Nov. 25, 1958 D. M. UPDEGRAFF ET AL 2,861,921

MICROBIOLOGICAL PETROLEUM PROSPECTING METHOD

Filed Jan. 25, 1954

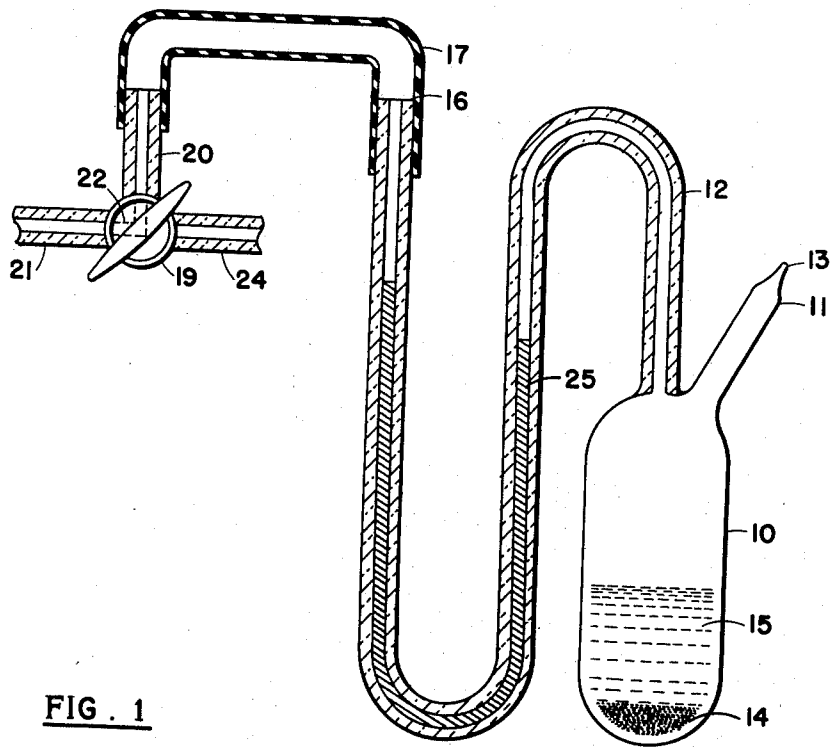


FIG. 1

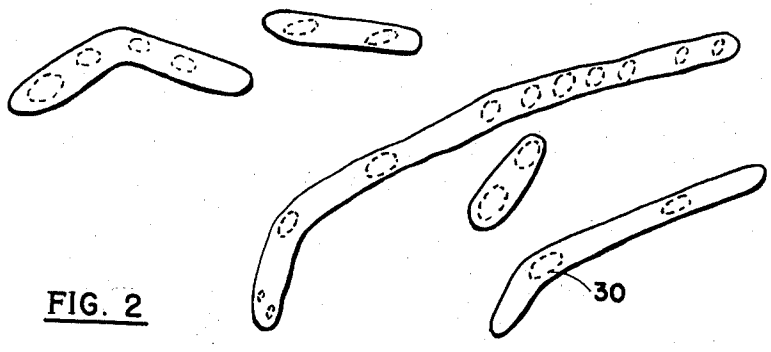


FIG. 2

DAVID M. UPDEGRAFF
HUBERT H. CHASE
INVENTORS

BY Frederick E. Desmoulin

ATTORNEY

United States Patent Office 2,861,921 Patented Nov. 25, 1958

2,861,921

MICROBIOLOGICAL PETROLEUM PROSPECTING METHOD

David M. Updegraff and Hubert H. Chase, Dallas, Tex., assignors, by mesne assignments, to Socony Mobil Oil Company, Inc., a corporation of New York

Application January 25, 1954, Serial No. 405,882

14 Claims. (Cl. 195—3)

This invention relates to prospecting for subterranean petroleum deposits and relates more particularly to a microbiological method for locating such deposits.

