospheric oxygen. The salinity of the brine used in the preparation of this medium should correspond roughly to the salinity of the formation in which the bacteria are to be used. This medium may also undergo an incubation period of several days in darkness at a temperature of between 70° F. and 180° F., temperature of incubation being approximately that which exists in the subterranean deposit for which the culture is being developed. If it is desired to promote the growth of sulfate-reducing bacteria which cannot assimilate hydrocarbons, I find it advisable to add a little organic matter such as a peptone or a lactate to the medium.

After a few days incubation of any of the culture mediums of the above examples at a temperature within the range mentioned, there is evidence that the calcium carbonate is being dissolved and that the calcium sulfate is being attacked. It will be found that carbon dioxide is being liberated and if the medium is covered with paraffin wax the gas pressure slowly forces the layer of wax upwardly in the container. In the case of the medium of Example 3 it will be found that the mineral oil which was used in impregnating the paste is being released from the medium. When these conditions exist the culture is ready for introduction into an inoculating medium for inoculating a subterranean formation.

The bacteria cultures obtained by the procedure of any of the foregoing examples should be introduced into an inoculating medium or carrier to be used in inoculating the subterranean formation. A suitable inoculating medium or carrier may be prepared from the following constituents:

Sea water	ml	750
Tap water	ml	250
Potassium phosphate (K_2HPO_4)	gm	0.2
Magnesium sulfate ($MgSO_4.7H_2O$)	gm	0.2
Ammonium chloride (NH_4Cl)	gm	0.1
Sodium sulfate (Na_2SO_4)	gm	1.0
Sodium sulfite (Na_2SO_3)	gm	0.5
Calcium carbonate ($CaCO_3$)	gm	0.2
Ferrous ammonium sulfate ($(NH_4)_2SO_4.FeSO_4.6H_2O$)	gm	0.1
Ascorbic acid ($C_5H_8O_6$)	gm	0.1
Sodium lactate ($NaC_3H_5O_3$)	gm	3.0

The reaction of the medium is adjusted to pH 7.0 by the addition of sodium hydroxide or hydrochloric acid as required. Sodium formaldehyde sulfoxylate or sodium sulfide may be substituted for the ascorbic acid for reducing the oxidation-reduction potential. Sodium citrate, sodium succinate or the salts of similar organic acids may be substituted for sodium lactate. The salinity of the medium should be adjusted to approximately that of the reservoir fluid into which the culture is to be introduced by the addition of sodium chloride, or preferably brine from the reservoir fluid can be substituted for sea water in the above formula. A culture isolated according to Example 1, 2 or 3 is then added to the inoculating medium.

Examples 1 to 3 above have reference to the isolation of *Desulfovibrio halohydrocarbonoclasticus*, that is the types having salinity requirements. The inoculating medium above is also restricted in its use to *Desulfovibrio halohydrocarbonoclasticus*. These are the more important of the bacteria for the reason that brine is present in many of the subterranean oil-bearing formations. The bacteria mentioned above which are not salt tolerant may be isolated by substituting fresh water for the brine solutions or otherwise eliminating the salt from the mediums of Examples 1 to 3. Inoculating mediums of these bacteria may be prepared by using fresh water instead of sea water or brine in the inoculating medium described above.

The subterranean formation may be inoculated with the bacteria by any procedure found most convenient and my invention is not limited to any particular method of introduction. The inoculating medium may be introduced into the formation through an existing well by means of a dump bailer or the culture may be pumped into the well. Any of the procedures which have been found practical for the introduction of acids into subterranean formations may be used. The cultures may also be introduced into formations with brine or water which is being injected into a subterranean formation for the purpose of a water drive.

I have demonstrated the ability of the bacteria to dissolve calcium carbonate and magnesium carbonate, including limestone and dolomite. Acids or acidic substances are formed during sulfate reductions. So far as is now known, carbon dioxide is always produced and this unites with water to form carbonic acid which converts the insoluble magnesium or calcium carbonates into soluble or unstable bicarbonates. There is evidence that the bacteria oxidize complex organic matter to form certain organic acids, probably acetic acid, propionic acid and butyric acid. These organic acids react with the calcium carbonate or magnesium carbonate to yield organic salts, carbon dioxide and water.

It has also been found that detergents or surface-active substances are produced by the bacteria. The identity of these detergents is not presently known. They may be the fatty acids mentioned above as resulting from the partial oxidation of organic matter or they may be sulfonated higher alcohols or esters.

Some of the sulfate is apparently reduced to sulfur, while some is further reduced to hydrogen sulfide. The hydrogen sulfide which is produced is not generally regarded as an acid and is not as acidic as the sulfate ion which is reduced, but it is effective to assist in the dissolution of the calcareous materials. This appears to be due to the fact that in subterranean formations many of the sulfates are insoluble and hence have no direct effect on the hydrogen-ion concentration or the dissolution reaction.

Unsaturated hydrocarbons are adsorbed and retained more tenaciously by sedimentary materials in producing horizons than are the saturated hydrocarbons. The sulfate-reducing bacteria appear to preferentially attack the unsaturated hydrocarbons and it further appears that

the adsorbed unsaturated hydrocarbons are attacked more readily than those which are free. There are indications that the adsorbed unsaturated hydrocarbons are split at the site of the double bond with liberation from the sedimentary material of lighter, more mobile hydrocarbons. There is also evidence that the bacteria split saturated long chain hydrocarbons into shorter chains with a resulting increase in the ability of the hydrocarbons to flow through the formation.

It is recognized that the chemical reactions induced by the bacteria are highly technical and that the precise nature of the compounds resulting from the reactions is not thoroughly understood. The foregoing explanation is necessarily somewhat general and is given only for the purpose of setting forth my present belief regarding the manner in which the bacteria function. The nature of the reactions and the circumstances under which they are carried out render it extremely difficult to make analytical determinations. For these reasons, the invention should not be construed as limited to the theories which I have outlined above regarding the reactions and the resulting compounds. Irrespective of whether those theories are correct, I desire to call attention to certain observed results of the use of the bacteria. The ability of the bacteria to dissolve calcium carbonate and magnesium carbonate and to release oil adsorbed in the carbonates has been demonstrated. The production of gaseous carbon dioxide during the dissolution of the carbonates has been proven. When the bacteria are cultivated in a liquid medium in the absence of adsorbents such as sand, the surface tension of the medium has been reduced, presumably by the production of detergents or surface-active substances. When the bacteria are cultivated in a liquid medium in the presence of adsorbents such as sand, the surface tension of the fluid medium is not perceptively reduced, presumably for the reason that the detergents are adsorbed by the adsorbent. When the adsorbents present in the medium contain oil, the oil is released from the adsorbent. Athabaska tar sands from the Athabaska region of Canada have been found to release oil when subjected to contact with a medium of the bacteria. The bacteria attach themselves so tenaciously to solid surfaces that they cannot be flushed away by the ordinary flow of fluids in a formation.

The bacteria attack hydrocarbons of high molecular weight but molecules having less than ten carbon atoms are not attacked. For example, hentriacontane ($C_{31}H_{64}$) is attacked but the bacteria have no converting action on octane (C_8H_{18}) or hexane (C_6H_{14}). In the process of decomposition the higher molecular weight hydrocarbons are converted into successively smaller molecules. Since molecules smaller than decane are not attacked and since methane has been detected during decomposition of higher molecular weight hydrocarbons, it seems probable that the decomposition is a hydrocarbon-splitting action.

There are indications that the bacteria may continue to grow indefinitely. The extent to which their proliferation causes them to spread through the formation or the rate of such spreading has not been definitely observed, but in the laboratory it has been found that they will grow through a one-half inch thickness of unglazed porcelain within a few hours and that they will permeate tightly packed sand for relatively long distances in a short time.

Unsaturated and long-chain hydrocarbons are slowly assimilated by the bacteria, although the organisms preferentially assimilate more complex organic matter. The assimilation of the hydrocarbons is a bacterial oxidation reaction and is self-limiting for the reason that the oxidizing activities of the organisms are inhibited by hydrogen-ion concentrations lower than pH 6.0, and the oxidation of relatively small quantities of hydrocarbons are required to produce sufficient carbon dioxide in solution to lower the pH to 6.0. For the foregoing reasons, it appears probable that the amount of hydrocarbons in a formation which are actually assimilated by the bacteria is negligible.

The bacteria require water for their growth and activity, but it appears that capillary or connate water is always available in formations in sufficient quantity. Oxidized sulfur compounds such as sulfates, sulfites, or thiosulfates appear to be essential for the growth of the bacteria and for this reason it would not appear to be advisable to introduce the bacteria into subterranean deposits in which all of the sulfates or other oxidized sulfur compounds have been reduced. By preparing the bacteria cultures in any of the manners which I have outlined above, they may be acclimatized to grow and exhibit activity at temperatures as high as 180° F. Activity of the bacteria has not been observed at temperatures higher than 190° F., so the bacteria should not be expected to perform their intended functions in formations in which the temperature substantially exceeds 180° F.

From the foregoing it will be seen that my invention favorably influences the several factors which control the extent or facility of recovery of valuable fluids from subterranean formations. Much of the foregoing discussion has had particular reference to the recovery of hydrocarbons from formations containing the same. It is in this field that all of the enumerated advantages of the invention find utility. However, it will be obvious that the increase in porosity of the formation, the production of surface tension depressing agents, and the increase in gas pressure in the formation are advantageous in the recovery of other fluids.

Having thus described my invention, I claim:

1. The method of treating a fluid-bearing earth formation to facilitate or increase the recovery of fluids therefrom which comprises subjecting the formation to the action of *Desulfovibrio hydrocarbonoclasticus.*

2. The method of treating a subterranean formation to facilitate or increase the recovery of valuable fluids therefrom which comprises subjecting the formation to the action of *Desulfovibrio hydrocarbonoclasticus.*

3. The method of treating a hydrocarbon-bearing earth formation to facilitate the recovery of hydrocarbons therefrom which comprises subjecting the formation to the action of *Desulfovibrio hydrocarbonoclasticus.*

4. The method of increasing or facilitating the recovery of oil from a subterranean oil-bearing formation which comprises subjecting the formation to the action of *Desulfovibrio halohydrocarbonoclasticus.*

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Patented June 9, 1953

2,641,566

UNITED STATES PATENT OFFICE

2,641,566

RECOVERY OF HYDROCARBONS

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No Drawing. Application May 15, 1948, Serial No. 27,363

5 Claims. (Cl. 195—3)

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This invention relates to the treatment and recovery of hydrocarbons, bituminous substances, and the like in underground deposits, shales and similar formations.

The recovery and improvement of oil or so-called kerogen, supposedly a complex bituminous mixture from shale, is a major problem of long standing because of the close association of the kerogen with the inorganic mineral aggregate and the necessity of handling such large quantities of the latter. Processes such as destructive distillation, solvent extraction at high temperatures and high pressures, and hydrogenation at high temperatures and pressures in the presence of a catalyst have been practiced to some extent but are generally regarded as unsatisfactory because of the relatively low yields obtained and the large quantities of aggregate that must be handled and brought up to the required high temperatures. In some cases the mineral aggregate may run as high as 75% or more.

A similar problem is encountered in the so-called secondary recovery of the more viscous petroleum oils in underground deposits wherein the oil is intimately associated with mineral aggregate including rock and sand. Even in cases where the oil is relatively free of the aggregate, it cannot be pumped to the surface because of its relatively high viscosity.

