

X 1287 T  
X 1033 C X 1550 W  
X 1028 C

Sept. 1, 1942.

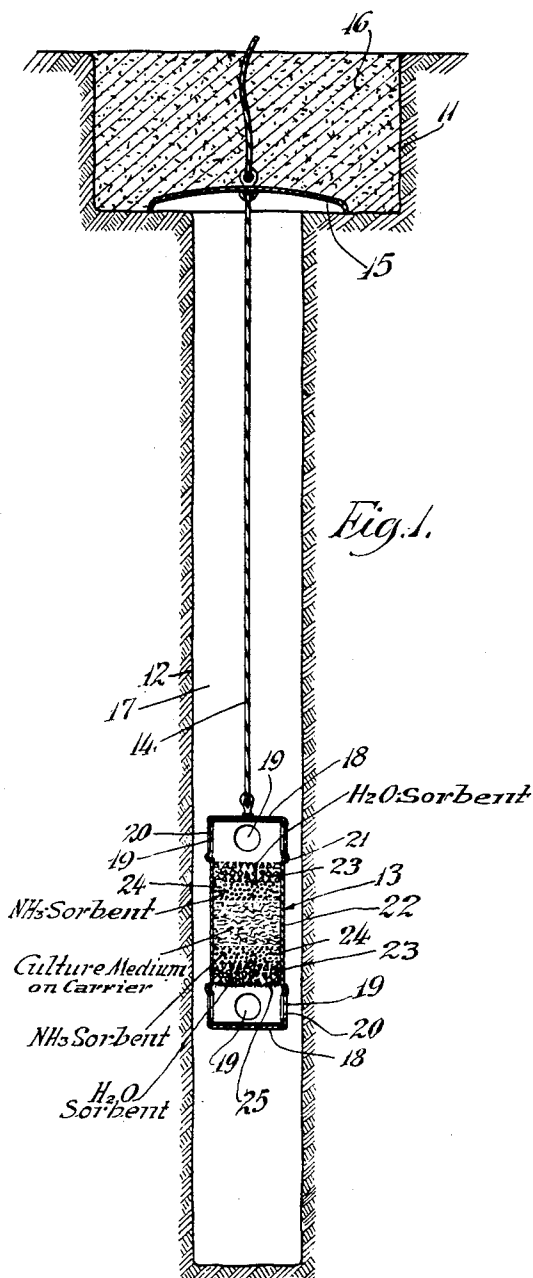
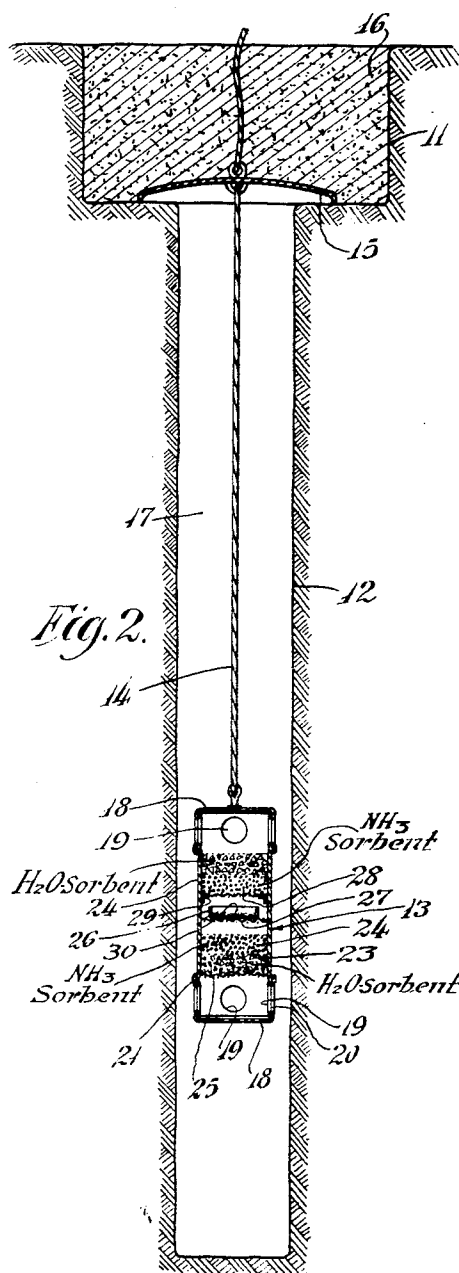
R. T. SANDERSON

2,294,425

GEOMICROBIOLOGICAL PROSPECTING

Filed May 22, 1940

2 Sheets-Sheet 1



Inventor  
Robert Thomas Sanderson  
By Geo L Parkhurst  
Attorney

Sept. 1, 1942.