It has been postulated that hydrocarbons emanate from a subterranean petroleum oil or gas deposit upwardly through the earth overlying the deposit and that the presence of these hydrocarbons or their reaction products in the earth is indicative of the underlying deposit. Accordingly, various methods based on the concept of emanation or migration of hydrocarbons from the subterranean deposit have been proposed for the purpose of locating the deposit. Among such methods are those which involve the detection of hydrocarbon consuming bacteria in the earth, it being taken that such bacteria exist and thrive in the earth by virtue of their ability to utilize as nutrient, or as an energy source, the hydrocarbons emanating from the underlying petroleum deposit, and that the presence of such bacteria is thereby indicative of the presence of the underlying petroleum deposit. In one microbiological method of this sort, earth samples are collected over a prospect area and maintained in an atmosphere containing a petroleum derived hydrocarbon. Any hydrocarbon-consuming bacteria which may have been contained in the earth samples will consume the hydrocarbon and the rate at which the hydrocarbon is consumed has been believed to be a measure of the number of hydrocarbon-consuming bacteria originally in the samples. The number of bacteria thus determined is then related to the location of the sampling points to detect the presence of anomalous variations in the number of bacteria per unit amount of earth which are indicative of the presence of an underlying petroleum deposit. However, this method of measuring the number of bacteria in the earth samples is tedious, requiring a long period of time for the attainment of rates of consumption that may be measured with accuracy. Further, it is not certain that the rate of consumption is a dependable measure of the number of hydrocarbon-consuming bacteria. Additionally, hydrocarbon-consuming bacteria which have been utilizing one particular type of hydrocarbon present in the earth may be capable of adapting themselves to consume other types of hydrocarbons. Thus, since hydrocarbons other than petroleum derived hydrocarbons may be contained in the earth and thereby account for the presence of hydrocarbon-consuming bacteria, the fact that an accurately measurable rate of consumption of petroleum derived hydrocarbon is attained cannot validly be regarded as indicative of the proximity of an underlying petroleum deposit, even assuming that the rate of consumption is a dependable measure of the number of bacteria.

It is an object of this invention to provide a petroleum prospecting method. It is another object of this invention to provide a more rapid microbiological method for prospecting for petroleum oil and gas. It is another object of this invention to provide a microbiological method for prospecting for petroleum oil and gas which is free of error arising from ability of bacteria to adapt themselves to consume petroleum derived hydrocarbon during testing.

It is another object of this invention to provide a more dependable method for measuring the number of hydrocarbon-consuming bacteria in an earth sample. These and other objects of the invention will become apparent from the following description thereof.

In accordance with our invention, earth samples are collected from a prospect area, the samples are admixed with an aqueous inorganic salt medium, the mixtures are contacted with a petroleum hydrocarbon nutrient, and the time required for consumption of the hydrocarbon to begin is compared with the time required for known numbers of hydrocarbon-consuming bacteria to begin consumption of the same petroleum hydrocarbon. By this comparison, a measure of the number of hydrocarbon-consuming bacteria in each sample is made. Thereafter, the numbers of bacteria in each sample may be plotted or mapped relative to the sampling points in the prospect area to determine location of any anomalously high numbers indicative of the location of a petroleum deposit. In order to eliminate the effects arising from the ability of any bacteria contained in the earth samples, whose presence may be due to the availability of nonpetroleum hydrocarbon or other nutrient in the earth, to adapt themselves to consume the petroleum hydrocarbon employed and thereby give rise to the erroneous conclusion that the earth contained petroleum hydrocarbons indicative of a petroleum deposit, all samples wherein the time for initiation of consumption of the hydrocarbon nutrient employed is longer than the adaptation time are eliminated from consideration. We have discovered that this adaptation time is ordinarily greater than 25 days and therefore all samples which do not show initiation of hydrocarbon consumption within 25 days are regarded as being indicative of not having contained hydrocarbon migrating from a petroleum reservoir and therefore indicative of the non-proximity of a hydrocarbon reservoir.