One object of the invention is the provision of a novel process of treating hydrocarbons, bituminous materials, and like substances, normally held in close association with an inorganic mineral aggregate, so that they can be separated therefrom and recovered.

Another object of the invention is the provision of a novel process wherein oil shale can be hydrogenated at a relatively low temperature to effect both recovery of the oil contained therein and improvement of the properties of that oil.

Other objects and advantages of the invention will appear from the following description and claims.

In brief, the present invention may be said to involve the processing of hydrocarbons such as petroleum oils, and bituminous mixtures such as shale oils and the like, wherein the viscosities of the oils are apparently reduced or their characteristics otherwise altered so that the converted oils and their products of alteration can be recovered from retaining inorganic aggregate such as that of oil sands or shale, and, if, underground, be transferred to the surface and recovered. Additionally, the process is carried out at relatively low or atmospheric temperatures by hydrogenation in the presence of microbiological or enzymatic catalysts whereby the properties of the recovered oils are improved.

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A suitable microbiological catalyst for the process is hydrogenase, an enzyme produced by a number of different bacteria, which has been found to catalyze the reduction of a number of different substances by molecular hydrogen. While the action of the enzyme is not entirely understood, it is believed to convert molecular hydrogen to a more active form of hydrogen, termed by some "active" or nascent hydrogen, which finds an acceptor in a number of constituents of shale and petroleum oils such as carbon and sulfur.

It is to be understood that another enzyme or catalyst is usually required in addition to the hydrogenase, the other enzyme serving to activate the substrate. The same enzyme complex may perform both functions or two separate entities may be required. In other words, some bacteria which produce hydrogenase also produce enzymes which activate the reducible substrate, but this is not true of all hydrogenase-producing bacteria. Many substrates will react directly with the hydrogen activated by hydrogenase in the absence of a second enzyme or catalyst. Typical of such substrates are certain olefins, diolefins, acetylenes and amines. Nitrate is an example of such an inorganic substrate. However the majority of substrates require another enzyme or catalyst, separate and distinct from hydrogenase to bring about the reaction with active hydrogen produced from hydrogenase. The reduction of carbon dioxide to methane and the reduction of sulfates to sulfides are in this class as is the reduction of sulfur in cyclical sulfur compounds. The sulfur in cysteine (—C—SH) may be reduced to hydrogen sulfide by active hydrogen in the absence of another enzyme or catalyst but cystine (—C—S—C—) is not so reduced under ordinary conditions of temperature, pressure and pH.

"Substrate" may be defined as the substance or compound acted upon, i. e., the substance which accepts hydrogen and is reduced, a substance from which hydrogen may be produced by hydrogen-producing bacteria, or a substance from which hydrogenase may be produced by hydrogenase-producing bacteria.

Hydrogenase is believed to be an iron porphyrinprotein complex which is active only in the reduced or ferrous states.

Hydrogenase is produced by several microbial species and can be used to catalyze the reaction

of hydrogen with a number of substances including fumarates, malates, formaldehyde, methyl alcohol, carbon monoxide, carbon dioxide, carbonates, nitrates, sulfates, sulfites, thiosulfates and a number of other compounds, thus enabling the method of this invention to be used in a number of different processes for treating various substances. A preferred hydrogenase for reducing complex sulfur compounds in oil is *Desulfovibrio desulfuricans* or related species although others may be used.

Hydrogenase produced by methane bacteria can be used to catalyze the reaction of molecular hydrogen with carbon monoxide and carbon dioxide. It can also be used to catalyze the reaction of molecular hydrogen with formic acid, formaldehyde, and methyl alcohol, the reactions being believed to be generally as follows:

$$CO+3H_2\rightarrow CH_4+H_2O$$
$$CO_2+4H_2\rightarrow CH_4+2H_2O$$
$$HCOOH+3H_2\rightarrow CH_4+2H_2O$$
$$HCHO+2H_2\rightarrow CH_4+H_2O$$
$$CH_3OH+H_2\rightarrow CH_4+H_2O$$

The production of methane by the reduction of carbon dioxide with hydrogen can also be catalyzed by *Methanobacterium omelianskii, Methanosarcina methanica, Methanosarcina barkerii* and *Methanobacterium formicicum.* The two last mentioned species produce hydrogenase capable of reducing carbon monoxide to methane in the presence of hydrogen. The reduction enabled by the present process is capable of use in still further reactions such as the hydrogenation of carbon dioxide to form formic acid, a suitable catalyst being the hydrogenase produced by *Escherichia coli.* While this reaction is normally reversible, an increase in the partial pressure of the carbon dioxide appears to cause the reaction to proceed to completion. The yield may be increased by raising the partial pressure of the hydrogen. The reaction is believed to be generally as follows:

$$CO_2+H_2\rightarrow HCOOH$$

In the presence of hydrogen, hydrogenase and other enzymes or catalysts produced by the following microorganisms may be used to effect the reactions indicated:

Clostridium aceticum and *Clostridium thermoaceticum.*—

$$2CO_2+4H_2O \longrightarrow CH_3COOH+2H_2O$$
(Acetic acid)

Butyribacterium rettgeri.—

$$2CH_3COOH+2H_2 \longrightarrow CH_3CH_2CH_2COOH+2H_2O$$
(butyric acid)

Hydrogenomonas pantotropha.—

$$CO_2+2H_2 \longrightarrow (HCHO) \longrightarrow \text{carbohydrates, etc.}+H_2O$$

Escherichia formica.—Reduction of fumarate and methylene blue by molecular hydrogen.

Proteus vulgaris.—Hydrogenation of fumarate, maleate, malate, carbonates and oxygen.

Clostridium sporogenes.—Catalyzes hydrogenation of a number of organic compounds including acetaldehyde, acetic acid, acetyl methyl carbinol, acrolein, arginine, aspartic acid, cysteine, cystine, diacetyl, glutaric acid, glyceric aldehyde, glycine, glyoxal, hydroxylamine, malonic acid, methionine, ornithine, oxalic acid, proline, tryptophane and tyrosine.

Clostridium welchii, Bacillus hydrogenes, Hydrogenomonas minor, Escherichia formica and *Escherichia coli* catalyze hydrogenation of nitrates, nitrites, or other reducible nitrogen compounds:

$$HNO_3+4H_2\rightarrow NH_4OH+2H_2O$$
$$HNO_2+3H_2\rightarrow NH_4OH+H_2O$$
$$NH_2OH+H_2\rightarrow NH_4OH$$

Certain species of *Desulfovibrio, Sporovibrio,* and other sulfate reducers catalyze reduction of sulfates by hydrogen:

$$SO_4^{=}+4H_2 \longrightarrow S^{=}+4H_2O$$

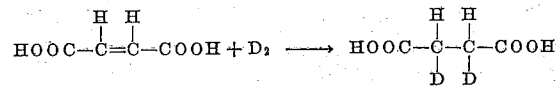
Some species also catalyze reduction of sulfites and thiosulfates to hydrogen sulfide.

Catalysts for the reduction of sulfur-containing compounds can be isolated also from marine sediments, the cultures apparently being best in an aqueous medium of the composition of sea water enriched by:

0.02% calcium lactate
0.02% ascorbic acid
0.02% ferrous ammonium phosphate
0.05% sodium bicarbonate
0.5% potassium sulfate

Except when oxygen is being intentionally reacted with hydrogen, it is desired that only anaerobic species be used to avoid the presence of oxygen and consequent undesired consumption of hydrogen.

Deuterium or heavy hydrogen (D_2) can be substituted for hydrogen in some reducing reactions, for example, *Escherichia coli* is capable of catalyzing the reaction of hydrogen with fumaric acid to form dideuterosuccinic acid.



Some other examples of reactions are:

$$CO_2+4D_2\rightarrow CD_4+2D_2O$$
$$CO_2+D_2\rightarrow DCOOD$$

Thus the present method provides a relatively simple and inexpensive process for the manufacturing of special chemicals wherein deuterium is employed.

Kerogen, found in shales, is considered to be formed, primarily, of complex bituminous substances. It contains hydrocarbons, phenols, labile bituminous materials, sulfur and nitrogen complex compounds. In the presence of a catalyst such as hydrogenase, other enzymes or catalysts usually being required, hydrogen combines with such compounds to reduce the phenols, react with any labile materials, remove the nitrogen and sulfur, reduce the overall viscosity of the oil and in general, convert the original substantially heavy oil to lighter oil of more desirable characteristics. The gaseous products produced may include some hydrocarbons, such as methane, hydrogen sulfide and ammonia. These reactions also take place with other hydrocarbons such as the petroleum oils found in underground deposits, the principal advantage of the process in that connection being the separation of the oil from the oil sand or other aggregate and apparent reduction of viscosity of the oil so that the oil can be transferred to the surface as by pumping.

One application of the invention can be explained in connection with the processing of oil shales which are notoriously difficult and expensive to process and secure a reasonable hydrocarbon yield therefrom. The shale is first ground, preferably to relatively fine particle size, and placed in a suitable receptacle. Preferably, the latter is substantially gas tight. Such substantial sealing of the receptacle is preferred in order

that any oxygen-containing gases can be excluded therefrom since the hydrogen in the presence of a catalyst such as hydrogenase finds a ready acceptor in oxygen and is lost as water as far as the oil shale is concerned. Preferably, the upper portion of the receptacle in which the gaseous reaction products are collected and removed is blanketed with hydrogen or some relatively inert gas before the actual processing is begun.

The shale particles in the receptacle are covered with a nutrient medium, principally water, containing either the enzyme hydrogenase or hydrogenase-producing microorganisms, another enzyme or catalyst usually being required. One specific type of such microorganism such as *Clostridium sporogenes*, previously described, can be used to limit the reaction to only one constituent of the shale of the shale oil or a mixture of such organisms can be used to effect the reaction of a plurality of constituents. Various types of hydogenase capable of catalyzing the hydrogenation of different substances have already been discussed. It is to be understood that such organisms may be used singly or in combination, it also being understood that when some combinations are attempted, one type of organisms may eventually become much more active than another and substantially eliminate the effect of the second, a phenomenon well known to microbiologists.

The type of nutrient medium employed will vary with the type or types of microorganisms being handled as is well known in the microbiological art. A mineral-salt type medium is usually used, the above described medium being typical.

The nutrient medium is charged with either hydrogenase or hydrogenase-producing bacteria and another enzyme or enzymes or the bacteria producing them and the temperature, pressure and pH maintained in ranges most conducive to the catalytic action of the microorganisms including the product enzymes thereof. Preferably the temperature is maintained in the range of 20° to 75° C., depending upon the types of microorganisms being used, the pressure at substantial atmospheric or relatively low pressures, and the pH in the range of 6.5 to 7.5. Optimum temperatures may be raised appreciably by hydrostatic pressure. The total pressure can be varied from atmospheric to high pressure in the order of 300 atmospheres. Preferably, the pressure, because of engineering and economic considerations, is kept below 10 atmospheres.

Molecular hydrogen is then charged to the receptacle in a manner whereby it is uniformly distributed throughout the nutrient medium and the shale. Means is preferably employed to keep the slurry agitated, thus avoiding settling of the shale and insuring contact of the hydrogen and the enzyme with all the shale particles. Such agitation can be secured by proper distribution of the hydrogen charged to the receptacle.