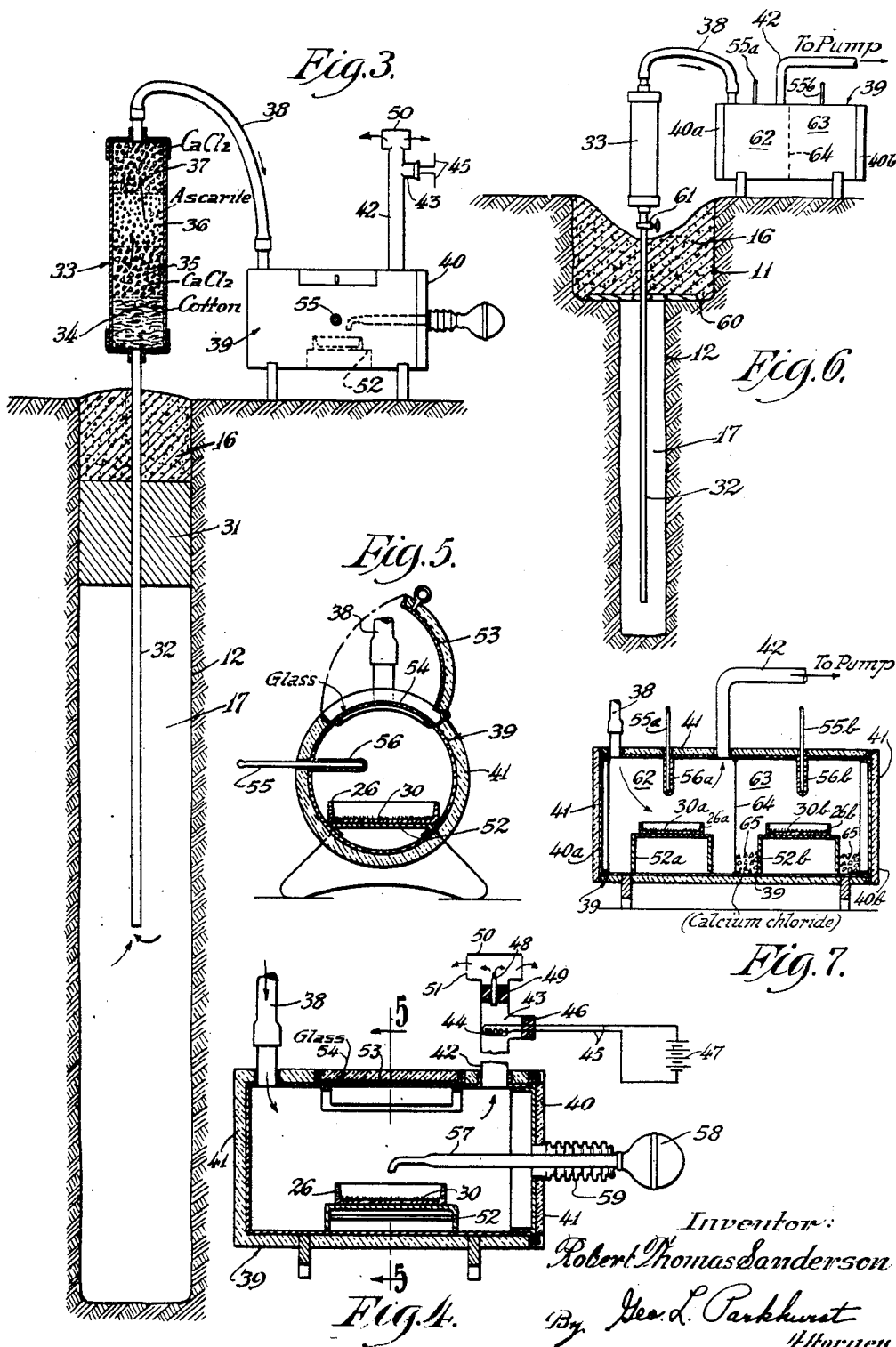
R. T. SANDERSON

2,294,425

GEOMICROBIOLOGICAL PROSPECTING

Filed May 22, 1940

2 Sheets-Sheet 2



Inventor:  
Robert Thomas Sanderson  
By Geo. L. Parkhurst  
Attorney.

## UNITED STATES PATENT OFFICE

2,294,425

## GEOMICROBIOLOGICAL PROSPECTING

Robert Thomas Sanderson, Sierra Madre, Calif.,  
 assignor to Stanolind Oil and Gas Company,  
 Tulsa, Okla., a corporation of Delaware

Application May 22, 1940, Serial No. 336,652

4 Claims. (Cl. 23—230)

This invention relates to prospecting for mineral deposits and more particularly to prospecting for hydrocarbon deposits such as oil and gas. In accordance with my invention such prospecting is carried on by the use of bacteria and other microbiological organisms which have an effect on or are affected by substances found in the soil and/or other relatively shallow geological formations, which substances are related to the relatively deep-seated mineral deposits.

A method of prospecting for hydrocarbon deposits known as geochemical prospecting has recently come into rather widespread use. Geochemical prospecting is carried on by taking samples of the soil at horizontally spaced survey stations or by drawing off samples of the soil gas at similarly spaced survey stations and then analyzing each of these samples of either soil or soil gas for constituents having a genetic relationship to deep-seated hydrocarbon deposits.