The time required for initiation of consumption of the nutrient hydrocarbon, or lag time, is taken as a measure of the number of hydrocarbon-consuming bacteria in the earth sample. We have discovered that, in earth samples containing small numbers of bacteria which have been consuming petroleum derived hydrocarbon, the consumption of the added hydrocarbon nutrient is undetectable at first but that this undetectability of consumption is not evidentiary of the absence of bacteria significant with respect to the proximity of a petroleum reservoir. We have further discovered that, after a characteristic lag time, the bacteria in the earth samples begin to consume the added hydrocarbon nutrient and that the beginning of consumption is readily detected. Additionally, we have discovered that this lag time is inversely proportional to the logarithm of the number of bacteria in the earth sample and by comparison with lag times determined with known numbers of bacteria, the number of bacteria in each earth sample which have been consuming petroleum derived hydrocarbon may be determined with improved accuracy and dependability.

Figure 1 is a vertical cross-section view of a preferred form of apparatus for detecting the consumption of hydrocarbon nutrient by hydrocarbon-consuming bacteria in an earth sample.

Figure 2 is a diagrammatic view of typical cells of *Agrobacterium ethanicus*, a hydrocarbon-consuming bacteria.

In carrying out the invention, earth samples are collected at spaced intervals over an area to be prospected for an underlying petroleum deposit. The samples are taken from points below the surface in order to avoid samples which have been affected by weathering, contamination, or other factors rendering them not uniform or not representative of the prospect area. Generally, samples taken at depths greater than about one foot will

be satisfactory although local conditions may require that the sampling be made at greater depths. However, since the significant bacteria are aerobic, the samples must be taken at points where the earth contains sufficient oxygen to enable these bacteria to survive and therefore the samples must be taken at depths not greater than the water table. Sterile tools and containers must be employed for sampling in order to prevent contamination of the individual samples and the techniques for handling the samples thereafter should also be such as to prevent contamination.

To determine the number of significant bacteria per unit amount of earth, a portion of each sample which may be of any convenient weight, for example, about one gram, is first weighed and then admixed with a suitable amount of an aqueous inorganic salt medium. Preferably, the medium should be employed in an amount of at least about 9 times the weight of the portion of earth sample. The use of the aqueous inorganic salt medium with the portion of earth sample is essential since a homogeneous environment is required for each sample and the effect of interfering substances in the earth samples consuming or taking up the subsequently employed hydrocarbon nutrient or one or more of its non-hydrocarbon components must be minimized. Further, the aqueous salt medium furnishes the inorganic salts required for bacterial growth. Such salts include those furnishing ammonium, ferrous or ferric, calcium, magnesium, phosphate, and sulphate ions. The concentrations of the salts may vary widely although the concentrations must be below those which will have an inhibitory or poisoning effect on the bacteria. Thus, the concentrations of iron should not be greater than about 10 parts per million while the concentration of calcium may be as high as 1000 parts per million. In addition, the pH of the medium must be suitable for the growth of the significant bacteria, namely, between about 7.2 and 7.6, and adjustment of the pH of the aqueous medium may be made by addition of acid or base as required.

A suitable aqueous medium has the following composition:

Ingredient:	Parts by weight
KH_2PO_4	0.5
$(NH_4)_2SO_4$	0.5
$MgSO_4$	0.2
$CaCl_2$	0.01
$FeSO_4.7H_2O$	0.005
$MnSO_4$	0.002
$Na_2MoO_4.2H_2O$	0.002
Na_2CO_3	0.1
Distilled water	1000

The medium is prepared by dissolving the salts in the water and sterilizing by heating; for example, in an autoclave with steam at 20 pounds per square inch gage for 20 minutes. After sterilization, the pH of the medium is adjusted to a value between 7.2 and 7.6 by addition of a sterile solution of sodium hydroxide whose concentration may be about one normal.