The hydrogen is apparently converted to a more active form by action of the hydrogenase and reacts with the so-called kerogen or complex bituminous material in the shale to apparently reduce its viscosity and effect its disengagement from the solid aggregate. At the same time, the characteristics of the kerogen are improved in that sulfur is removed and the labile compounds are hydrogenated. Some of the material is exhaustively hydrogenated in some cases to methane. The resultant gaseous products including methane, hydrogen sulfide, and ammonia are collected at the top of the receptacle and withdrawn. Any hydrogen removal in this manner may be recovered and recycled through the shale.

The released oil tends to collect on the surface of the medium and may be withdrawn by a series of valve-controlled take-offs positioned at different levels in the tank. Separation of the oil from the medium is a relatively simple task by reason of the difference in their specific gravities. After recovery of the oil, the residual aggregate is removed from the receptacle and a new charge of shale substituted therefor. Preferably fresh inoculated nutrient is charged with each batch of shale.

If hydrogen is not available or not desirable for use, the nutrient medium may be charged with hydrogen-producing bacteria and a suitable substrate from which hydrogen for use in the process can be produced directly in the nutrient medium. Polyhdric compounds such as glycerol, sugar, starch and cellulose, are especially good substrates. From an economical point of view, cheaper materials such as agricultural wastes, offal, etc. are preferred.

This alternate procedure has an advantage in that the receptacle containing the shale can be charged with the several types of bacteria and the process carried out with a minimum of attention

Some substrates from which hydrogen can be produced by different bacteria are listed in the following table:

Substrate	Microorganism
Glucose	*Aerobacter aerogenes.*
	Aerobacter cloacae.
	Bacillus acetoethylicus.
	Bacillus polymyxa.
	Clostridium acetobutylicum.
	Clostridium butylicum.
	Clostridium butyricum.
	Clostridium botulinum.
	Clostridium oedematis-maligni.
	Clostridium sporogenes.
	Clostridium tetani.
	Clostridium tetanomorphum.
	Clostridium thermosaccharolyticum.
	Clostridium welchii.
	Escherichia coli.
	Proteus mirabilis.
	Proteus vulgaris.
	Sarcina maxima.
	Sarcina ventriculata.
	Serratia marcescens.
Cellulose	Algae such as Scenedesmus.
	Bacillus polymyxa.
	Clostridium hydrogenicus.
	Clostridium celluloslytigue.
	Clostridium cellulosolvens.
	Clostridium dissolvens.
	Clostridium fossicularum.
	Clostridium werni.
Glycerol	*Aerobacter aerogenes.*
	Clostridium butylicum.
	Clostridium sporogenes.
	Clostridium tetanomorphum.
Mannitol	*Aerobacter aerogenes.*
	Escherichia coli.
Acetic acid	*Aerobacter indologenes.*
Citric acid	Do.
Succinic acid	Do.
Amygdalin	*Clostridium acetobutylicum.*
	Clostridium butylicum.
	Clostridium butyricum.
Cellobiose	*Clostridium acetobutylicum.*
	Clostridium butylicum.
	Clostridium butyricum.
Galactose	*Clostridium acetobutylicum.*
	Clostridium butyricum.
Fructose	*Clostridium acetobutylicum.*
	Clostridium butyricum.
	Clostridium botulinum.
	Clostridium oedematis-maligni.
	Escherichia coli.
	Sarcina maxima.
	Sarcina ventriculata.
Lactose	*Clostridium acetobutylicum.*
	Clostridium butyricum.
	Clostridium sporogenes.
	Clostridium welchii.
Maltose	*Clostridium acetobutylicum.*
	Clostridium butylicum.
	Clostridium butyricum.
	Clostridium botulinum.
	Clostridium oedematis-maligni.
	Clostridium tetanomorphum.
	Escherichia coli.

Substrate	Microorganism
Mannose	*Clostridium acetobutylicum.*
	Clostridium butyricum.
	Clostridium botulinum.
	Clostridium oedematis-maligni.
	Escherichia coli.
Salicin	*Clostridium acetobutylicum.*
	Clostridium butyricum.
	Clostridium botulinum.
	Clostridium oedematis-maligni.
	Clostridium sporogenes.
	Escherichia coli.
Sucrose	*Clostridium acetobutylicum.*
	Clostridium butylicum.
	Clostridium butyricum.
	Clostridium botulinum.
	Clostridium oedematis-maligni.
	Clostridium sporogenes.
	Escherichia coli.
Arabinose	*Clostridium acetobutylicum.*
	Clostridium butylicum.
	Clostridium butyricum.
	Escherichia coli.
Gluconic acid	*Clostridium acetobutylicum.*
Mannitol	Do.
Aesculin	*Clostridium butylicum.*
Dextrin	Do.
Dimethyl glucoside	Do.
Inositol	Do.
Inulin	*Clostridium butylicum.*
	Clostridium sporogenes.
Melibiose	*Clostridium butylicum.*
Raffinose	*Clostridium butylicum.*
	Pseudomonas rathonis.
Rhamnose	*Clostridium butylicum.*
Starch	*Clostridium butylicum.*
	Clostridium sporogenes.
	Algae such as Scenedesmus.
Pyruvic acid	*Clostridium butylicum.*
	Clostridium butyricum.
	Clostridium botulinum.
	Clostridium tetanomorphum.
Trehalose	*Clostridium butylicum.*
Xylose	*Clostridium butylicum.*
	Clostridium thermosaccharolyticum.
	Clostridium butyricum.
Acetic acid	*Clostridium butyricum.*
Butyric acid	Do.
Lactic acid	Do.
Valeric acid	Do.
Pectin	Do.
Peptone	*Clostridium botulinum.*
	Clostridium sporogenes.
Sorbitol	*Clostridium oedematis-maligni.*
	Escherichia coli.
Aspartic acid	*Clostridium tetani.*
	Clostridium teanomorphum.
Glutamic acid	*Clostridium tetani.*
	Clostridium tetanomorphum.
Serine	*Clostridium tetani.*
	Clostridium tetanomorphum.
Cystine	*Clostridium tetanomorphum.*
	Proteus vulgaris.
Fumaric acid	*Clostridium tetanomorphum.*
Methionine	Do.
Histidine	Do.
Malic acid	Do.
Tyrosine	Do.
Galactose	*Escherichia coli.*
Formic acid	Do.
Glycolic acid	*Escherichia coli.*
	Salmonella enteritidis.

The gases, oils or hydrocarbons produced may be subsequently processed and refined as desired in accordance with recognized petroleum refining and synthetic hydrocarbon manufacturing practices.

While the foregoing example is concerned with shale, it is to be understood that the process is applicable also to the treatment of underground oils that may be bound in aggregate such as the aggregate of oil sands or the like and be incapable of recovery as by pumping. Such methods of recovery, usually termed secondary-recovery, can be broadly considered as the recovery of oil, gas or oil and gas by any method involving artificial flowing or pumping through the joint use of two or more well bores. Water flooding, one of such secondary-recovery operations, involves the introduction of water into an oil formation for the purpose of increasing the oil recovery.

It is considered that the oil, incapable of primary recovery as by artificial or natural flow, is held within the aggregate in the pores thereof by capillary forces and adsorbed on the surface of the grains, such as are found in oil sand, as a film. A typical water flow accomplishes its result by actual displacement, i. e., the forcing of the oil ahead of the water toward a producing well. Ordinarily, the practice comprises the application of water under pressure to the oil bearing formation by means of water intake wells so located that the bank of oil, which is ahead of the advancing water as the latter permeates the aggregate, is forced toward producing wells. The arrangements of the intake wells and the producing wells vary widely in accordance with the formations being processed. A typical arrangement is the so-called 5-spot arrangement in which each producing well is located at the center of a square and is surrounded by 4 intake wells at the corners of the square. An alternative is the so-called 7-spot in which each producing well is located at the center of a hexagon and is surrounded by 6 intake wells at the corners. An excellent discussion of water flooding and the customary practices followed therein will be found in "Secondary Recovery of Oil in the United States," published by American Petroleum Institute, 50 West 50th Street, New York, N. Y., in 1942. Reference is made thereto for further explanation of the various methods that have been practiced.

One of the factors which effect the efficiency of such flooding is viscosity. Oils of relatively high viscosity are difficult to flood satisfactorily. In the application of the process of this invention to water flooding and like operations, the problem of viscosity is considerably reduced since the reactions of hydrogen in its more active form in the presence of hydrogenase tends to decrease the viscosity of the oil.

In practicing the invention in connection with water flooding, the water to be charged to the water intake wells is preferably utilized as the nutrient medium, the general composition thereof being varied in accordance with the microorganisms to be charged. The compositions of such mediums are well known to the art. The water is also charged or inoculated with hydrogenase-containing bacteria and hydrogen-producing bacteria in combination with a suitable substrate, and other enzymes or bacteria producing the same that may be required to activate the various substrates encountered. If the water originally contains bacteria or other microorganisms that may be harmful in the processing, it may be sterilized before the nutrient constituents and microorganisms are added.

When the resultant composition is charged through the water intake wells, not only is the expected flooding achieved, but also a marked decrease in the viscosity of the oil and a release of the oil from its association with the oil sand or other aggregate. The oil which is then displaced by means of its lowered viscosity and by bacterial or enzymatic action is pushed along with the water and eventually separated therefrom at the producing well. It will be understood that the action of the hydrogen in the presence of the hydrogenase is substantially the same as already described as in connection with the treatment of oil shale.

It is to be understood that optimum conditions for the metabolism of the charged bacteria or the catalytic action of the enzymes cannot always be maintained underground by reason of the various temperatures encountered and certain formations such as limestone which may tend to change the pH of the nutrient. The various pressures encountered are not considered to be

limiting factors, inasmuch as the bacterial action apparently continues despite the radical changes in the pressure. Proper tests will usually determine the presence or absence of any formation that may alter the pH radically and the initial composition of the water medium determined accordingly.

The amount of oil thus recovered by waterflooding is increased. Any increase in such recovery is a material advantage particularly in view of the present-day shortage of oil. This process admits of the recovery of large quantities of oil hitherto considered not recoverable. One such reserve is found in the Athabaska sands of Northern Alberta, Canada, wherein it is estimated that the oil bearing sands cover an area of from 10,000 to 30,000 square miles and constitute the largest known deposit of oil in the world. While the total reserve in this field is estimated to be 100 million barrels, only about 1% is recoverable with present-day methods.

The term "microorganisms" as used herein is intended to include bacteria, their enzymes and other products as well as related fungi and molds.

Obviously, many modifications and variations of the invention as hereinabove set forth may be made without departing from the spirit and scope thereof and therefore only such limitations should be imposed as are indicated in the appended claims.

I claim:

1. A method of treating hydrocarbons and bituminous materials such as those encountered in combination with inorganic aggregate underground deposits and shales and similar formations for the purpose of facilitating separation and recovery of such substances which comprises the steps of contacting said substances with a substantial quantity of oxygen-free hydrogen in the presence of hydrogenase-producing microorganisms selected from the group consisting of *Desulfovibrio desulfuricans* and *Sporovibrio*, effecting said contact in the presence of a microbiological nutrient medium and removing the resulting products.

2. A method according to claim 1 in which said hydrogen is generated by the action of *Clostridium* microorganisms on carbohydrates.