The constituents of prime interest in this connection are hydrocarbon gases, particularly hydrocarbon gases heavier than methane. However, liquid hydrocarbon or quasi-hydrocarbons and solid hydrocarbons or quasi-hydrocarbons, the latter being known as soil waxes, also serve as indicators of deep-seated hydrocarbon deposits. Other substances such as hydrogen, carbon monoxide, etc., are likewise believed to be significant.

A great variety of analytical procedures for determining the presence and amount of these various indicator substances in soils and soil gases have been worked out. In general, however, these analytical procedures are either time-consuming and expensive or are of questionable value.

It is an object of my invention to provide a particularly rapid and convenient method of analysis for constituents such as hydrocarbons, hydrogen, carbon monoxide, etc., found in soil and soil gas.

Another object of my invention is to provide a prospecting method in which the analysis by means of chemical or physical properties is replaced by analysis based on the effect of indicator substances found in soil or soil gas on various types of micro-organisms, notably bacteria.

A further object of my invention is to provide prospecting methods in which the determination of indicator substances having genetic relationships to deep-seated hydrocarbon deposits can be made in the field without the use of bulky, delicate and expensive apparatus.

Still another object of my invention is to provide methods for correcting the results obtained in geochemical prospecting in order to eliminate

the effect of certain factors unrelated to the possible presence and location of deep-seated hydrocarbon deposits.

Other and more detailed objects, advantages and uses of my invention will become apparent as the description thereof proceeds.

Various types of microbiological organisms, for instance bacteria, are known to be affected by the presence of hydrocarbons and other oxidizable substances, for instance hydrogen and carbon monoxide. These micro-organisms in general act upon such substances by oxidizing them to carbon dioxide and/or water.

The phenomena can be used in accordance with my invention in either or both of two general ways. First, the presence or growth of such micro-organisms acting on hydrocarbon and/or oxidizable gas substrates can be determined and used as an indication of the presence and relative amounts of these indicator substances in soils or soil gases. Second, a geochemical survey can be conducted in any of the various manners now known or later developed and concomitantly the presence of micro-organisms of the type described can be determined in order to provide a check on, and/or correction for, the results of the geochemical survey.

In the latter connection, if low values for certain indicator substances are found by chemical analysis and simultaneously it is found that soils from substantially these same sampling points are very high in micro-organisms adapted to convert and destroy the indicator substances concerned, this fact can be used to correct the results of the survey. For example, if two survey stations or sets of survey stations are found to give approximately equal values for some indicator substance or group of indicator substances, say hydrocarbon gases heavier than methane, and it is also found that one of these survey stations or groups of survey stations corresponds with very high counts of soil bacteria adapted to convert and destroy such hydrocarbons, a corrected map or other correlation can be worked out by increasing the experimentally determined hydrocarbon values in the case of that survey station or group of survey stations where the high hydrocarbophilic microbiological counts are found.

Such corrections can be made on an arbitrary basis or they can be made on a more scientifically sound quantitative basis by experimental determination of the effect of these micro-organisms. Thus a culture of a micro-organism of the type concerned can be planted in the soil in part of a given area and then the relative hydrocarbon

contents above such micro-organisms and also in a similar and adjacent soil area from which such organisms are absent (or present in much lower concentrations) can be determined to provide experimental data which can be used as a basis for quantitatively correcting the results of the geochemical survey. For instance, if it is found that a given concentration  $W$  of a particular soil bacterium of a hydrocarbophagic type reduces the soil hydrocarbon content by a factor  $X$  the values  $Y$  for soil hydrocarbons at various survey stations can be corrected by multiplying these values  $Y$  by

$$\frac{XZ}{W}$$

where  $Z$  is the concentration of the same soil bacteria at the survey station for which the value  $Y$  was determined.

Not only are micro-organisms known to bacteriologists, botanists and biologists which act upon and convert such indicator substances as hydrocarbons to other substances, notably carbon dioxide and/or water, but other micro-organisms, particularly saprophytic bacteria, are likewise known which act upon non-hydrocarbon substrates such as putrescent, animal and particularly vegetable tissues to convert such substrates to a greater or lesser extent into hydrocarbons. In other words, there are not only hydrocarbophagic but also hydrocarbogenetic bacteria and other microbiological organisms.

Thus high hydrocarbon contents found at certain survey stations or groups of survey stations may not actually correspond with deep-seated hydrocarbon deposits which it is desired to locate but may rather be due wholly or at least to a substantial extent to the presence of such hydrocarbogenetic micro-organisms in abnormally high concentrations in the soil. By accompanying a geochemical survey with a geomicrobiological survey, particularly directed to the counting of such hydrocarbogenetic organisms, checks and/or corrections can be made to apply to the results of the geochemical survey. If a very high hydrocarbon content or so-called hydrocarbon count is found at certain locations and these locations are likewise found to contain large concentrations of soil bacteria of a hydrocarbogenetic type, the geochemical results will be of little or no value as a positive indication of the presence of related deep-seated deposits of hydrocarbons.