The mixture of earth sample and aqueous inorganic salt medium is next contacted with a petroleum hydrocarbon nutrient and maintained in contact with the nutrient under temperature conditions suitable for growth of bacteria, such as 30° C., for at least such time, less than the adaptation time, that consumption of the hydrocarbon nutrient is detected. Any type of petroleum hydrocarbon nutrient is suitable provided that such petroleum hydrocarbon is one that could be present in the earth as a result of emanation from a petroleum reservoir such as methane, ethane, propane, etc. Preferably, however, we employ ethane since ethane can be present in the soil only as a result of emanation from a petroleum reservoir, the beginning of consumption can be readily detected, and the lag time measured is highly representative of the number of significant bacteria in the sample. The ethane

is employed in admixture with oxygen and nitrogen to promote growth of the bacteria and, while various concentrations of the gases may be employed, a suitable composition consists of the following, in percentages by volume:

	Percent
Ethane	40
Oxygen	18
Nitrogen	42

Contact of the sample and the aqueous medium with the nutrient petroleum hydrocarbon may be made by maintaining an atmosphere of the nutrient petroleum hydrocarbon in a suitable vessel containing the sample and aqueous medium. Where the petroleum hydrocarbon nutrient is gaseous, such as ethane, the beginning of consumption of the nutrient may be detected by noting the decrease in volume or the decrease in pressure of the gas, i. e., a change in the volume-pressure ratio, and any suitable apparatus may be employed for this purpose. A preferred type of apparatus for maintaining the sample and aqueous medium in contact with the hydrocarbon nutrient and detecting the beginning of consumption of the hydrocarbon nutrient is illustrated in Figure 1.

Referring now to Figure 1, the apparatus comprises a chamber **10** provided with a filling tube **11** and a manometer tube **12**. The apparatus may be constructed of any suitable material but glass is preferred. The filling tube **11** narrows at its end to form a tip **13** which, as shown, is closed. Prior to introduction of the earth sample **14** and the aqueous inorganic salt medium **15**, the tip **13** is open but thereafter is closed by sealing with a flame. By means of this feature, and by making the manometer tube **12** integral with the chamber **10**, the use of joints, stoppers, etc., which may give rise to leaks or require lubricants in which the hydrocarbon nutrient would dissolve with consequent error in measuring the time for consumption of the hydrocarbon nutrient to begin, is eliminated. To reuse the apparatus, the tip may be broken and after the apparatus is refilled the tip may be resealed.

For operation, the apparatus is first cleaned and sterilized. A portion of the earth sample is weighed on sterile paper and is then introduced through the open tip **13** by means of a sterile powder funnel. Thereafter, a measured amount of aqueous inorganic salt medium is introduced by means of a sterile pipette, through the open tip **13**. Alternatively, the weighed portion of the earth sample may be mixed with a measured amount of the aqueous medium and the mixture introduced by means of a pipette through the open tube **13**. Following introduction of the earth sample and the aqueous medium, the tip **13** is flame sealed.

The petroleum hydrocarbon nutrient is then contacted with the mixture in chamber **10**. This can be effected by evacuating the chamber **10** and permitting the gaseous nutrient mixture to enter the evacuated chamber. The chamber can be evacuated by connecting the open end **16** of manometer tube **12** to tubing **17** leading to line **20** on three-way valve or stop cock **19** provided with line **21** leading to a vacuum pump or other means for obtaining a low pressure. After a suitably low pressure has been obtained in chamber **10**, the valve **19** is turned so as to break the connection between lines **20** and **21** through channel **22** in the valve. Gaseous nutrient mixture is then permitted to enter the chamber by turning valve **19** so as to position the channel **22** between line **20** and line **24** leading to a source of the gaseous nutrient mixture. The pressure of the nutrient mixture should preferably be slightly above atmospheric to prevent entrance of air to the chamber **10** upon disconnecting the tubing **17** from the manometer tube **12**. The tubing **17** is disconnected from the manometer tube **12** and mercury or other liquid in which the hydrocarbon nutrient is not soluble is poured into the open end **16** of the manometer