3. A method of effecting the separation and recovery of hydrocarbons from oil shale which comprises the steps of immersing said shale in the microbiological nutrient medium, charging said mixture of shale and nutrient medium with a substantial amount of oxygen-free hydrogen and with hydrogenase-producing microorganisms selected from the group consisting of *Desulfovibrio desulfuricans* and *Sporovibrio*, and separating the resulting products.

4. A method according to claim 3 in which said shale is reduced to relatively fine particles prior to its contact with hydrogen.

5. A method according to claim 3 in which said hydrogen is generated by the action of *Clostridium* microorganisms on carbohydrates.

CLAUDE E. ZoBELL.

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June 9, 1953 C. E. ZOBELL 2,641,564

PROCESS OF REMOVING SULFUR FROM PETROLEUM HYDROCARBONS AND APPARATUS

Filed March 31, 1948 2 Sheets-Sheet 1

Fig. 1.

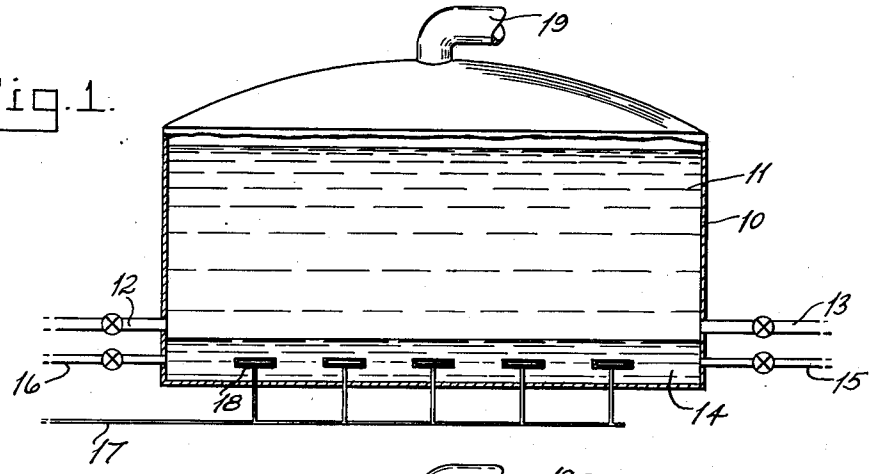


Fig. 2.

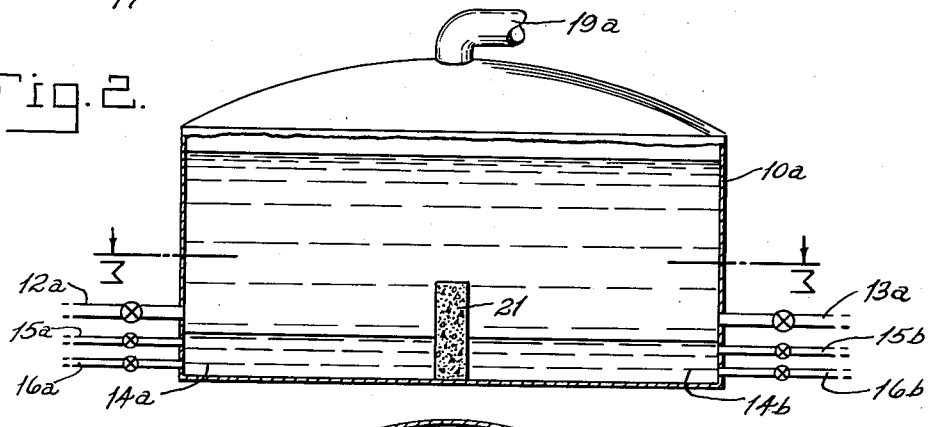
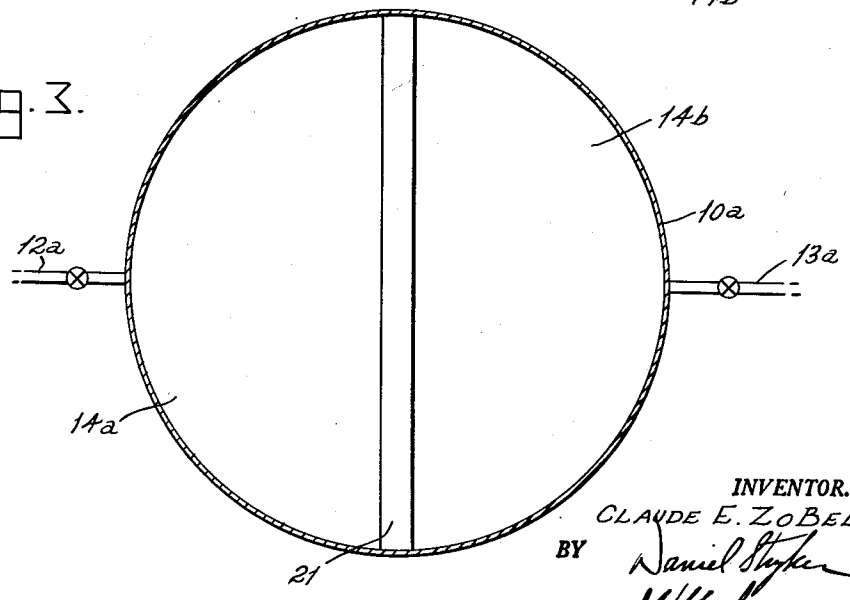


Fig. 3.



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PROCESS OF REMOVING SULFUR FROM PETROLEUM HYDROCARBONS AND APPARATUS

Filed March 31, 1948 2 Sheets-Sheet 2

Fig. 4.

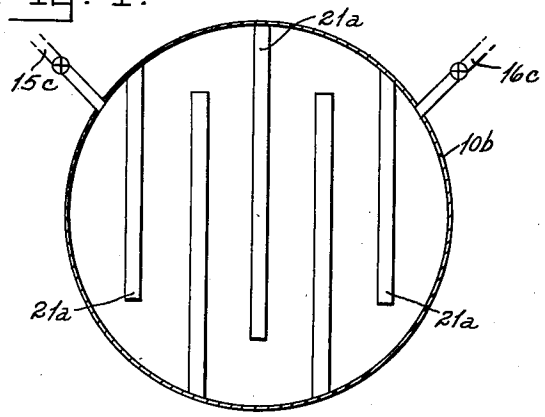
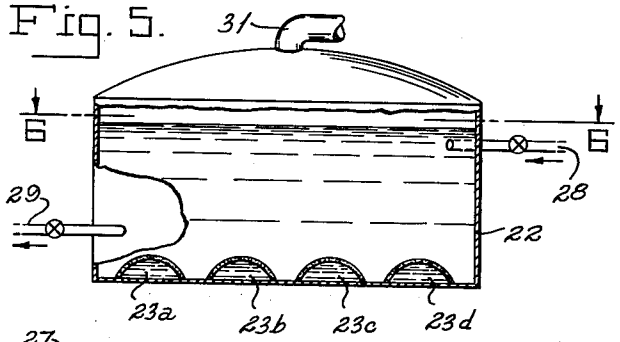


Fig. 5.



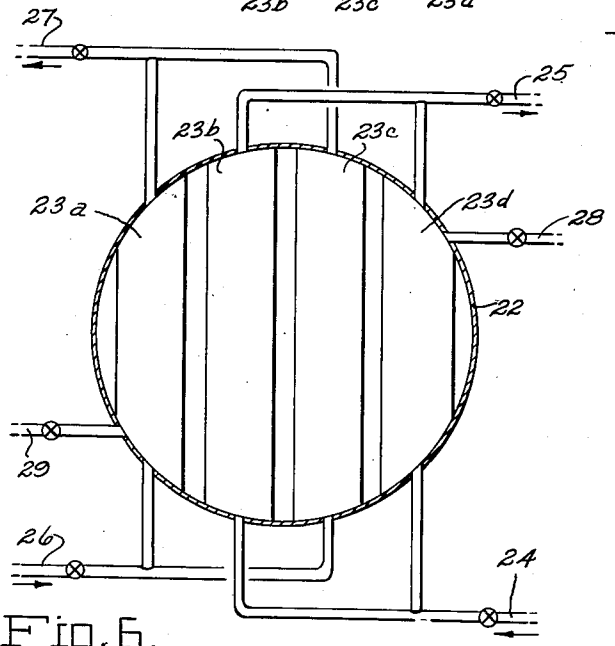
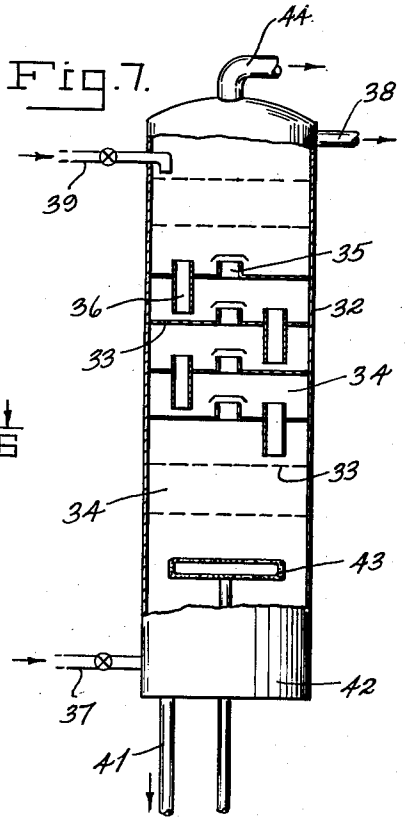


Fig. 6.

Fig. 7.



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Patented June 9, 1953

2,641,564

UNITED STATES PATENT OFFICE

2,641,564

PROCESS OF REMOVING SULFUR FROM PETROLEUM HYDROCARBONS AND APPARATUS

Claude E. ZoBell, La Jolla, Calif., assignor to Texaco Development Corporation, New York, N. Y., a corporation of Delaware

Application March 31, 1948, Serial No. 18,275

6 Claims. (Cl. 195—3)

1

This invention relates to the purification, hydrogenation, and like processing of substances, including hydrocarbons and other types of compounds, by the use of microbiological catalysts, and more particularly to the reduction and removal of hydrogen-reducible compounds such as sulfur-bearing complexes or complex sulfur compounds from petroleum hydrocarbons by the use of such catalysts.

In many cases, it is desirable to purify or alter the composition of a substance such as a petroleum hydrocarbon by hydrogenation, oftentimes without subjecting the hydrocarbon to the relatively high temperatures normally encountered in conventional catalytic hydrogenation. Such selective purification or alteration in many cases cannot be accomplished feasibly and practically by chemical methods, even without the temperature problem. For example, crude oil, in some instances, is characterized by the presence of objectionable quantities of sulfur compounds, mostly in complex forms, which it is desirable to remove or substantially reduce in amount, dependent upon the intended use of the final products. Sulfur removal eliminates possible corrosive and other objectionable characteristics of such products when put in use. Such sulfur compounds are further objectionable in such final products as fuels for internal combustion engines in that they reduce the efficiency of antiknock compounds such as tetraethyl lead and may, when used alone, reduce the octane number of the fuel. It is not desirable to process such crudes by conventional refining means because of the corrosive effect of the sulfur compounds. Modifications of the usual refining processes plus additional steps are required in the refining of such crudes either to abstract the sulfur compounds, to reduce them to unobjectionable amounts or to modify them chemically to noninterfering types. Such process modifications require expensive and special apparatus, involve corrosion problems, and add materially to the cost of the final products. Desulfurization by hydrogenation, as previously known to the art, requires either special catalysts, extremely high temperatures or pressures, or combinations thereof, and consequently has not found wide application. The poisoning of the catalysts by the resultant hydrogen sulfide is particularly disadvantageous. Problems of equal extent for sulfur-free materials and compounds are often encountered in other types of hydrogenations.