As in the case of applying corrections by the use of hydrocarbophagic micro-organisms, the correction based on hydrocarbogenetic micro-organisms can likewise be on either a semi-qualitative basis or can be rendered quantitative by experimental determination of the effect on indicator substance content obtained by planting such micro-organisms in soils similar to those encountered in the geochemical survey.

Experimental determinations of the effect of these microorganisms on either a hydrocarbophagic or a hydrocarbogenetic type can be made in the laboratory instead of in the field.

While geomicrobiological prospecting methods can thus be applied by using them for the correction or checking of the results of geochemical surveys, I prefer to use the geomicrobiological technique to replace rather than to supplement the geochemical methods.

There are various ways in which my geomicrobiological methods can be used in prospecting for deep-seated mineral deposits and particularly for deep-seated hydrocarbon deposits. One

method is by analyzing soil samples for particular micro-organisms. Another is by planting micro-organisms in the soil or in communication with the soil and after a suitable period observing such factors as growth, spore formation, etc. in order to estimate or measure the effect of hydrocarbons and other soil or soil gas components on the micro-organisms. A third, and in many instances preferable, method of applying my invention is by subjecting a culture of hydrocarbon-sensitive, hydrogen-sensitive and/or carbon monoxide-sensitive micro-organisms under carefully standardized conditions to gases withdrawn from the soil and then observing the effect of such soil gases on these micro-organisms.

The first of the methods discussed in the preceding paragraph can be applied as when microbiological studies are made for the purpose of correcting or checking geochemical surveys. In other words, samples of the soil can be taken and the presence therein of hydrocarbon-sensitive and particularly hydrocarbophagic bacteria can be determined. Such hydrocarbophagic bacteria include such organisms as *Methanomonas methanica*, *Bacterium aliphaticum liquefaciens*, etc., as well as bacteria known as the methane bacterium and the paraffin bacterium. As is well known to bacteriologists, a census of such bacteria can be made by incorporating a sample of the soil in a suitable culture medium such, for instance, as agar agar and beef broth, incubating them for a suitable period, then killing the micro-organisms and observing them microscopically or otherwise to count the number of colonies of the particular distinctive types corresponding to the micro-organisms of interest.

Incubation can be conducted under conditions particularly conducive to the reproduction and growth of the particular micro-organism or micro-organisms which are of significance, notably the hydrocarbophagic bacteria. One method of doing this is to use a non-nutrient culture medium—or, preferably, a culture medium containing only the necessary inorganic nutrients—and to incubate the cultures in the presence of hydrocarbons of some particular type. In the case of *Bacterium aliphaticum liquefaciens* a small amount of a hydrocarbon such as pentane, hexane, heptane, octane, nonane or decane can be used as the sole substrate in the culture medium so that the growth of this particular bacterium is promoted. In the case of the paraffin bacterium solid hydrocarbons can be incorporated in the culture medium whereas when using the methane bacterium or *Methanomonas methanica*, methane, ethane, hydrogen, carbon monoxide or similar gaseous reducible compounds containing either hydrogen, carbon or both can be present in the atmosphere above the culture medium during the incubation period (preferably in the absence of other nutrient substrate). The concentration of such nutrient gas can suitably be from 0.001% to 50%, preferably from 0.01% to 10%.

When samples of soils are thus taken at spaced survey stations, for instance stations located at one-tenth mile intervals and the relative number of colonies produced from standard amounts of soil taken at each of these sampling points is compared, an indication which is significant with regard to the possible presence of deep-seated hydrocarbon deposits is obtained. In other words, high concentrations of one or more hydrocarbophagic bacteria and/or other microbiolog-

ical organisms in the soil is indicative of the fact that such soil has been subjected to diffusion of hydrocarbons therethrough and this is in turn a useful indication of the presence of deep-seated hydrocarbon deposits.

I prefer in work of this nature to utilize bacteria which are sensitive to hydrocarbons heavier than methane since methane is by no means as good an indicator of deep-seated hydrocarbon deposits as are the heavier hydrocarbons. This latter fact is due to the formation of methane by the action of other bacteria and in various bacteriological or non-bacteriological putrefactive transformations. Thus *Bacterium aliphaticum liquefaciens* is particularly suitable for use in connection with my invention and the paraffin bacterium is likewise suitable.

It is, of course, important when conducting work of this sort to avoid contamination of soil samples and they should be obtained by the use of sterile tools and conveyed to the laboratory under sterile conditions.

Since factors such as temperature, light and moisture are of great importance in influencing bacterial growth, the samples of soil taken for bacteriological analysis should in so far as possible be taken at locations of equivalent moisture content at equivalent depths and below the level to which light penetrates. It is also, of course, important to take such samples from undisturbed soils and from soils free from any type of cultural contamination. Samples should likewise be taken in the same season of the year.

The level from which the soil samples are taken depends on the particular conditions encountered and the particular micro-organisms chosen but it is preferred to take these samples at a depth of at least an inch or two and in general not more than three or four feet.