tube. The apparatus is then maintained at a temperature of about 30° C. as by placing in an incubator, water bath, or by other suitable means. Readings of the two levels of the mercury column **25** are made frequently and the time at which the levels begin to change at a more rapid rate than the levels in a control apparatus containing sterile aqueous medium and gas mixture only, indicating the beginning of consumption of the hydrocarbon nutrient by the bacteria in the sample in chamber **10**, is noted. However, as previously mentioned, since the bacteria in the sample may adapt themselves to consume the petroleum hydrocarbon nutrient even though they may have been consuming a nonpetroleum hydrocarbon or other nutrient in the earth in place, said nutrient accounting for their presence in the earth in place, the sample is regarded as being free of bacteria significant with respect to the proximity of a petroleum reservoir if the levels of the mercury column do not change, as compared with those in the control apparatus, within 25 days.

Knowing the time required for consumption of petroleum hydrocarbon nutrient to begin, the amount of bacteria in the portion of earth sample taken is determined by comparison with the time required for consumption of identical nutrient under identical conditions where the number of bacteria is known. A known number of bacteria, as determined by direct microscopic count or other means, which have been consuming petroleum hydrocarbon are introduced into chamber **10** of an identical apparatus used for the earth samples and after sealing, evacuating, and introducing identical nutrient at identical pressure, are incubated at the same temperature, and the time required for consumption of nutrient to begin, as compared with a control, is noted. A series of these determinations are made, each with a different known number of bacteria, whereby a curve may be drawn indicating the number of bacteria for each time required for initiation of consumption of nutrient.

Any suitable species of bacteria which consume petroleum hydrocarbons may be employed for determining the time required for initiation of consumption by known numbers of bacteria. However, it is preferred for this purpose to employ bacteria of the species *Agrobacterium ethanicus*. As shown in Figure 2, *Agrobacterium ethanicus* are slender rod shaped bacteria showing granular inclusions **30** in the cells and identification of these bacteria may be assisted by reference to the drawing. They are found in the earth where ethane is present and cultures thereof for use in determining times for initiation of consumption with known numbers of bacteria may be made by taking earth samples known to contain ethane, admixing the samples with an aqueous inorganic salt medium, and incubating at about 30° C. in the presence of ethane nutrient previously described. If *Agrobacterium ethanicus* are present, the medium becomes turbid and a yellow pellicle composed of these bacteria is formed on the surface. A portion of the pellicle is streaked on a mineral agar plate and incubated at the same temperature and with the same nutrient gas employed for the earth sample. Yellow colonies of bacteria will form on the agar plate and a portion of a yellow colony is streaked on another agar plate. The second plate is similarly incubated and the yellow colonies of bacteria produced will be substantially pure cultures of *Agrobacterium ethanicus*. A known number of these bacteria may then be admixed with aqueous inorganic salt and the time required for initiation of consumption of hydrocarbon nutrient measured.

This application is a continuation-in-part of our copending application Serial No. 86,372, filed April 8, 1949, now abandoned.

Having thus described our invention, it will be understood that such description has been given by way of illustration and example only and not by way of limitation, reference for the latter purpose being had to the appended claims.

We claim:

1. A microbiological method of prospecting for a subterranean petroleum deposit comprising collecting earth samples at spaced locations over a prospect area, admixing a known portion of each of said samples with aqueous inorganic salt medium for bacteria, contacting the resulting mixture with a nutrient comprising a petroleum hydrocarbon, and determining the time required for consumption of said nutrient by bacteria in each of said earth samples to begin as a measure of the number of bacteria in each of said samples whereby the number of bacteria per unit amount of sample may be correlated with sample locations to detect anomalous variations in the number of bacteria indicative of the proximity of a subterranean petroleum deposit.

2. A microbiological method of prospecting for a subterranean petroleum deposit comprising collecting earth samples at spaced locations over a prospect area, admixing a known portion of each of said samples with aqueous inorganic salt medium for bacteria, contacting the resulting mixture with a nutrient comprising a gaseous petroleum hydrocarbon, and determining the time required for consumption of said nutrient by bacteria in each of said earth samples to begin as a measure of the number of bacteria in each of said samples whereby the number of bacteria per unit amount of sample may be correlated with sample locations to detect anomalous variations in the number of bacteria indicative of the proximity of a subterranean petroleum deposit.

