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Research Article

MICROBIOLOGY IN PETROLEUM EXPLORTATION

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ABSTRACT

Petroleum microbiology is an interdisciplinary area involving microbiologists, biochemists, chemists, chemical engineers, physicists and geologists. A wide range of studies have dealt with processes like biotransformation, biodegradation and bioremediation of petroleum hydrocarbons. Petroleum is a complex mixture of hydrocarbons and other organic compounds, including some organo metallo constituents, most notably complexing vanadium and nickel. Petroleum recovered from different reservoirs varies widely in compositional and physical properties. Long recognized as substrates supporting microbial growth, these hydrocarbons are both a target and a product of microbial metabolism.

Practically all geologists agree that petroleum has an organic marine sedimentary origin, but the mode of its formation is not known. Bacterial activity has undoubtedly been involved in petroleum genesis, but the extent to which bacteria have contributed to the formation of petroleum is debatable. Attempts to demonstrate hydrocarbon formation by bacteria under highly artificial conditions have yielded only small amounts of paraffinic hydrocarbons other than methane and practically none of the other myriad compounds present in petroleum. The conservative viewpoint is that bacterial action is limited to producing reduced organic matter more closely resembling petroleum than the original material and that the final stages of petroleum genesis are physicochemical.

INTRODUCTION

The microbiologist within the past decade has joined the many other technologists serving the petroleum industry. His endeavors are not as highly specialized as might be presumed. In time, certain aspects within this scope will likely be pursed with much greater intensity of effort. Today, the microbial conversion of certain petroleum constituents into useful products receives little attention. On the other hand, although the role of microorganisms in petroleum genesis (the process by with petroleum is formed in nature) has been a long range study of interest to geologists for more than twenty years, this subject has yet to pass beyond the realm of speculation.

Exploration for petroleum deposits was pioneered by the rank wildcatter who was followed and surpassed by the geologist. The geophysicist followed the geological and added fruitful physical techniques. On the heels of the geophysicist came the geochemist, who, in turn, is followed by the geomicrobiologist. While the geochemist searches for chemical evidence of petroleum in the surface soils, the geomicrobiologist investigates the effects of microbial activity upon these chemicals and, in addition, looks for specific microorganism which feed upon hydrocarbons emanating from petroleum reservoirs.

Geomicrobiological Prospecting for Petroleum

Soil microorganims as indirect indices of petroliferous emanations.

Sohngen, one of the first bacteriologists to become interested in hydrocarbon oxidizing microorganisms, in 1906 described an enrichment method of isolating methane oxidizing bacteria from soil (*Sishngen, N. L. 1906*). Hydrocarbon oxdizing bacteria in soil have since been patented (*Hassler, G. L. 1943 & Strawins1ki, R. J. 1954*) and assigned to petroleum companies. The premise is that detection of hydrocarbon oxidizers will serve as an index of hydrocarbons in the soil. Gaseous hydrocarbons are believed to emanate from subsurface petroleum reservoirs into the soil.

In 1943, Hassler obtained the first U. S. patent (*Hassler, G. L. 1943*), and in 1954, Strawinski obtained the latest U. S. patent (*Strawins1ki, R. J. 1954*) describing methods of prospecting for oil based upon measuring gas uptake by hydrocarbon oxidizing bacteria in systems containing soil, gaseous hydrocarbons and oxygen. Russian workers, particularly the geologist

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Mogilevskii, had proposed in 1940 the utilization of data obtained in bacteriological studies of the subsoil for the purpose of detecting and contouring gas emanating areas *(Mogile Vsii, G. A. 1940*). Bacterial surveys of oil and gas fields were made by Mogilevskii and co-workers during the years 1937-1939 in conjunction with gas surveys. The Russian microbiologist, V. S. Butkevich, head of the Microbiology Department of the Timiryazev Agricultural Academy, participated in this work in which a total of more than 3,000 soil samples was studied. Gas surveys previously carried out by the Russians had established that only negligible concentrations of gaseous hydrocarbons could be found in the soil, even over known gas deposits, and they questioned whether these gases could serve as a medium for bacteria. Furthermore, as pointed out by Mogilevskii, the bacterial surveys, like the gas surveys, were complicated by the presence of mete in the surface soi layers, the result of organic matter decomposition rather than seepage from crude oil and gas reservoirs.

Some of the physiological properties of the methane oxidizing bacteria (found in the subsoil layers at a depth of two to three meters) were studied under the direction of Professor Butkevich. The bacteria were capable of developing in an atmosphere containing methane and oxygen in the presence of moisture and mineral salts. Hence, it was concluded that a low concentration of methane, in a steady supply, is the determining factor making it possible for methane oxidizing bacteria to grow in the subsoil.