As previously indicated, the second method of applying my invention by using geomicrobiological prospecting as an independent tool rather than for the checking or correction of the results of geochemical surveys is by planting in the soil cultures of hydrocarbon-sensitive micro-organisms, notably hydrocarbophagic organisms of the type already discussed, allowing them to remain in situ for a suitable period and then removing such cultures and counting the bacteria by any of various well known methods in order to determine the influence of soil or soil gas hydrocarbon content on such factors as growth, reproduction, spore formation, etc.

In applying my invention cultures are prepared which are free from components adapted to supply the same nutrient effect as the indicator substances. These cultures can be mounted, if desired, on sterile fibrous material or on granular sterile materials or can preferably be placed between two layers of such sterile material which permits penetration of soil gas into contact with the culture. The micro-organism chosen being sensitive to the presence of hydrocarbons, particularly hydrocarbon gases, and also in many instances to such significant components as hydrogen and carbon monoxide, its growth will be dependent on the content of such materials and the measurement of growth of the organism or, in other words, increase of the bacteria or other micro-organism count, provides a quantitative indication of the presence of such oxidizable substances in the soil.

The cultures referred to can be buried in direct contact with the soil at depths from one inch or ten inches to two or fifteen feet or more, preferably

bly at least four or five feet deep in order to get below the region of hydrocarbogenic and other naturally-occurring soil bacteria and allowed to remain in place for a suitable period which may be as short as a day but is preferably longer depending upon temperature and other factors. The cultures can then be removed, taken to the laboratory and examined as above described and by various other procedures well known to bacteriologists.

However, as previously indicated, it is preferable to bury these cultures between sterile layers of permeable material and keep them out of contact with the soil, thus confining the effect obtained from soil components to gaseous or vaporous materials such as the lighter hydrocarbons, hydrogen, carbon monoxide, etc.

Alternatively, the culture of hydrocarbophagic bacteria or other hydrocarbon-sensitive, hydrogen-sensitive and/or carbon monoxide-sensitive micro-organisms can be placed in a Petri dish or other container which can be placed in a cavity in the earth, covered with sterile fibrous material (for instance, sterile asbestos) and then buried. Still better a solid culture in a deep Petri dish or the like can be placed face down on the ground, preferably on a surface freshly exposed by the use of a sterile tool, protected from contamination by cotton or other means, buried, left in place for a suitable period, and then removed and examined.

The various cultures used at spaced survey stations should be kept free from any possible contamination in taking them into the field and from the field to the laboratory or to the examining station and they should be subjected to contact with the soil or soil gases for standardized lengths of time under standardized conditions in so far as possible.

Since certain of the micro-organisms useful in connection with my invention, for instance, *Methanomonas methanica*, are sensitive to ammonia and other basic or acidic soil components as well as to hydrocarbons and the like, it is often highly desirable to prevent access of such components to the culture planted in the soil. Thus, for instance, the gases before reaching the culture can be caused to pass through a material adapted to the removal of ammonia and other basic components before coming into contact with the cultures. Likewise, the moisture content can be controlled.

Other aspects of this second method of applying my invention as an independent prospecting tool rather than as a means for correcting or checking geochemical prospecting and also the third of these methods to which reference has been made above will be further described in connection with the accompanying drawings which form a part of this specification and in which:

Figure 1 is a vertical cross section through one portion of the soil illustrating one type of apparatus for use in connection with my invention;

Figure 2 is a vertical cross section through one portion of the soil illustrating a modification of the apparatus of Figure 1;

Figure 3 is an elevation partly in section showing an alternative type of apparatus for use in connection with my invention;

Figure 4 is a sectional elevation illustrating in more detail part of the apparatus shown in Figure 3;

Figure 5 is a sectional elevation taken along the line 5—5 of Figure 4;

Figure 6 is an elevation partly in section showing a still further modification of apparatus which can be used in applying my invention; and

Figure 7 is an elevation partly in section showing in more detail part of the apparatus of Figure 6.

Referring now more particularly to Figure 1, instead of burying the culture of hydrocarbon-phagic bacteria or other hydrocarbon-sensitive, hydrogen-sensitive or carbon monoxide-sensitive micro-organism in the soil, such a culture can be suspended in a bore hole and sealed from contact with atmospheric air. Thus in Figure 1 a cavity 11 is formed with a spade or otherwise. This cavity, for example, can be about two feet wide and one foot or so deep. From the bottom of cavity 11 a bore hole 12 is drilled to a suitable depth, for instance five or ten feet although much deeper holes can be used if desired. A post hole auger can be used to drill this hole. Container 13 is suspended within bore hole 12 by a cord or chain 14 from a cover 15. This cover is located at the bottom of the cavity 11 at the top of bore hole 12. After installation of the apparatus earth 16 is packed into excavation 11 in order to seal the bore hole from atmospheric air. Thus a soil gas collecting space 17 is formed. The walls of this space should be made up wholly or largely of soil and, as previously indicated, access of atmospheric air should be prevented. It is desirable that this sample collecting space 17 be disposed entirely below the surface of the earth.

