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RESEARCH ARTICLE

MICROBIOLOGY IN PETROLEUM GENESIS

Akshay George^{1*}, Palaniswamy E², Vignesh J³ and Maithili T⁴

^{1,3} Department of Petroleum Engineering

²Excel College of Engineering and Technology, Komarapalayam

⁴Department of Applied Geology, Periyar University, Salem

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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.

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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 pursued 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 which 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.

Modification of Organic Marine Sedimentary Material

Oxidative processes

It is axiomatic that bacteria will oxidize sedimentary organic matter for the purpose of gaining energy as long as physicochemical conditions permit. The most efficient means of gaining energy from organic compounds is for the bacteria to oxidize them in the presence of oxygen, the carbon compounds becoming completely oxidized to carbon dioxide and water, thus yielding the maximum of energy. Such oxidation can take place only at the surface of marine sediments since below the first few centimeters most sediments rich in organic matter are depleted of oxygen. The bacteria which are active in the oxidation of sedimentary organic material in the presence of free oxygen are common forms found in soil and fresh water, usually facultative anaerobes such as *Pseudomonas*, *Achromobacter*, *Flavobacterium*, and *Spirillum* (Stone, R.w., and Wight, K.M., 1952).

*Corresponding author: Akshay George
Department of Petroleum Engineering

In the absence of free oxygen strictly anaerobic bacteria are active as well as the facultative anaerobes. Certain anaerobic bacteria such as the *Desulfovibrio* have been given much attention regarding their role in petroleum genesis, especially by ZoBell (ZoBell, C.E 1953). These bacteria oxidize organic compounds in sediments and concomitantly reduce oxidized forms of sulfur, using them as hydrogen acceptors. This process takes place in the absence of oxygen resulting in oxidized compounds, energy for the *Desulfovibrio* and hydrogen sulfide. Because hydrogen sulfide reacts with metals to give a black sulfide precipitate, the blackening of organic sediments is usually an indication of the activities of *Desulfovibrio*. Other anaerobic bacteria may be active in sediments, but little attention has been given them. Anaerobes other than *Desulfovibrio* oxidize organic compounds in the absence of oxygen by using other organic compounds as hydrogen acceptors rather than sulfur compounds.

The hydrolysis products of protein and carbohydrate materials are the most rapidly metabolized compounds, yielding CO₂, NH₃, H₂S, CH₄ and fatty acids depending upon the bacteria and the conditions involved (Stone, R.w., and Wight, K.M., 1952). Other materials such as chitin and lignin are more slowly decomposed by bacterial action and form the basis for the accumulation of marine humus (Waksman, S.A, 1938). Marine humus, like soil humus, is chemically ill-defined and may be described simply as a colloidal residual of undecomposed organic matter which because of its resistance to oxidation very slowly succumbs to bacterial decomposition processes.

Marine sediments are somewhat analogous to soil in the sense that the bacterial flora and consequently bacterial activity are regulated by the type of organic material available and the conditions existing at a given time. The bacteria function in both soil and marine sediments as a biochemical means of regenerating the elements concerned with the carbon, nitrogen, sulfur, and Phosphorus cycles of nature, thereby prohibiting the accumulation of dead organic matter on the Soil surface as well as on the ocean floor.

Formation of hydrocarbons in marine sediments

The formation of petroleum hydrocarbons in recent marine sediments by bacteria has not been demonstrated although it is known that the sediments do contain methane producing bacteria (Thayer, L.A 1931), and certain bacteria found in sediments contain minute amounts of hydrocarbon as a part of their cell substance (Stone, R.w., and Wight, K.M., 1952 & Zobell. C.E 1952). Trask and Wu (Trask, P.D., and Wu, C.E 1930) were unable to detect liquid hydrocarbons in sediments twenty years ago but reported small amounts of solid hydrocarbons. Smith (Smith, P.V. 1952) recently has detected small amounts of hydrocarbons in marine sediments using chromatographic methods. Smith extracted sediments of the Gulf of Mexico with fat solvents and obtained about 0.031 per cent extractables which contained from 16 to 25 per cent paraffin hydrocarbons besides other hydrocarbons. Trask and Wu extracted sediments of the Florida Bay and obtained 0.062 per cent extractables which contained 8.9 per cent "paraffinaceous" material, and another of their sediment samples yielded 0.087 per cent extractable material containing

27 per cent paraffin. Trask and Wu apparently were looking for liquid petroleum in the sediments and did not attach much significance to their findings. Smith, on the other hand, with the modern methods of chromatography has been able to study the characteristics of the sediment extracts and has found them actually to resemble petroleum, although, admittedly, not identical with it. The role of bacteria in the formation of these hydrocarbons is not known, but it is known that bacterial cells contain very small amounts of hydrocarbons. In Stone's laboratory (Stone, R.w., and Wight, K.M., 1952) 400 grams of one bacterial cell mass yielded 0.25 per cent hydrocarbons, but analysis of 10 kilograms of another mass of bacteria revealed only 0.03 per cent hydrocarbons. ZoBell in 1951 (99) reported an "oily" material produced by the anaerobe *Desulfovibrio*, but so little of the material was available that an accurate identification of it was impossible. In 1952, Dr. Hanson of the Mellon Institute examined a small amount of unsaponifiable material (67 milligrams) submitted to him by ZoBell, which was described as having been produced by *Desulfovibrio* as it grew autotrophically in a synthetic medium consisting of carbonate, sulfate and other mineral salts in a hydrogen gas atmosphere. Dr. Hanson remarked: "Although it was necessary to forego some of the usual techniques employed in handling materials of this type because of the small amount available, some information on the chemical constitution of this oily extract was obtained. Chromatography made possible the separation of the total material into five distinct fractions. Although the first of these fractions could not be analyzed further, it seems likely that it was composed entirely of hydrocarbon material.