It is an object of this invention to provide a novel process for the purification, hydrogenation

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and similar processing of hydrocarbons and other types of compounds wherein microorganisms, their enzymes, or other microbiological catalysts are employed.

A more specific object of the invention is the purification of petroleum hydrocarbons by the reduction of complex sulfur compounds therein and the removal of the resultant sulfur-containing products.

A further object of the invention is the provision of a novel process of the type herein described wherein any hydrogen sulfide produced functions in support of the catalyst.

Still another object of the invention is the provision of novel apparatus for carrying out the process of the invention.

Other objects and advantages of the invention will appear from the following description and claims taken in connection with the attached drawings wherein:

Fig. 1 is a diagrammatic showing of apparatus for practicing the invention in a batch process.

Fig. 2 is a modification of the apparatus of Fig. 1.

Fig. 3 is a horizontal section taken on the line 3—3 of Fig. 2.

Fig. 4 is a view similar to that of Fig. 3 of a modification.

Fig. 5 is a further modification of the apparatus of Fig. 1.

Fig. 6 is a section taken on the line 5—5 of Fig. 4.

Fig. 7 is a diagrammatic showing of apparatus for practicing the invention in a continuous process.

In brief the present invention involves the reaction of selected compounds or mixtures thereof under controlled conditions by contact with hydrogen in the presence of certain microbiological catalysts. More specifically, the invention involves the removal of selected compounds, such as complex sulfur compounds, from petroleum hydrocarbons under controlled conditions by the reaction of such compounds with hydrogen in the presence of a microbiological catalyst such as hydrogenase, which activates molecular hydrogen, and other enzymes or catalysts, which activate the substrate.

It is to be understood that two or more enzymes or catalysts are usually involved in such reactions, first, the hydrogenase, which activates hydrogen, and second, the enzyme or catalyst which activates the substrate or other reactant. The same enzyme complex may perform both functions, or

two separate entities may be required. In other words, some bacteria which produce hydrogenase also produce enzymes which activate the reducible substrate, but this is not true of all hydrogenase-producing bacteria. Many substrates will react directly with the hydrogen activated by hydrogenase in the absence of a second enzyme or catalyst. Typical of such substrates are certain olefins, diolefins, acetylenes and amines. Nitrate is an example of such an inorganic substrate. However, the majority of substrates require another enzyme or catalyst, separate and distinct from hydrogenase to bring about the reaction with active hydrogen produced by hydrogenase. The reduction of carbon dioxide to methane and the reduction of sulfates to sulfides are in this class as is the reduction of sulfur in cyclical sulfur compounds. The sulfur in cysteine (—C—SH) may be reduced to hydrogen sulfide by active hydrogen in the absence of another enzyme or catalyst but cystine (—C—S—C—) is not so reduced under ordinary conditions of temperature, pressure and pH.

"Substrate" may be defined as the substance or compound acted upon, i. e., the substance which accepts hydrogen and is reduced, a substance from which hydrogen may be produced by hydrogen-producing bacteria, or a substance from which hydrogenase may be produced by hydrogenase-producing bacteria.

Generally speaking, the substance to be treated with hydrogen is contacted with hydrogen from any suitable source in the presence of microbiological catalysts capable of activating the substrate and rendering the hydrogen sufficiently active to react with and effect the reduction or hydrogenation of selected compounds of the system. One type of microorganism or the enzyme complex therefrom may perform both functions on different types, each capable of performing different functions, may be used. In some cases, hydrogenase alone is sufficient as previously described.

Hydrogenase, an enzyme believed to be an iron porphyrin-protein complex, is an example of a microbiological catalyst capable of activating hydrogen and can be utilized directly as an enzyme or be produced in and from the substance being processed as by hydrogenase-producing bacteria in the presence of a suitable nutrient medium. In some cases, another enzyme or catalyst is not required. In other cases, other microorganisms must be used as substrate activators. Such an enzyme or combinations thereof are capable of catalyzing a number of different types of reactions, some of which are hereinafter discussed.

When materials are being processed wherein it is desired to react the hydrogen with elements or radicals other than oxygen, the process is preferably carried out in the presence of an oxygen-free gas and in the absence of any oxygen-containing compounds from which the oxygen might be liberated to combine with hydrogen. Such combination of hydrogen and oxygen would represent a loss of hydrogen.

While the action of the catalyst such as hydrogenase is not entirely understood, it is believed that it functions to catalyze the conversion of the molecular hydrogen, originally charged to or generated in the substance to an active form of hydrogen, termed by some "active" or nascent hydrogen, which finds an acceptor in the compounds to be hydrogenated. In the removal of sulfur complexes from crude petroleum, the active hydrogen apparently combines with the sulfur of the sulfur complexes to form mercaptans and/or hydrogen sulfide which are readily removed and collected. The reactions are believed to be generally as follows:

$$R—S—R'+H_2 \rightarrow RSH+R'H \text{ (or } RH+R'SH)$$
$$R—S—R'+2H_2 \rightarrow RH+H_2S+R'H$$

Any hydrogen collected with the gaseous products of the reaction may be processed by the removal of such products and recycled. While the hydrogen in some processes such as in the treatment of crude petroleum may tend to combine with and remove other elements, the loss thereby is relatively slight and the product is usually of a nature that can be collected and marketed or otherwise utilized to advantage. For instance, any carbon removed from crude petroleum by hydrogen is usually recoverable as methane which can be considered a valuable by-product.

The process can be carried out in either a batch or a continuous method as explained hereinafter and can be controlled by varying the partial pressure of the hydrogen, temperature, pH, and like factors. The partial pressure of the hydrogen is preferably maintained within a range of 25% to about 100% of the total pressure but is capable of being varied to control the rate of the reaction. The temperatures and pH of the reactants are preferably maintained under optimum conditions as regards the action of the hydrogen and the functioning of the hydrogenase-producing bacteria and hydrogen-producing bacteria if such be used. The temperature is preferably maintained between 20° and 75° C., depending upon the types of microorganisms being used, and the pH between 6.5 and 7.5. The hydrogenase produced by some varieties of *Desulfovibrio* has an optimum temperature between 60° and 65° C. Others function best between 20° and 25° C. Optimum temperatures may be raised appreciably by hydrostatic pressure. The total pressure can be raised from atmospheric to high pressure in the order of 300 atmospheres. Preferably, the pressure, because of engineering and economic considerations, is kept below 10 atmospheres.

Generally, the reaction is exothermic and some cooling may be required to remove the generated heat.

When hydrogenase is used alone in the absence of any other enzyme or catalyst, a buffer in the form of a mineral salt solution having a pH in the range of 6 to 8 is used, the exact pH depending upon the particular hydrogenase complex.

If microorganisms be employed to generate the hydrogenase or the hydrogen or both in the actual processing, a suitable nutrient medium is employed. In some instances where both types of bacteria may be used and the bacteria are maintained physically separated from one another, different nutrient mediums best suited to the bacteria are employed. Since such mediums are of conventional compositions and well known to the art, further explanation thereof is not required. It is believed sufficient to state that each particular organism has its own specific requirements for a nutrient medium. This fact is well known to the art and examples thereof are available in the literature.

Hydrogen for the process can be generated elsewhere and charged directly to the substance to be treated or can be generated directly in the substance being processed as by microbial action. Some substrates from which hydrogen can

be produced by different bacteria are listed in the following table:

Substrate	Microorganism
Glucose	*Aerobacter aerogenes.*
	Aerobacter cloacae.
	Bacillus acetoethylicus.
	Bacillus polymyxa.
	Clostridium acetobutylicum.
	Clostridium butyricum.
	Clostridium butyricum.
	Clostridium botulinum.
	Clostridium oedematis-maligni.
	Clostridium sporogenes.
	Clostridium tetani.
	Clostridium tetanomorphum.
	Clostridium thermosaccharolyticum.
	Clostridium welchii.
	Escherichia coli.
	Proteus mirabilis.
	Proteus vulgaris.
	Sarcina maxima.
	Sarcina ventriculata.
	Serratia marcescens.
	Algae such as Scenedesmus.
Cellulose	*Bacillus polymyxa.*
	Clostridium hydrogenicus.
	Clostridium cellulolytique.
	Clostridium cellulosolvens.
	Clostridium dissolvens.
	Clostridium fossicularum.
	Clostridium werni.
Glycerol	*Aerobacter aerogenes.*
	Clostridium butylicum.
	Clostridium sporogenes.
	Clostridium tetanomorphum.
Mannitol	*Aerobacter aerogenes.*
	Escherichia coli.
Acetic acid	*Aerobacter indologenes.*
Citric acid	Do.
Succinic acid	Do.
Amygdalin	*Clostridium acetobutylicum.*
	Clostridium butylicum.
	Clostridium butyricum.
Cellobiose	*Clostridium acetobutylicum.*
	Clostridium butylicum.
	Clostridium butyricum.
Galactose	*Clostridium acetobutylicum.*
	Clostridium butyricum.
Fructose	*Clostridium acetobutylicum.*
	Clostridium butyricum.
	Clostridium botulinum.
	Clostridium oedematis-maligni.
	Escherichia coli.
	Sarcina maxima.
	Sarcina ventriculata.
Lactose	*Clostridium acetobutylicum.*
	Clostridium butyricum.
	Clostridium sporogenes.
	Clostridium welchii.
Maltose	*Clostridium acetobutylicum.*
	Clostridium butylicum.
	Clostridium butyricum.
	Clostridium botulinum.
	Clostridium oedematis-maligni.
	Clostridium tetanomorphum.
	Escherichia coli.
Mannose	*Clostridium acetobutylicum.*
	Clostridium butyricum.
	Clostridium botulinum.
	Clostridium oedematis-maligni.
	Escherichia coli.
Salicin	*Clostridium acetobutylicum.*
	Clostridium butyricum.
	Clostridium botulinum.
	Clostridium oedematis-maligni.
	Clostridium sporogenes.
	Escherichia coli.
Sucrose	*Clostridium acetobutylicum.*
	Clostridium butylicum.
	Clostridium butyricum.
	Clostridium botulinum.
	Clostridium oedematis-maligni.
	Clostridium sporogenes.
	Escherichia coli.
Arabinose	*Clostridium acetobutylicum.*
	Clostridium butylicum.
	Clostridium butyricum.
	Escherichia coli.
Gluconic acid	*Clostridium acetobutylicum.*
Mannitol	Do.
Aesculin	*Clostridium butylicum.*
Dextrin	Do.
Dimethyl glucoside	Do.
Inositol	Do.
Inulin	*Clostridium butylicum.*
	Clostridium sporogenes.
Melibiose	*Clostridium butylicum.*
Raffinose	*Clostridium butylicum.*
	Pseudomonas rathonis.
Rhamnose	*Clostridium butylicum.*
Starch	*Clostridium butylicum.*
	Clostridium sporogenes.
	Algae such as Scenedesmus.
Pyruvic acid	*Clostridium butylicum.*
	Clostridium butyricum.
	Clostridium botulinum.
	Clostridium tetanomorphum.