Container 13 is provided with caps 18 equipped with openings 19 which cooperate with openings 20 in container 13 itself so that by turning caps 18 the container can be opened or closed. Caps 18 and container 13 are provided with cooperating beads 21. The soil gases gain access to the culture through openings 19 and 20.

The cultures used can be of any of the types previously described. As shown in Figure 1, the culture medium carrying a culture of the desired micro-organism can suitably be mounted on a sterile porous carrier 22 made of any desired type of fibers or of active carbon or other material which will permit penetration of soil gases. Asbestos fibers which have been heated to render them sterile or activated charcoal which has similarly been heated are satisfactory carriers.

Before the soil gases obtain access to the micro-organisms they must pass through materials adapted to prevent or control the access of such gases and vapors as ammonia, water and other substances which would affect the growth of the bacteria or other micro-organism used. Thus, for instance, layers 23 can be layers of calcium chloride and layers 24 can be potassium acid sulfate. The calcium chloride or other drying agent, of course, serves to keep the moisture content at a low and constant level. If desired, the humidity can be controlled at a high level, for instance by water in an open dish. The potassium acid sulfate or any other suitable acidic sorbent serves to prevent the access of ammonia and other basic gases or vapors to the micro-organism. This is highly desirable since various hydrocarbon-sensitive microorganisms are similarly sensitive to ammonia. Sorbents for acidic and other contaminants can likewise be used. The materials in container 13 of Figure 1 are supported on screen 25.

The apparatus as shown in Figure 1 is left in place for a suitable period as above discussed, for instance several weeks, and it is then removed and taken to the laboratory under con-

ditions to avoid contamination. If desired, the bacterial action can be terminated at the time the apparatus is removed from the ground by sterilization, staining or otherwise so as to prevent further growth prior to laboratory examination. In the laboratory the growth of the micro-organism is measured by any of the various means well known to bacteriologists and botanists and other examinations can likewise be made to provide an indication of the presence and content of hydrocarbons, hydrogen, carbon monoxide, etc., in the soil gases.

Instead of using a culture medium suspended on a fibrous or porous carrier, the culture medium can be used in the ordinary manner familiar to bacteriologists by designing container 13 so as to carry a Petri dish 26 as shown in Figure 2. It will be noted that this Petri dish is carried by a perforated partition 27 and that layers 23 and 24 are disposed above and below it as in Figure 1 for the purposes already described. Upper layers 23 and 24 are carried by a removal screen 28 resting on brackets 29. Petri dish 26 carries an inoculated culture medium 30 which is examined after exposure to determine the effect of soil gases.

In discussing the previous methods of applying my invention as a direct method of prospecting and not merely as a correction to be applied to the results of a geochemical survey, I indicated that one of the preferred procedures was to withdraw soil gases and then subject hydrocarbon-phagic bacteria or other hydrocarbon-sensitive, hydrogen-sensitive or carbon monoxide-sensitive micro-organisms to an atmosphere of these soil gases in order to determine the effect on such micro-organisms. This can be done by withdrawing the soil gases in any manner and taking the soil gases to the laboratory where the microbiological assay can be performed. It is, however, often desirable to conduct this microbiological assay in the field.

This can be done, for instance, as shown in Figure 3. Bore hole 12 is drilled to a suitable depth, for instance five or ten feet or considerably deeper, and the lower portion of the cavity thus formed is isolated from atmospheric air by the use of packer 31 (shown diagrammatically) above which earth or other sealing material 16 can be packed in order to seal the cavity further. Tube 32, preferably a small bore tube, passes through earth 16 and packer 31 and preferably extends to a low level in cavity 17.

Soil gases are withdrawn slowly through this tube and are then passed through container 33 which can contain any desired purifying substances. Thus, for instance, lower layer 34 within container 33 can be made of sterile cotton or other mechanical filtering medium, layer 35 can be calcium chloride, layer 36 can be potassium acid sulfate or other acidic sorbent and layer 37 can be a second layer of calcium chloride. It will be understood, of course, that other sorbents can be used either in connection with the apparatus of Figure 3 or in connection with any of the other forms discussed. Thus, for instance, if acidic components which might have an effect on the micro-organisms are likely to be encountered, a basic sorbent such as potassium hydroxide or the like can be incorporated in the sorption train.

From sorbent container 33 of Figure 3 the gases pass through tube 38 to microbiological assay chamber 39. This chamber is shown in more detail in Figures 4 and 5. As shown it consists of a cylindrical metallic chamber with an

open end which is adapted to be closed by cover 40. The cylindrical chamber 39 and cover 40 are maintained at constant temperature by insulation 41 (Figures 4 and 5). In practice the chamber should be protected from the sun.