3. A microbiological method of prospecting for a subterranean petroleum deposit comprising collecting earth samples at spaced locations over a prospect area and at depths at which aerobic, petroleum hydrocarbon-consuming bacteria can survive, admixing a known portion of each of said samples with aqueous inorganic salt medium for such bacteria, contacting the resulting mixture with a gaseous petroleum hydrocarbon, and determining the time, less than the adaptation time of hydrocarbon-consuming bacteria, required for consumption of said nutrient by bacteria in each of said earth samples to begin as a measure of the number of bacteria in each of said samples whereby the number of bacteria per unit amount of sample may be correlated with sample locations to detect anomalous variations in the number of bacteria indicative of the proximity of a subterranean petroleum deposit.

4. The method of claim 3 wherein the gaseous petroleum hydrocarbon is ethane.

5. In the determination of the number of hydrocarbon-consuming bacteria in an earth sample by measurement of the time required for the hydrocarbon-consuming bacteria in the earth sample to begin consumption of a hydrocarbon nutrient the method of providing a standard whereby the time required for consumption of hydrocarbon nutrient to begin is related to the number of hydrocarbon-consuming bacteria in the earth sample comprising preparing a substantially pure culture of *Agrobacterium ethanicus*, admixing separately groups of known numbers of said *Agrobacterium ethanicus* with aqueous inorganic salt medium, and measuring the time required for each of said groups of known numbers of *Agrobacterium ethanicus* to begin consumption of a hydrocarbon nutrient.

6. A microbiological method of prospecting for a subterranean petroleum deposit comprising collecting earth samples at spaced locations over a prospect area at a depth not less than one foot but not greater than the water table, admixing a known portion of each of said samples with aqueous inorganic salt medium for bacteria, said medium having a pH between 7.2 and 7.6 and containing not more than 10 parts per million of iron, contacting the resulting mixture with a nutrient comprising a light, gaseous petroleum hydrocarbon, and determining the time required for consumption of said nutrient by bacteria in each of said earth samples to begin as a

measure of the number of bacteria in each of said samples whereby the number of bacteria per unit amount of sample may be correlated with sample locations to detect anomalous variations in the number of bacteria indicative of the proximity of a subterranean petroleum deposit.

7. A microbiological method of prospecting for a subterranean petroleum deposit comprising collecting earth samples at spaced locations over a prospect area at a depth not less than one foot but not greater than the water table, admixing a known portion of each of said samples with at least about 9 parts by weight of aqueous inorganic salt medium for bacteria, said medium having a pH between 7.2 and 7.6 and containing not more than 10 parts per million of iron, contacting the resulting mixture with a nutrient comprising a light, gaseous petroleum hydrocarbon, and determining the time required for consumption of said nutrient by bacteria in each of said earth samples to begin as a measure of the number of bacteria in each of said samples whereby the number of bacteria per unit amount of sample may be correlated with sample locations to detect anomalous variations in the number of bacteria indicative of the proximity of a subterranean petroleum deposit.

8. A microbiological method of prospecting for a subterranean petroleum deposit comprising collecting earth samples at spaced locations over a prospect area at a depth not less than one foot but not greater than the water table, admixing a known portion of each of said samples with at least about 9 parts by weight of aqueous inorganic salt medium for bacteria, said medium having a pH between 7.2 and 7.6 and containing not more than 10 parts per million of iron, contacting the resulting mixture with a mixture of oxygen and a nutrient comprising a light, gaseous petroleum hydrocarbon, and determining the time required for consumption of said nutrient by bacteria in each of said earth samples to begin as a measure of the number of bacteria in each of said samples whereby the number of bacteria per unit amount of sample may be correlated with sample locations to detect anomalous variations in the number of bacteria indicative of the proximity of a subterranean petroleum deposit.