Substrate	Microorganism
Trehalose	*Clostridium butylicum.*
Xylose	*Clostridium butylicum.*
	Clostridium thermosaccharolyticum.
	Clostridium butyricum.
Acetic acid	*Clostridium butyricum.*
Butyric acid	Do.
Lactic acid	Do.
Valeric acid	Do.
Pectin	Do.
Peptone	*Clostridium botulinum.*
	Clostridium sporogenes.
Sorbitol	*Clostridium oedematis-maligni.*
	Escherichia coli.
Aspartic acid	*Clostridium tetani.*
	Clostridium tetanomorphum.
Glutamic acid	*Clostridium tetani.*
	Clostridium tetanomorphum.
Serine	*Clostridium tetani.*
	Clostridium tetanomorphum.
Cystine	*Clostridium tetanomorphum.*
	Proteus vulgaris.
Fumaric acid	*Clostridium tetanomorphum.*
Methionine	Do.
Histidine	Do.
Malic acid	Do.
Tyrosine	Do.
Galactose	*Escherichia coli.*
Formic acid	Do.
Glycolic acid	*Escherichia coli.*
	Salmonella enteritidis.

Hydrogenase can be used to catalyze the reaction of hydrogen with a number of substances including fumarates, malates, formaldehyde, methyl alcohol, carbon monoxide, carbon dioxide, carbonates, nitrates, sulfates, sulfites, thiosulfates and a number of other compounds, thus enabling the method of this invention to be used in a number of different processes for treating various substances. A preferred hydrogenase-producing organism for reducing complex sulfur compounds in crude oil is *Desulfovibrio desulfuricans* or related species although others may be used.

Hydrogenase produced by methane bacteria can be used to catalyze the reaction of molecular hydrogen with carbon monoxide and carbon dioxide. It can also be used to catalyze the reaction of molecular hydrogen with formic acid, formaldehyde, and methyl alcohol, the reactions being believed to be generally as follows:

$$CO+3H_2 \rightarrow CH_4+H_2O$$
$$CO_2+4H_2 \rightarrow CH_4+2H_2O$$
$$HCOOH+3H_2 \rightarrow CH_4+2H_2O$$
$$HCHO+2H_2 \rightarrow CH_4+H_2O$$
$$CH_3OH+H_2 \rightarrow CH_4+H_2O$$

The production of methane by the reduction of carbon dioxide with hydrogen can also be catalyzed by *Methanobacterium omelianskii, Methanosarcina methanica, Methanosarcina barkerii* and *Methanobacterium formicicum.* The two last-mentioned species produce hydrogenase and other enzymes and catalysts capable of reducing carbon monoxide to methane in the presence of hydrogen. The reduction enabled by the present process is capable of use in still further reactions such as the hydrogenation of carbon dioxide to form formic acid, suitable catalysts being the hydrogenase and other enzymes produced by *Escherichia coli.* While this reaction is normally reversible, an increase in the partial pressure of the carbon dioxide appears to cause the reaction to proceed to completion. The yield may be increased by raising the partial pressure of the hydrogen. The reaction is believed to be generally as follows:

$$CO_2+H_2 \rightarrow HCOOH$$

In the presence of hydrogen, hydrogenase and other enzymes or catalysts produced by the fol-

lowing microorganisms may be used to effect the reactions indicated:

Clostridium aceticum and Clostridium thermoaceticum.—

$$2CO_2+4H_2\rightarrow CH_3COOH+2H_2O$$
(acetic acid)

Butyribacterium rettgeri.—

$$2CH_3COOH+4H\rightarrow CH_3CH_2CH_2COOH+2H_2O$$
(butyric acid)

Hydrogenomonas pantotropha.—

$$CO_2+2H_2\rightarrow(HCHO)\rightarrow \text{carbohydrates, etc.}+H_2O$$

Escherichia formica.—Reduction of fumarate and methylene blue by molecular hydrogen.

Proteus vulgaris.—Hydrogenation of fumarate, maleate, malate, carbonates and oxygen.

Clostridium sporogenes catalyzes hydrogenation of a number of organic compounds including: acetaldehyde, acetic acid, acetyl methyl carbinol, acrolein, arginine, aspartic acid, cysteine, cystine, diacetyl, glutaric acid, glyceric aldehyde, glycine, glyoxal, hydroxylamine, malonic acid, methionine, ornithine, oxalic acid, proline, tryptophane and tyrosine.

Clostridium welchii, Bacillus hydrogenes, Hydrogenomonas minor, Escherichia formica and *Escherichia coli* catalyze the hydrogenation of nitrates, nitrites, or other reducible nitrogen compounds:

$$HNO_3+4H_2\rightarrow NH_4OH+2H_2O$$
$$HNO_2+3H_2\rightarrow NH_4OH+H_2O$$
$$NH_2OH+H_2\rightarrow NH_4OH$$

Certain species of *Desulfovibrio, Sporovibrio,* and other sulfate reducers catalyze reduction of sulfates by hydrogen:

$$SO_4^{=}+4H_2\rightarrow S^{=}+4H_2O$$

Some species also catalyze reduction of sulfites and thiosulfates to hydrogen sulfide.

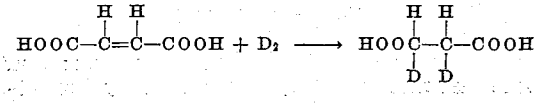
Catalysts for the reduction of sulfur-containing compounds can be isolated also from marine sediments, the cultures apparently being best in an aqueous medium of the composition of sea water enriched by:

0.02% calcium lactate
0.02% ascorbic acid
0.02% ferrous ammonium phosphate ($FeNH_4PO_4$)
0.05% sodium bicarbonate
0.5% potassium sulfate

Except when oxygen is being intentionally reacted with hydrogen, it is desired that only anaerobic species be used to avoid the presence of oxygen and consequent undesired consumption of hydrogen.

In lieu of hydrogen from usual commercial sources, hydrogen can be secured by the bacterial fermentation of organic matter such as sewage, agricultural waste products, offal, etc., or by the alpha radiation of compounds containing hydrogen.

Deuterium or heavy hydrogen (D_2) can be substituted for hydrogen in some reducing reactions, for example, *Escherichia coli* is capable of catalyzing the reaction of hydrogen with fumaric acid to form dideuterosuccinic acid:



Some other examples of reactions are:

$$CO_2+4D_2\rightarrow CD_4+2D_2O$$
$$CO_2+D_2\rightarrow DCOOD$$

Thus the present method provides a relatively simple and inexpensive process for the manufacturing of special chemicals wherein deuterium is employed.

As an example of an application of the process of the invention to a batch method, attention is directed to Fig. 1 wherein a reaction chamber **10** in a form resembling a storage tank is shown. Chamber **10** is provided with a valve-controlled inlet **12** for the material to be processed and a valve-controlled outlet **13** for the processed material. By way of illustration, it is assumed that the substance **11** being processed is crude petroleum hydrocarbons and it is desired to remove or reduce the complex sulfur compounds therein. It will be noted that both inlet **12** and outlet **13** are disposed somewhat above the bottom of the storage tank. A layer **14** of nutrient medium containing both hydrogen-producing bacteria, a hydrogen-producing substrate, and bacteria which produce hydrogenase and other enzymes and catalysts, is maintained in the bottom of the tank, the medium with its contained bacteria being charged through a valve-controlled inlet **15** and discharged through a valve-controlled outlet **16**. By reason of the difference in the specific gravities of the material being processed such as crude oil and the nutrient medium which is principally water, there is no substantial mixing of the two liquids. Obviously this method of practicing the invention is not desirable where the material being processed and the nutrient medium are of such specific gravities that they tend to mix, unless the two can be separated later without too much trouble. Since inlet **12** and outlet **13** are disposed above the layer of medium, the material being processed can be charged and discharged without disturbing the layer of medium.

In practicing the process in this apparatus, the material to be processed is charged through inlet **12**. The nutrient medium with its bacteria content is charged through inlet **15** and maintained as the bottom layer. It functions to produce the necessary hydrogen and microbiological catalysts. Connections **15** and **16** enable the medium to be circulated or to be replaced as desired or found necessary. It is to be understood that hydrogen from some other source may be charged to chamber **10** in which case the nutrient medium in layer **14** will contain hydrogenase and other enzymes and catalysts or bacteria which produce same. When hydrogen is charged directly as through a manifold **17**, it is distributed preferably by porous diffusers **18**, usually of ceramic material, to insure optimum distribution. If desirable, hydrogenase and other activating enzymes and catalysts can be charged directly in a buffered mineral salts solution.

Oil **11** remains in the tank until its processing is complete whereupon it is withdrawn through outlet **13** and a new batch of oil charged through inlet **12**. Preferably a new charge of hydrogenase or hydrogenase-producing bacteria, other enzymes or catalysts, and nutrient is charged with each succeeding batch of oil.

The resultant gaseous products of reaction are removed through an outlet **19**.

Fig. 2 shows a modification wherein a tank **10***a* is modified by the provision of a central par-

tition 21 of relatively impermeable material, such as that of the tank, and of sufficient height to rise substantially above layers 14a and 14b of nutrient media. In this case, layer 14a of nutrient medium is charged with only one of the bacteria such as the hydrogen-producing bacteria and a hydrogen-producing substrate, the layer 14b being charged with microbiological catalysts or bacteria capable of producing such catalysts. This arrangement insures against the bacteria contacting one another in a manner that might be detrimental to one or the other since it is recognized that in some mixtures of bacteria there is a tendency for only one to survive and dominate, the other species gradually disappearing. Partition 21 is arranged to extend completely across the bottom of the tank as shown in Fig. 3. It is to be understood that the number of partitions may be increased and placed in different positions so that a multiplicity of separate nutrient-containing cells can be formed in any desired design.

The operation of this modification is the same as that of Fig. 1 with the added advantage that the bacteria are maintained separate from one another but in contact with the substance being processed. The valved connections shown permit of charging and discharging the substance being treated and the nutrient media, the latter being capable of being continuously recirculated, if desired.

Fig. 4 is a horizontal section like Fig. 3 illustrating a modification wherein a series of offset partitions 21a are provided in the bottom of the tank 10b in a manner similar to partition 21 of Figs. 2 and 3. Nutrient medium containing hydrogenase or any types of bacteria can be charged at 15c and discharged at 16c. The medium can be charged periodically or continuously. In the latter case, fresh medium at the interface between the medium and the oil is assured.

Figs. 5 and 6 illustrate another modified form of apparatus capable of use in the present process, a tank 22 being provided on its bottom with a series of longitudinally extending chambers 23a to 23d, inclusive, the walls of such chambers being sufficiently porous to permit the passage of hydrogen and microbiological catalysts therethrough. It is to be understood that chambers 23 can be formed in any desired number and in any shape, dependent upon the desired distribution of the reactants and the catalyst.

Preferably the hydrogen-producing bacteria is charged through manifold 24 to chambers 23b and 23d and removed through manifold 25. The catalysts or catalyst-producing bacteria are charged through manifold 26 to chambers 23a and 23c and removed through outlet manifold 27. Thus the two types of organisms are maintained entirely separate from one another while their products are permitted to circulate freely through the material being processed in the tank. The walls of chambers 23a to 23d, inclusive, are preferably formed of unglazed porcelain or sintered glass, the pores therein being of sufficient size to retain the bacteria and a major portion of the nutrient medium while allowing the escape of hydrogen and the catalyst. While some nutrient medium and bacteria may pass through the walls of the chambers and mix with the substance being treated, no substantial harm is done since the medium is usually of a much higher specific gravity than the substance being processed and will tend to collect in the bottom of the tank from which it can be drained. The substance being treated is charged through inlet 28 and removed, after processing, through outlet 29. The gaseous products are removed through an outlet 31.