At the suggestion of Butkevich, Mogilevskii had the soil samples analyzed for both methane oxidizing bacteria and cellulose decomposing bacteria (ostensibly methane forming bacteria). Particularly significant were those samples which contained methane oxidizers, in the absence of cellulose decomposers. The ceilulose decomposers were detected by observation of paper decomposition in a mineral salts medium together with the soil samples during a prescribed incubation period. The determination of methane oxidizing bacteria was likewise qualitative. Samples of soil were added to test tubes with a mineral salts medium and the tubes placed under a bell jar. A water seal was used through which methane was introduced in admixture with oxygen. Incubation at 34-35 C lasted for 12-14 days, and methane oxidizing bacteria, when present, characteristically formed a pellicle on the surface of the mineral medium.

In spite of its simplicity, use of the method resulted in detecting anomalies of methane oxidizing bacteria in the subsoil which were asociated with gas and oil producing areas. Some of these bacterial surveys preceded drilling operations. Mogilevskii (*Mogile Vsii, G. A. 1940*) concluded the method had promise, but that a development of a quantitative interpretation was desired. A study of bacterial indicators for higher hydrocarbons was suggested as well as a determination of the optimum depths for soil sampling.

Later Russian workers followed the lead of Mogilevskii. In 1947 Bokova *et al*. (*Bobova, E. N., Kuznetsova, V. A., And Kuznetsov, S. I. 1947*) and Subbota (*Subbota, M. I. 1947*) described experiments and field surveys involving methane oxidizing bacteria as well as other gaseous hydrocarbon oxidizing bacteria. Subbota continued to compare the cellulose decomposing bacterial flora with the methane oxidizing bacterial flora as had Mogilevskii. Bokova and co-workers isolated not only methane oxidizing bacteria from the soil but also ethane and propane oxidizing bacteria. These workers were particularly interested in specificity relative to the particular hydrocarbons which could be utilized by the different bacteria. They reported that all methane oxidizing bacteria isolated failed to utilize ethane or propane. These they classified as *Methanomonas methanica* despite former reports, e.g., of Tausz and Donath (*Tausz, J., And Donath, P. 1930*), that this organism was capable of utilizing these hydrocarbons. Bokova and coworkers also reported the isolation of an ethane oxidizing bacterium which could not utilize methane, and a propane oxidizing bacterium which could not utilize ethane or methane.

Subbota (*Subbota, M. I. 1947*) pointed out that the bacterial method of oil prospecting proposed by geologist Mogilevskii recently came into use in oil exploration in Russia, in conjunction with gas surveys. It was also used independently by a specialized office of the Central Department of Eastern Oil Exploration and All-Union Scientific Research Institute of Hydraulic Geology and Geological Engineering. Bokova and his associates (*Bobova, E. N., Kuznetsova, V. A., And Kuznetsov, S. I. 1947*) reported the discovery of a gas field in Stavropol Kavkaz and an oil pool in Ikhta, the results of drilling to check bacteriological prospecting data.

German workers, Schwartz and Mueller, have likewise reported that bacteriological prospecting for petroleum has promise and claim some success using a quantitative dilution method. In a review *(Schwartz, W., And Mueller, A. 1952*) they refer only briefly to their own unpublished observations, and no details are given.

Another approach toward exploitation of bacteria in petroleum prospecting has been proposed by Sanderson (*Sanderson, R. T. 1942*), namely, the planting of hydrocarbon oxidizing bacteria in the soil and observing their growth in response to emanating hydrocarbons. He maintained that it was preferable to bury pure cultures at a depth of four or five feet between sterile layers of permeable material (e.g., asbestos) and keep them out of contact with the soil. Technical difficulties of such a procedure, particularly in view of the slow rate of growth of the bacteria in the presence of the minute amounts of emanating hydrocarbons, would be anticipated.' Varying water level in the soil because of unpredictable seasonal rains would likely inundate planted bacterial cultures in many areas where such a method is employed. Practical success in detecting hydrocarbon gas emanation by the Sanderson method has not been reported in the scientific literature. For that matter, success in geomicrobiological prospecting or petroleum on a commercial basis has not been reported in scientific journals apparently, except by the Russians already referred to.