9. The method of claim 8 wherein the light, gaseous petroleum hydrocarbon is ethane.

10. A microbiological method of prospecting for a subterranean petroleum deposit comprising collecting earth samples at spaced locations over a prospect area and at a depth not less than one foot but not greater than the water table, admixing a known portion of each of said samples with aqueous inorganic salt medium for bacteria, said medium having a pH between 7.2 and 7.6 and containing not more than 10 parts per million of iron, maintaining the resulting mixture in an atmosphere of oxygen and a light, gaseous petroleum hydrocarbon for a time less than the adaptation time of hydrocarbon-consuming bacteria, and, after saturation of said medium with said atmosphere, measuring the time required for a change in the volume-pressure ratio of said atmosphere to begin as a measure of the number of bacteria in each of said samples whereby the number of bacteria per unit amount of sample may be correlated with sample locations to detect anomalous variations in the number of bacteria indicative of the proximity of a subterranean petroleum deposit.

11. The method of claim 10 wherein the light, gaseous petroleum hydrocarbon is ethane.

12. A microbiological method of prospecting for a subterranean petroleum deposit comprising collecting earth samples at spaced locations over a prospect area at a depth not less than one foot but not greater than the water table, admixing a known portion of each of said samples with at least about 9 parts by weight of aqueous inorganic salt medium for bacteria, said medium having a pH between 7.2 and 7.6 and containing not more than 10 parts per million of iron, contacting the resulting mixture with a mixture of oxygen and a light, gaseous petroleum hydrocarbon selected from the group consisting of methane, ethane, and propane, for a time less than the adaptation time of hydrocarbon-consuming bacteria, and, after saturation of said medium with said atmosphere, measuring the time required for a change in the volume-pressure ratio of said atmosphere to begin as a measure of the number of bacteria in each of said samples whereby the number of bacteria per unit amount of sample may be correlated with sample locations to detect anomalous variations in the number of bacteria indicative of the proximity of a subterranean petroleum deposit.

13. In the determination of the number of hydrocarbon-consuming bacteria in an earth sample by measurement of the time required for the hydrocarbon-consuming bacteria in the earth sample to begin consumption of a hydrocarbon nutrient, the method of providing a standard whereby the time required for consumption of hydrocarbon nutrient to begin is related to the number of hydrocarbon-consuming bacteria in the earth sample, comprising preparing a substantially pure culture of *Agrobacterium ethanicus*, admixing separately groups of known numbers of said *Agrobacterium ethanicus* with aqueous inorganic salt medium for bacteria, said medium having a pH between 7.2 and 7.6 and containing not more than 10 parts per million of iron, and measuring the time required for each of said groups of known numbers of *Agrobacterium ethanicus* to begin consumption of a hydrocarbon nutrient.

14. In the determination of the number of hydrocarbon-consuming bacteria in an earth sample by measurement of the time required for the hydrocarbon-consuming bacteria in the earth sample to begin consumption of a hydrocarbon nutrient, the method of providing a standard whereby the time required for consumption of hydrocarbon nutrient to begin is related to the number of hydrocarbon-consuming bacteria in the earth sample comprising preparing a substantially pure culture of *Agrobacterium ethanicus* with aqueous inorganic salt medium for bacteria, said medium having a pH between 7.2 and 7.6 and containing not more than 10 parts per million of iron, maintaining the resulting mixture in an atmosphere of oxygen and a light, gaseous petroleum hydrocarbon nutrient selected from the group consisting of methane, ethane, and propane, and measuring the time required for each of said groups of known numbers of *Agrobacterium ethanicus* to begin consumption of said hydrocarbon nutrient.

References Cited in the file of this patent

UNITED STATES PATENTS

1,034,400	Bunzel	July 20, 1912
2,234,637	Taggart	Mar. 11, 1941
2,269,889	Blau	Jan. 13, 1942
2,294,425	Sanderson	Sept. 1, 1942
2,349,472	Taggart	May 23, 1944

OTHER REFERENCES

Catalogue, Fisher Scientific Company, Pittsburgh, Pa., April 19, 1935, page 535.