In all the batch methods previously described, it is desirable to agitate the reactants continuously as by a propeller or other well known means or by proper distribution of the hydrogen when the latter be charged directly as a gas.

As an illustration of an application of the process of this invention in a continuous method, attention is directed to Fig. 7 of the drawing wherein a contact tower 32 of relatively simple design is shown. Tower 32 is preferably of the zonalized type in that it is divided into zones by a number of plates 33, the several zones 34 being connected by bubble caps 35 and overflow pipes 36 in conventional manner. Tower 32 is provided with an inlet 37 for the material to be processed and an outlet 38 for the processed material. The microbiological catalysts are charged through an inlet 39 and removed at 41, the lower portion of the tower at 42 serving as a settling or separating chamber. The reactant gas such as hydrogen is charged through a diffusion plate 43, preferably of porous ceramic material, to insure the gas being evenly distributed in the form of very fine globules. Any unused hydrogen is discharged through an outlet 44 with the gaseous products of the reaction, the hydrogen being subsequently recovered and recycled to the tower. If desired, outlets may be provided at different levels on the tower to remove the product material at different stages during its processing.

Assuming that a hydrocarbon such as crude petroleum containing complex sulfur compounds is to be processed, the crude is charged through inlet 37 to tower 12, and passed upwardly from zone to zone through bubble caps 35. Hydrogen, preferably of a purity of 25% to about 100% is charged through distributor 43 and intimately mixed with the crude in its passage through the tower. Hydrogen of high purity can be used if a little CO_2 is present and the diluting gas is relatively inert, such as nitrogen. The small amount of CO_2 is desirable since heterotropic bacteria require a small amount of CO_2 for their growth and reproduction. After they begin to multiply and respire, they produce their own CO_2 by the oxidation of carbon compounds. Autotrophic bacteria obtain their carbon for the synthesis of all substance from CO_2. The necessary CO_2 may be provided by carbonates or bicarbonates in solution in accordance with well known dissociation equilibria. The microbiological catalysts, in this case a hydrogenase-producing bacteria or hydrogenase and other activating enzymes and catalysts with their accompanying medium, are charged to the tower through inlet 39, a maximum level thereof being maintained on each plate by the several overflow pipes 36. The medium and the catalyst eventually pass into zone 42 and out of outlet 41.

It will be noted that the catalyst, the substance being processed, and the hydrogen are brought into intimate contact with one another in the tower, the processed material eventually being discharged through outlet 38.

It is desirable, as with the other apparatus disclosed herein that, before the process is initiated the space at the top of the tower be filled with hydrogen or some relatively inert gas since gas

containing oxygen in this space would tend to dissolve in the liquid and tend to react with the hydrogen in the presence of certain catalysts to form water.

While the invention has been principally discussed herein with reference to the purification of substances, it has an equally important position in the preparation of valuable products such as methane, various organic acids, etc. In practically every case of purification or hydrogenation, the gaseous by-products are of value.

It is to be noted that the process herein disclosed is relatively simple and inexpensive to operate, particularly when the hydrogen is obtained by bacterial action on a relatively cheap substrate. The process requires a minimum of attention and is of a type that its rate can be varied over a wide range. A particularly valuable advantage is achieved by reason of the fact that any hydrogen sulfide produced does not, in most cases, poison such catalysts as are herein disclosed. For instance, hydrogenase, in most reactions, functions best in the presence of hydrogen sulfide.

The term "microorganism" as used herein, is intended to include molds, fungi, and bacteria as well as the microbiological enzymes and other products produced thereby.

Obviously many modifications and variations of the invention as above set forth may be made without departing from the spirit and scope thereof, and therefore only such limitations should be imposed as are indicated in the appended claims.

I claim:

1. Apparatus for effecting the reaction between a liquid material and hydrogen in the presence of enzymatic catalysts comprising a reaction chamber, at least two physically-separated tank-like compartments in the bottom of said chamber arranged to contain liquid microbiological nutrient medium of greater density than the liquid to be processed, the medium in one of said compartments being charged with an enzymatic catalyst and the medium in another compartment being charged with a hydrogen-producing bacteria, a fluid connection to charge liquid to be treated to said reaction chamber in a manner to contact the medium in said compartments, and a fluid connection to remove resultant products from said reaction chamber.

2. The apparatus of claim 1 wherein fluid connections are provided to each compartment to charge the medium therein independently of the medium in another compartment.

3. Apparatus for effecting reaction between a liquid and a gas in the presence of enzymatic catalysts comprising a reaction chamber, means to charge liquid thereto, means to charge gas thereto, means to remove resultant products therefrom, and a closed chamber within said reaction chamber for containing catalyst-producing bacteria and a nutrient medium therefor, the walls of said chamber being sufficiently permeable to enable the passage of the generated catalyst therethrough without permitting escape of substantial amounts of bacteria.

4. Apparatus for effecting reaction between a liquid material and microbiologically-produced hydrogen in the presence of microbiological catalysts comprising a reaction chamber, a fluid connection to charge said liquid material and catalyst thereto, and a closed chamber within said reaction chamber for containing hydrogen-producing microorganisms and nutrient medium therefor, the walls of said chamber being sufficiently permeable to enable the passage of produced hydrogen therethrough without permitting escape of substantial amounts of bacteria.

5. A method of removing sulfur from petroleum hydrocarbons containing relatively complex sulfur compounds which comprises subjecting said hydrocarbons to contact with a substantial amount of hydrogen in the presence of hydrogenase-producing microorganisms selected from the group consisting of *Desulfovibrio desulfuricans* and *Sporovibrio*, effecting said contact in the presence of a nutrient medium for said microorganisms whereby sulfur is split from said complex compounds in the form of gaseous products and removing said gaseous products therefrom.

6. A method of removing sulfur from petroleum hydrocarbons containing relatively complex sulfur compounds which comprises subjecting said hydrocarbons to contact with a substantial amount of hydrogen in the presence of hydrogenase-producing microorganisms selected from the group consisting of *Desulfovibrio desulfuricans* and *Sporovibrio*, producing said hydrogen in situ by the reaction of *Clostridium* microorganisms on carbohydrates, said contact resulting in the splitting of sulfur from said complex compounds in the form of gaseous products and removing said gaseous products from said reaction.

CLAUDE E. ZoBELL.

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2,742,398

METHOD OF REMOVING DEPOSITS OF WAX AND LIKE MATERIALS

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No Drawing. Application June 9, 1951, Serial No. 230,853

5 Claims. (Cl. 195—3)

This invention relates to the dissolution and removal of waxes such as paraffin wax and like deposits from oil wells, pipe lines for conveying hydrocarbons, and similar locations where such waxes tend to collect and either halt or materially impede circulation.

This application is a continuation-in-part of my application Serial No. 21,307, filed April 15, 1948, now abandoned.

The deposition and collection of waxes such as paraffin wax in pipe lines, oil wells and the like has been a material problem in the petroleum industry. Such deposits are most always in inaccessible locations or in such forms as very small pore plugs, the latter being prevalent in oil wells. Consequently the deposits are both difficult to reach and practically impossible to remove. While it is known that certain microorganisms will attack paraffin and effect its decomposition into readily removable products, such organisms are effective only under certain optimum conditions which are obviously impossible to secure and maintain in an oil well that may pass through strata of widely different compositions and temperatures or in a pipe line that may extend cross-country for many miles. For this and other reasons, their use in pipe lines, oil wells, etc. has been considered impractical.

An object of this invention is to provide a novel process wherein such microorganisms or enzymes thereof can be efficiently utilized in removing paraffin wax from pipe lines, oil wells and the like and inhibit further depositions of such wax therein.

Another object of the invention is the provision of a novel process for removing and inhibiting the formation of such paraffin deposits wherein the process, once initiated, is capable of continuing with a minimum of attention and little expense.

In its broader aspect, the present invention involves the simultaneous use of a number of different species of paraffin-consuming microorganisms or enzymes thereof, each species being capable of consuming paraffin under different conditions as to pH, temperature and other factors. More specifically, the invention involves the use of such microorganisms or enzymes thereof in combination with wax particles, such as flaked or granulated paraffin, whereby the ability of the microorganisms or enzymes thereof to adhere to the wax-coated surfaces of the pipe line or oil well is increased, the transplantation of the microorganisms being improved and the period of incubation, required for the microorganisms to manifest their wax-dissolving propensities, reduced. The invention contemplates the use of such microorganisms or their enzymes in the fluid being conveyed or otherwise handled, in the form of slugs or charges that may be fed periodically through a pipe line, or in the form of static charges to a pipe line when temporarily out of use.

As already stated, it is known that certain individual species of microorganisms and the enzymes thereof will assimilate paraffin wax. Typical of such microorganisms are species of Actinomyces, Nocardia, Mycobacterium,

Pseudomonas, Micromonospora, Corynebacterium, Aspergillus, Penicillium, and Torula.

A number of such organisms are listed in the following table in the order in which they are considered most effective:

Nocardia (Proactinomyces) paraffinae
Nocardia corallina (Proactinomyces agrestis)
Mycobacterium rubrum
Mycobacterium hyalinum
Aspergillus versicolor
Aspergillus oryzae
Aspergillus flavus
Micrococcus paraffinae
Botrytis cinerea

All the microorganisms in the foregoing table, as well as other species of these genera, assimilate paraffin wax best in the presence of free oxygen. A good many, however, will utilize paraffin slowly in the absence of free oxygen in media which provide oxygen from such compounds as nitrates, nitrites, carbohydrates, alcohols, etc.

Other microorganisms which are facultative aerobes i. e. grow best in the presence of free oxygen but will grow in appropriate medium in the absence of free oxygen, include *Nocardia minima, Nocardia rubropertincta* and *Nocardia veridis.* Microaerophilic organisms, which require at least a trace of free oxygen for their growth, may also be used in the invention.

Examples of a microorganism capable of assimilating paraffin in the total absence of free oxygen are species of Desulfovibrio, Sporovibrio and other sulfate reducers. *Desulfovibrio hydrocarbonoclasticus* and *Desulfovibrio halohydrocarbonoclasticus* are typical species of Desulfovibrio. Another such microorganism is *Nocardia salmonicolor.* Such sulfate reducing bacteria are preferred because of their ability to assimilate paraffin wax in the absence of free oxygen which is the usual circumstance in a pipe line or oil well. Moreover, the various varieties thereof are active over a total temperature range from near 0° to 85° C. and higher, although no one strain is active throughout this range. One strain may be limited to a maximum of 25° C.; another may exhibit maximum activity between 32° and 45° C. While the range of any one strain or variety may be narrow, a selected combination of the strains covers an exceedingly wide range. In addition, it is to be understood that most strains are susceptible to training or acclimatization to different temperatures. The microorganism can also be acclimatized to environmental factors such as salinity and osmotic pressure.