Several factors influence results of soil analysis for hydrocarbon oxidizing bacteria. Methane oxidizing bacteria, particularly, have been observed and their function described in ecological relationships totally unrelated to petroliferous emanations (*Aiyers, P. A. S. 1920*). Anomalies in the abundance of methane oxidizing bacteria in the soil must therefore be scrutinized carefully before they are given significance as an index of petroleum-gas emanation. The adaptive ability of bacteria to utilize organic compounds, including hydrocarbons, must likewise be considered. Therefore, the detection in the soil of bacteria which can oxidize the various hydrocarbons in natural gas is not necessarily an index of natural gas emanation. Seasonal fluctuations in the soil bacterial flora, including the hydrocarbon oxidizing flora, must likewise be considered, as pointed out by Subbota (*Subbota, M. I. 1947*).

Bactrial products as indices of petroliferous emanations

In 1942, Blau (*Blau, L. W. 1942*) described a method for detecting a "color change" in the soil as anindex of bacterial action upon hydrocarbon gases emanating from subterranean petroleum deposits. The best reagent used for this purpose was reported to be sodium peroxide although avariety of reagents were employed. According to Blau, the "color change" resulted with soil samples containing hydrocarbon consuming bacteria which converted hydrocarbons into polymerized and oxidized compounds of high molecular weight that appeared to be carboxylic acids. He intimated that bacterial cells them could account for the color reaction, described as "deep red to light yellow", depending upon the reagent employed.

In 1943, he pointed out further that these "bodies of high molecular weight" apparently fluoresce under the influence of ultraviolet light (*Blau, L. W. 1943*). Slavina (Slavina, G. P. 1948) more recently studied the fluorescence of certain soil bacteria including hydrocarbon oxidizers. Bacterium aliphaticum liquef which utilized pentane, hexanes, and heptane fluoresced brilliant green, while Methanomonas methanica reportedly active on methane, ethane, and propane did not fluoresce in ultraviolet light. Evidence of practical success utilizing the above methods as prospecting parameters of petroliferous emanation apparently has not been published.

A manifestation of surface soils, described as "paraffin dirt", has long been associated with certain oil and gas producing areas by petroleum geologists. One assumption prevailed that a deposition of high concentrations of paraffin emanating from petroleum deposits resulted in Milner (Milner, H. B. 1925) gave a good description of this peculiar material and pointed out its low hydrocarbon content more than twenty-five years ago. Recent studies by Davis (Davis, J. B. 1952) on a "paraffin dirt" bed in Texas confirmed suspicions of other observers that microorganisms were responsible for a conversion of hydrocarbon gases into microbial cell material, thus accounting for the waxy appearance of the soil in a localized area. Analyses of a representative "paraffin dirt" sample showed the dried soil to contain 17.6 per cent organic carbon, 1.2 per cent organic nitrogen, 0.27 per cent lipid (organic matter soluble in CCII), and 0.0038 per cent saturated hydrocarbons. Microscopic examination of the soil revealed an abundance of microorganisms including protozoa, filamentous fungi, yeasts, actinomycetes and bacteria. Among the bacteria, especially, were varieties capable of utilizing methane and other gaseous hydrocarbons as carbon sources. Mass spectrometer analysis of the soil gas collected about six feet below the surface of the "paraffin dirt" bed showed the presence of 1.4 per cent methane and 0.13 part per million of ethane. Traces of other gaseous hydrocarbons were indicated. It is believed that the organic matter of "paraffin dirt" consists largely of microbial cells, living and dead.

Laboratory experiments consisting of passing natural gas through two ordinary surface soils for a period of months resulted in a marked increase in organic content of the soils. The number of microorganisms also increased markedly as the gas flow continued. Both soils acquired a waxy, gummy appearance, and one of the soils upon microscopic (wide field binocular) examination was indistinguishable from specimens of "paraffin dirt" collected in the field. The other treated soil, while similar, was not identical in character with the field samples, primarily, it is believed, because of an original difference in soil texture.

The fixation of organic matter in the form of hydrocarbon oxidizing microbial cells as they consume the emanating hydrocarbons ostensibly results in a food source for other microorganisms. The latter thus feed indirectly upon hydrocarbon emanations. "Paraffin dirt" is a misnomer since the waxy appearance of the soil is not caused by paraffin, as is borne out by its low lipid and hydrocarbon content.