Soil gas is circulated through this chamber by any desired means, for instance by thermal circulation. Thus a tube or pipe section 42 rises from the end of chamber 39 opposite the inlet point and this tubing or pipe section carries at the upper end a T 43. A heating filament 44 having insulated wires 45 passing through a stopper 46 is inserted in this T as seen in Figure 4 and is heated electrically, for instance by battery 47 which is shown diagrammatically. Above this heating element is a capillary 48 carried by stopper 49 and this projects into or is in communication with an upper T 50, the ends of which are provided with screens 51. The result of this heating is to cause a thermosyphon effect, thus circulating soil gas slowly through chamber 39. Heating filament 44 should be located well above chamber 39 to avoid heating the culture.

Within the chamber is a shelf or platform 52 on which a Petri dish 26 carrying a culture medium 30 inoculated with the desired micro-organism can be placed. At the top of the chamber is a hinged insulated cover 53 below which is placed a glass window 54 for the purpose of viewing the interior of the chamber without opening it to the atmosphere.

Chamber 39 is likewise provided with a thermometer 55 projecting into a thermometer well 56 as best seen in Figure 5. Frequent observation of the temperature is desirable so that a correction can be made for the effect of temperature.

It will be understood that the apparatus of Figures 3, 4 and 5 can be utilized by placing in chamber 39 a Petri dish 26 or other container carrying a culture medium 30. This culture medium can be inoculated at any desired number of points with the desired bacteria or other micro-organism. End closure 40 can then be closed and soil gas can be circulated through the chamber as described for a suitable period, for instance, a few days. When the desired period has elapsed the micro-organisms can be killed, for instance by staining them with a suitable dye which can be dropped on the colony by means of pipette 57 which terminates in rubber bulb 58. As shown in Figures 3 and 4, this pipette can be mounted on a Siphon bellows 59 to permit manipulation.

Another desirable method of procedure is to inoculate the culture medium with a number of colonies of a suitable micro-organism in the laboratory and take the Petri dishes containing these inoculated culture media to the field.

After installing the Petri dish and the rest of the apparatus as shown in Figure 3, part of these colonies can be destroyed by dyeing, utilizing pipette 57 and window 54. Thus for instance, six colonies can be used and three can be destroyed immediately before starting the test, thereby providing a "blank" and other colonies can be destroyed at the end of the test and the amount of growth can thus be determined and used as a measure of the significant indicator substance content of the soil gases.

After the microbiological assay is carried out, the data thus obtained can be used to prepare tabulations, comparisons, maps or other forms of summarized information showing for each of a number of survey stations in the area concerned the relative values of the microbiological assay

in terms of bacterial growth or in any other desired terms. Since all that is desired is comparative values for the various survey stations, it is not at all essential to convert the microbiological assays into any such terms as percentage of hydrocarbons. This can be done, however, by experimental determinations of the effects of known quantities of hydrocarbons or other indicator substances on the micro-organism concerned under similar conditions and thus provide a calibration curve or a calibration table showing the relationship of bacterial growth or the like to hydrocarbon or other indicator substance content.

The apparatus shown in Figures 6 and 7 provides an alternative to that shown in Figures 3, 4 and 5. In Figures 6 and 7 the cavity 17 is of the general type shown in Figures 1 and 2 utilizing excavation 11 and bore hole 12. Figure 6 illustrates the use of a board or screen 60 to prevent access of the soil to the sample collecting cavity and also to serve as a support for soil gas withdrawal tube 32. At the top of this withdrawal tube is mounted stop-cock 61 and above it is sorbent container 33 which can be similar to that of Figure 3. Soil gases pass from this sorbent container to chamber 62 within an assay cylinder 39. From this chamber 62 the soil gases are withdrawn at a low circulation rate through pipe 42 by means of a pump not shown. Cylinder 39 is divided into the previously mentioned chamber 62 and a similar chamber 63 by a heat-conductive thin metal wall 64 and is adapted to be closed at both ends by covers 40a and 40b. The apparatus is provided with insulation 41 as shown. Chambers 62 and 63 are preferably provided with thermometers 55a and 55b fitting within thermometer wells 56a and 56b. They are both provided with shelves or platforms 52a and 52b on which Petri dishes 26a and 26b or other culture containers can be placed.

The purpose of the two chambers 62 and 63, as best seen in Figure 7, is to provide a "blank." Thus Petri dish 26a is subjected to atmospheric air at the same temperature as Petri dish 26b which is subjected to a soil gas atmosphere. The humidity in compartment 63 can be kept the same as that in compartment 62 by the use of calcium chloride 65. Thus the humidity in both compartments is that existing over calcium chloride. In other words, the apparatus of Figures 6 and 7 provides a set-up in which the only significant variable is the indicator substance content of the soil gases. When using this apparatus identically inoculated cultures 30a and 30b in Petri dishes 26a and 26b are installed in the two compartments 62 and 63 and after a suitable period, as previously discussed, these cultures can be removed from their compartments, sterilized and/or dyed, if desired, and taken to the laboratory for analysis or they can be examined microscopically in the field in order to measure the relative rate of growth of the micro-organism subjected to soil gases as compared with those subjected to atmospheric air. The ratio between the growth of the micro-organisms subjected to the soil gases and the growth of those subjected to the atmospheric air or the difference between the two amounts of growth can be used as an index of the indicator substance content of the soil gases and from these data for various survey stations maps or other comparisons can be made as previously suggested and as will be entirely familiar to those skilled in the art of geochemical prospecting.