Typical microorganism with their operating temperature range, the preferred range being shown in brackets, are listed below:

Nocardia (Proactinomyces) paraffinae 5° to 35° C. (25° to 30° C.)
Nocardia corallina (Proactinomyces agrestis) (22° to 25° C.)
Nocardia minima (22° to 25° C.)
Nocardia rubropertincta (20° to 37° C.)
Nocardia veridis (25° to 30° C.)
Nocardia salmonicolor (35° to 40° C.)
Mycobacterium rubrum 20° to 58° C. (35° to 40° C.)
Mycobacterium hyalinum 25° to 45° C. (25° to 30° C.)
Aspergillus versicolor 25° to 35° C.
Aspergillus oryzae 25° to 35° C.
Aspergillus flavus 25° to 35° C.
Micrococcus paraffinae 26° to 37° C.
Botrytis cinerea 30° to 35° C.

The temperature range is not the only factor to be considered in selecting or developing the cultures to be used.

ther environmental factors must also be considered in-
uding salinity, oxygen tension, the types of hydrocar-
ons present, the possibilities of iron corrosion or biofoul-
ıg, the material from which the conduit or well elements
re made, etc. Obviously it would not be desirable to use
n acid-producing strain in a concrete conduit or anaerobic
rains in the presence of free oxygen. All of the above
re obvious factors calling for culture selections equally as
bvious, such selections being well within the skill of the
ıicrobiologist.

Assuming that the conditions as to the above factors
ı a given pipe line are known and that a selection of
trains has been made to meet those conditions, the strains
re dispersed in a suitable nutrient medium, usually of the
nineral salt type. As is well known to the art, the compo-
itions of such nutrients are selected in accordance with
he requirements of the strain or strains used.

In the case of a pipe line, the inoculated medium may
›e charged directly into the line while the oil or other
luid is being conveyed thereby. The microorganisms are
listributed throughout the line and brought into contact
with the wax deposits. They function to dissolve and ef-
ect the removal of those deposits and tend to cling to
he walls of the pipe line in a manner to be readily avail-
able for any new deposits of wax.

As a specific example of such a method, an inoculated
microbiological nutrient medium can be prepared by in-
oculating a typical nutrient medium of the following com-
position with a number of different varieties of Desulfo-
vibrio. Sporovibrio and other sulfate reducers such as
*Desulfovibrio hydrocarbonoclasticus, Desulfovibrio halo-
hydrocarbonoclasticus*, varieties thereof being selected as
will be active over substantially the total temperature
range to which the pipe line is subject. Other microorgan-
isms such as *Nocardia salmonicolor, Nocardia minima,
Nocardia rubropertincta* and *Nocardia veridis* may also
be added.

NaCl	grams	60
$Ca(C_3H_5O_3)_2$	do	5.0
$CaCO_3$	do	40.0
$CaSo_4.2H_2O$	do	50.0
KH_2PO_4	do	0.2
$FeSO_4.7H_2O$	do	0.1
$(NH_4)_2HPO_4$	do	0.1
Sea water	ml	1000

In the treatment of a pipe line through which oil is being
conveyed, the inoculated medium can be metered into the
oil at the point of entry or it can be metered into the line
at some intermediate point. The quantity of inoculated
medium may be as much as from 5 to 10% of the oil, it
being understood that the medium will be diluted by the
oil. By reason of certain of the bacteria being thigmotac-
tic whereby they fix themselves to the internal surface of
the pipe line as hereinafter explained, the dilution prob-
lem is not too great a disadvantage.

As an example of treating an oil well, the same inocu-
lated medium as described above is charged down the cas-
ing at a rate of 5 to 6 gallons per day with the well being
produced through the tubing. A lubricator can be used
for this purpose, the medium being charged into the an-
nulus defined by the tubing and casing. The charging
may be continuous or by batch. When the well is being
pumped off, the continuous method is preferred.

However in such cases, the concentration of the micro-
organisms or their enzymes is reduced. Hence it is pre-
ferred to charge the inoculated medium as an entity into
the pipe line between charges of the fluid being conveyed
thereby. The medium then moves as a substantially solid
slug through the line, the microorganisms being brought
into contact with the paraffin deposits.

In this method of treatment, the oil fed into the pipe line
is interrupted, and inoculated medium of the above-de-
scribed type is charged into the line in a quantity to fill
100 to 200 feet of the line. The feed of oil is resumed

whereupon the inoculated medium moves as a substantial-
ly solid slug through the line, the thigmotactic bacteria
adhereing to the surface thereof. At some convenient
valving point along the line or at the terminus thereof,
the flow of oil from the line is shut off and the slug of me-
dium diverted and removed from the line. The approach
of the slug to such a valving point or terminus can easily
be determined by charging the slug with a small amount
of a radioactive substance whereby the approach of the
slug and its passage at any point can be determined by a
Geiger-Mueller detector or the equivalent outside the pipe
line. A second preferred method may be used when it is
possible to shut off the pipe line temporarily or the pipe
line is in intermittent use. In this case, the inoculated
medium is charged to the empty line as between two valve
controlled points and permitted to remain therein until
it is desired to put the line in use again. The charge is
then withdrawn and stored for use again since it is ca-
pable ordinarily of such reuse. If necessary, the nutrient
constituents may be replenished and fresh cultures added.

To illustrate this particular method, *Nocardia (Pro-
actinomyces) paraffinnae, Nocardia corallina (Proacti-
nomyces agrestis), Mycobacterium rubrum, Mycobacteri-
um hyalinum, Aspergillus oryzae, Micrococcus paraffinae
and Botrytis cinerea* are charged to a nutrient medium of
the following composition, the bacteria selected being of
types that will be effective over the temperature range en-
countered.

Distilled water	ml	1000
$NaH_2PO_4.H_2O$	gram	1
K_2HPO_4	do	2
NH_4NO_3	do	2
$MgCl_2.6H_2O$	do	0.25
$MnCl_2.4H_2O$	do	0.01
$CaCO_3$	do	0.01
$FeCl_2$	do	0.01

The addition of certain ions such as copper, mercury, zinc,
bismuth and iodine to the medium in trace amounts may
stimulate the activity of the microorganisms.

Assuming the pipe line is shut off and substantially
drained between two valved points, the inoculated medium
is charged to the empty section in a volume to substantial-
ly fill the section and air or any oxygen-containing medi-
um passed through the line from one shut-off point to the
other. This is continued as long as is necessary to clean
the line or as long as the line can be held inactive as re-
spects the transport of oil. The inoculated medium is
then withdrawn and stored for reuse or discarded as de-
sired. Since some of the bacteria such as *Proactinomyces
paraffinae* are thigmotactic, they will be usually retained
in the line in substantial quantity to continue their me-
tabolism.

Similar methods can be practiced in the cleaning of oil
wells, the medium being charged to the well and with-
drawn with the oil. Or the well may be charged with the
medium and closed until the well is cleaned. In the case
of wells being dug, the medium may be mixed with the
drilling mud and distributed throughout the well.

So-called "attachment or stalked bacteria", more re-
cently termed "thigmotactic bacteria" are preferred be-
cause of their ability to attach and fix themselves to sur-
faces such as the interior surfaces of a pipe line, oil tank
or oil well. *Desulfovibrio hydrocarbonoclasticus* is typ-
ical of such an anaerobe and *Proactinomyces paraffinae*
of such an aerobe.

The term "microorganism" as used herein is intended
to include bacteria and their enzymes as well as fungi.
Certain fungi such as *Botrytis cinerea*, an aerobe, is cap-
able of perforating and dissolving paraffin wax. The
vegetative hyphae of this fungus are capable of perfor-
ating paraffin wax to a depth of ½ inch or more. Such
fungi are capable of use only in the presence of free
oxygen.

In most instances, it is preferred to charge the in-

oculated medium with fine wax particles such as flaked or granulated paraffin, although the procedures outlined in the foregoing examples are satisfactory. Such particles are sufficiently fine to stay in suspension in the nutrient medium and function as mechanical carriers for the microorganisms. By reason of their waxy nature, they tend to cohere to the wax deposits in the pipe line or oil well, thus bettering the transplantation of the organism and shortening the incubation period.

It is to be understood that in some cases as in a short section of a pipe line or in some oil wells, the environment may remain substantially constant and be suited to one particular species or variety of wax-consuming microorganism. In such case, it may be necessary to use only single species or variety rather than a plurality thereof.

From the above, it is believed evident that the present process provides a simple and inexpensive method of removing wax from locations hitherto considered in accessible. Such locations are found in pipe lines, oil wells, storage tanks and other places. By reason of the tendency of the microorganisms, particularly when associated with paraffin in small particles or when thigmotactic varieties are used, to adhere to the interior surfaces of the pipe lines and other locations, the process continues to be effective in inhibiting further deposits of paraffin.

While reference has been made herein specifically to a wax such as paraffin wax, it is to be understood that the invention is applicable to deposits of any material susceptible to attack by microorganisms.

Obviously many modifications and variations of the invention, as hereinbefore set forth, may be made without departing from the spirit and scope thereof, and therefore only such limitations should be imposed as are indicated in the appended claims.

I claim:

1. A method of dissolving, removing, and inhibiting deposition of wax and like materials in pipe lines for hydrocarbons, oil wells and similar locations comprising the step of intermittently contacting said wax deposits with a microbiological nutrient medium having relatively fine particles of wax suspended therein and inoculated with a plurality of different species of wax-consuming microorganisms, each species being selected in accordance with its environmental characteristics so that one or more species is effective on said wax deposits throughout substantially the entire range of changes in environmental characteristics in said locations.

2. A method of dissolving, removing and inhibiting deposition of wax and like materials in pipe lines for hydrocarbons wherein there is normally a continuous flow of fluid therethrough comprising the steps of intermittently substituting a charge of a microbiologcial nutrient

medium for said fluid, said medium having relatively fine particles of wax suspended therein and inoculated with a plurality of different species of wax-consuming microorganisms, each species being selected in accordance with its environmental characteristics so that one or more species is effective on said wax throughout substantially the entire range of changes in environmental characteristics in said locations.

3. A method of dissolving, removing, and inhibiting deposition of wax and like materials in a pipe line for hydrocarbons comprising the steps of periodically discharging said line of hydrocarbons, charging said line with microbiological nutrient medium having relatively fine particles of wax suspended therein and inoculated with a plurality of different species of wax-consuming microorganisms, each species being selected in accordance with its environmental characteristics so that one or more species is effective on said wax throughout substantially the entire range of charges in environmental characteristics in said line, discharging said nutrient medium, and recharging said line with hydrocarbons.

4. A method of dissolving, removing, and inhibiting deposition of wax and like materials in pipe lines for hydrocarbons, oil wells and similar locations comprising the step of intermittently contacting said wax deposits with a microbiological nutrient medium inoculated with a plurality of different species of wax-consuming microorganisms, at least one of which is *Proactinomyces paraffinae*, a thigmotactic microorganism, each species being selected in accordance with its environmental characteristics so that one or more species is effective on said wax deposits throughout substantially the entire range of changes in environmental characteristics in said locations.

5. A method of dissolving, removing and inhibiting deposition of wax and like materials in pipe lines for hydrocarbons wherein there is normally a continuous flow of fluid therethrough comprising the steps of intermittently substituting a charge of a microbiological nutrient medium for said fluid, said medium being inoculated with a plurality of different species of wax-consuming microorganisms, at least one of which is *Proactinomyces paraffinae*, a thigmotactic microorganism, each species being selected in accordance with its environmental characteristics so that one or more species is effective on the wax deposits throughout substantially the entire range of changes in environmental characteristics in said locations.

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