Microbial Acivity as Related to Geochemical Prospecting for Petroleum

Visible seepages of hydrocarbon gases and crude oil at the surface of the earth have served man as an index of subsurface accumulation of petroleum for many years. Practically all of such seepages have been observed by this time, at least in this country. Invisible seepages which also may serve as a means of finding oil must be detected by technical means. Sokolov (Soikolov, V. A. 1936) and Laubmeyer (Iaubmeyer, G. 1932) were among the first to investigate methods of soil gas surveying as a means of geochemical prospecting for petroleum. Sokolov, in about 1930, began investigating gas surveying in Russia. Soil gas was assayed for gaseous hydrocarbons, including methane, using an intricate hot filament (combustion) means of measurement. Over known oil and gas deposits the range of hydrocarbons found was from 0.0001-0.2 per cent of the soil gas. It is significant that among Sokolov's collaborators was Mogilevskii who later, in 1937, proposed that bacterial surveys be made as a means of prospecting for petroleum. While Sokolov appreciated the fact that anaerobic bacteria in the soil produced methane which could mask the micro appearances of petroleum gases coming from subsurface reservoirs, it was his associate, Mogilevskii, who maintained that due to the preponderance of methane in natural gas, anomalies in methane oxidizing bacteria were significant if observed at depths ordinarily below organic matter decomposition in the soil (Mogile:vsii, G. A. 1940).

American investigators (*Horvitz, L. 1939, Mcdermo-T, E. 1939, Rosaire, E. E. 1939*) became interested in the observations of Sokolov and of Laubmeyer and began their own geochemical surveys. Rosaire (*Rosaire, E. E. 1939*) in particular was an active proponent of geochemical prospecting based upon soil analyses. He was especially interested in hydrocarbon gases, such as ethane, propane, and butane, which may be considered "direct" indices of petroleum because of their practically unique origin. He showed further interest in secondary products arising from the oxidation and polymerization of these emanating gases. Rosaire points out that these secondary products (called "soil waxes") resemble hydrocarbons but chemically they are not true hydrocarbons. Their molecular composition, mode or rate of formation has not been clarified.

It is interesting that Rosaire, Horvitz (*Horvitz, L. 1939*), and McDermott (*Mcdermo-T, E. 1939*) along with others (*Fash, R. H., et al. 1940*), in discussing factors affecting geochemical prospecting, did not consider microbial activity as a possible means of either modifying or destroying the index hydrocarbons.

Since it has long been agreed that methane in soil may have either a biological or a petroliferous origin, geochemists in the U. S. have had a tendency to shun measurements of methane as being nonsignificant. It should be pointed out, however, that there is no knowledge of the actual amounts of biomethane produced in ordinary soils. Rosaire (*Rosaire, E. E. 1939*) likewise referred to ethylene of biological origin (e.g., ripening fruits, plant tissues) as a factor to be considered in geochemical prospecting. More recently ethylene formation by filamentous fungi has been shown by Nickerson (*Tnickeson, W. J. 1948*) and Williamson (*Williamson, C. E. 1950*). Ethylene and other olefins have been observed in natural gases only rarely and in small amounts. Buswell and Mueller in 1952 reiterated that ethane and higher hydrocarbons had not been observed in bacterial methane fermentations and that if present must be in concentrations less than 20 parts per million of the partially purified methane.3 Thus, for all practical purposes one would assume that ethane in the soil is principally of petroleum origin and that ethylene has principally a biological origin. Interestingly enough, *McDermott* reported both ethane and ethylene in concentrations of 0.02 to 0.10 ppm by weight in the soil over oil fields.

Horvitz in discussing "soil wax" indicated that it was observed in a thousand to ten thousand fold greater concentrations in soil than the lighter constituents such as ethane, propane, and butane. While a true knowledge of "soil wax" was admittedly lacking, he maintained it was "empirically significant material", implying that it was a geochemical parameter of importance. Knowledge of the chemical characteristics of this organic material would be required before either speculation or experiments could relate it to microbial activity in soil.

CONCLUSION

Petroleum Microbiology, At least one university laboratory is engaged in such studies under a grant from a petroleum company, and it is hoped that other academic microbiologists will be attracted to this field in the future. An opportunity is here for fruitful fundamental research, which could provide a basis for applications in the refining and manufacturing of petroleum products. Although the petroleum companies do a certain amount of fundamental research, this is the type of information which must, at present, come principally from the academic laboratories, while in the petroleum industry microbiologists pursue information of a more applied nature. As time pass, more microbiologists should swell the thin ranks of those employed in the petroleum industry, and thus permit more fundamental work to be done, with results of mutual benefit to science and the petroleum industry.

Because of developments of possible competitive advantage in this little-known field, individual petroleum companies have restricted the publication of their research findings until they can be adequately protected by patents. Since patents require from two to five years to issue, many developments in petroleum microbiology are undoubtedly being retained in the confidential files of oil companies. The eventual publication of this material should immediately make certain aspects of this review obsolete.

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