My invention has been described with particu-

lar reference to the use of bacteria. Such bacteria as *Methanomonas methanica*, *Bacterium aliphaticum liquefaciens*, *Bacterium hidium* and *Bacillus oligocarbophilus* can be used.

Bacteria are likewise known to grow in petroleum and such bacteria have been isolated. (Nature, No. 3249, vol. 129, pages 204-5.) These particular facultative anaerobes can be employed in connection with my invention and are highly suitable, using anaerobic techniques.

When it is desired to use hydrogen as the indicator substance various bacteria of the genus *Hydrogenomonas* can be employed, while when carbon monoxide is the desired indicator substance *Carboxydomonas* bacteria can be utilized.

In place of making a bacteriological assay of the soil itself, similar bacteriological assays can be made in any desired manner on ground waters since the concentration of hydrocarbophagic and other hydrocarbon-sensitive, hydrogen-sensitive and carbon monoxide-sensitive micro-organisms in ground waters is an index of the gases which have been dissolved in, or have been in contact with, such ground waters. Either meteoric or deeper ground waters can be used.

Also, in connection with any of the methods herein described, instead of determining bacterial or other micro-organismic growth, determinations of motility, spore formation, mutations, etc., can be made and used as qualitative or quantitative indications of the presence of the indicator substances of interest. Determinations of the end products of the action of such micro-organisms on hydrocarbon and other indicator substance substrates can also be made and data thus obtained can be used as a basis for maps, or other comparisons.

Similarly the temperature rise resulting from the action of micro-organisms on indicator substance substrates can be determined to provide a measure of bacterial or other action, thus giving an indirect measure of indicator substance content. Such data can also be mapped or otherwise compared.

My invention has been described in connection with examination of surface soils at horizontally spaced survey stations. It will be apparent, however, that except for the matter of convenience similar observations can be made using soil gas and other soil components from deeper geological formations and subjecting these to microbiological assay as described. It will also be apparent that my invention can be applied to well logging by examination of cores, drill cuttings or the like for bacteria or other micro-organism content and also by subjecting such cores and drill cuttings or the like to various hydrocarbophagic and other related micro-organisms in order to determine the effect of hydrocarbon, hydrogen, carbon monoxide, etc., contained in such samples taken from vertically spaced points, thus providing data from which a well log can be made showing the direct results of bacterial assay or these results can be converted by the use of empirical correlations to figures significant with regard to the amount of indicator substances present and the latter can be used to provide a log. It will also be apparent that my invention

can be applied to the correction of the results of geochemical well logging in the same manner as it is applied to the correction of the results of geochemical surveying.

Extreme care must be taken in applying any of my methods to avoid any contamination which might influence the results.

My invention has been described with particular reference to the use of bacteria. However, other forms of micro-organisms sensitive to the indicator substances mentioned can be used, for example certain molds and fungi. Also biological assays can be made for any of the purposes herein described by observing the toxic or other effects of the indicator substances on plants of higher class, for instance various monocots and dicots.

In general, it will be apparent that while I have described my invention in connection with certain preferred embodiments thereof, these are by way of illustration and not by way of limitation and various improvements and modifications will be suggested by the foregoing description to those skilled in the art.

I claim:

1. A method of prospecting comprising analyzing earth components taken from spaced survey stations for at least one indicator substance selected from the group consisting of hydrocarbons, hydrogen and carbon monoxide, examining samples from substantially the same survey stations for at least one micro-organism capable of producing said indicator substance, and comparing the results of such analysis and examination.

2. A method of prospecting for sub-surface hydrocarbon deposits comprising determining the relative amount of at least one hydrocarbon contained in components taken from spaced survey stations, and determining the relative amount of at least one micro-organism, capable of producing at least one hydrocarbon thus determined, contained in soils taken from substantially the same spaced survey stations to provide a basis for more accurate correlation of data obtained by said hydrocarbon determinations.

3. In a method of prospecting for sub-surface hydrocarbon deposits comprising determining the relative hydrocarbon content of earth components taken from spaced survey stations, the improvement which comprises determining the relative content of at least one hydrocarbogenic micro-organism in soils taken from substantially the same spaced survey stations to provide a basis for more accurate correlation of data obtained by the determination of said hydrocarbon contents.

4. In a method of prospecting for sub-surface hydrocarbon deposits comprising determining the relative hydrocarbon content of earth components taken from spaced survey stations, the improvement which comprises determining the relative content of at least one saprophytic hydrocarbogenic bacterium in soils taken from substantially the same spaced survey stations to provide a basis for more accurate correlation of data obtained by the determination of said hydrocarbon contents.

R. THOMAS SANDERSON.