The second fraction, as shown by infrared absorption and the elementary analysis, is largely hydrocarbon of paraffinic character, and if any non-hydrocarbon components are present, they must make up a very small part of the cut. The third chromatographic fraction was the first to contain any amount of non-hydrocarbon constituents and these were largely oxygen-containing substances. Unfortunately, the remainder of the fractions could not be studied further because of the small amounts, but they are undoubtedly composed of non-hydrocarbon materials. If any nitrogen or sulfur components were present in the original sample, they must have been concentrated in the last fractions" (100). Thus, the material was apparently, in part, the hydrocarbon fraction of the bacterial cells, similar to that of the bacteria examined by Stone (Stone, R.w., and Wight, K.M., 1952).

This hydrocarbon material is synthesized by bacteria as part of the bacterial cell and, as such, very probably exists in sediments as a bacterially produced constituent of the hydrocarbon found there. Furthermore, bacterial flora under the reduced conditions of recent marine sediments would have a tendency to attack the more oxidized constituents of the sediments, thus preserving the more reduced organic material such as the lipid fraction including the hydrocarbons. Smith (Smith, P.V. 1952) recently has shown that the percentage of less polar (reduced) compounds increases with the depth of sediments; therefore, with time. Bacteria, because of their growth requirement for nitrogen, would be expected to attack preferentially the nitrogenous compounds; the sediments, therefore, become progressively less rich in nitrogenous compounds with time and depth of burial (Hammer, R.F.

1934). Trask in his extensive work (Trask, P.D. 1932) showed that ancient sediments contain a carbon/nitrogen ratio of about 14 whereas this ratio for recent sediments is 8.5. These observations may be considered as circumstantial evidence for bacterial activity, but the formation of petroleum by bacteria under adequately simulated or actual geological conditions has yet to be observed.

Treibs (Treibs, A. 1940), who has studied organically rich recent deposits such as are found in the Black Sea, is of the opinion that oil is generated from the nonlipid organic constituents in the sediments as well as from the lipid constituents. Treibs calculated petroleum generally to be 85.7 per cent carbon and 14.3 per cent hydrogen. The atom ratios are thus 7.15 to 14.3, and the empirical formula can be considered $(CH_2)_{1.1}$ for all practical purposes. Organic matter was calculated by Treibs to be 55 per cent carbon, 7 per cent hydrogen, 5 per cent oxygen and 3 per cent nitrogen (based upon a logical mixture of carbohydrate, protein and fat of which living things are composed). The atom ratios of carbon, hydrogen and oxygen then are 4.6-7-2.2. If one assumes that carbon dioxide is the most logical decomposition product of this organic mixture, the organic material thus becomes depleted of oxygen, and the ratio of carbon to hydrogen becomes 3.5 to 7 or $(CH_2)_{1.1}$, the same as the empirical formula for petroleum. It may be concluded with regard to bacterial action that it definitely can and does remove carbon dioxide from dead organic matter under anaerobic conditions and thereby contributes to its ultimate reduction making it more like petroleum.

Factors Which Affect Bacterial Activity in Sedimentary Rock

Depletion of nutrients

The first limiting factor of bacterial activity in organic sedimentary material is a lack of free oxygen. The oxygen demand of the sediments is apparently great enough to deplete free oxygen at an early stage in sedimentation (Emery, K.O. and Rittenberg, S.C. 1952). Lack of free oxygen results in the accumulation of sedimentary organic matter which otherwise would be oxidized (or mineralized) ultimately to carbon dioxide, minerals and water (Waksman, S.A. 1952). Bacterial decomposition under anaerobic conditions proceeds at a relatively slow rate, the hydrogen from the decomposable (oxidizable) organic compounds being transferred through the bacterial enzyme system to hydrogen acceptors such as oxidized organic compounds or to forms of oxidized sulfur. Thus, general anaerobic bacterial activity ultimately leads to an accumulation of more reduced organic material and hydrogen sulfide. As pointed out earlier, the activities of the sulfate reducing bacteria (*Desulfovibrio* spp.) have received a great deal of attention (ZoBell, C. E. 1946-1952) whereas other anaerobic bacteria which may be active in marine sediments have received little. *Desulfovibrio*, because of its peculiar metabolism, primarily reduces oxidized forms of sulfur rather than organic matter. If sulfate becomes limiting in the environs, activity of *Desulfovibrio* spp. ostensibly ceases. Connate waters associated with petroleum reservoirs are notably low in sulfate although there are many exceptions (Ginter, R. L. 1934).

Nitrogen in available form must be present in order for bacterial activity to proceed. As the bacteria incorporate nitrogen into their cells, it is largely converted into protein. Upon death of the cell and its subsequent decomposition the protein nitrogen is converted into ammonia and is therefore susceptible to dissipation. In this way the sediments could become depleted of available nitrogen, and the consequence would be a decrease in bacterial activity. Actually very little is known about the bacterial activity that ensues in recent marine sediments, and practically nothing is known of such activity in source beds productive of petroleum as we know it. The various stages of petroleum formation have yet to be clearly defined, and the bacterial flora, bacterial activity, or the nutritive factors influencing such activity have not been determined.

Thermodynamic considerations

It can be demonstrated in the laboratory that anaerobic bacteria convert fatty acids into methane although the production of significant amounts of higher paraffin homologs has not been accomplished (Buswell, A. M., and Mueller, H. F. 1952 & Thayer, L. A. 1931). This indicates that a bacteriological reduction of already relatively reduced organic matter in the *Desulfovibrio* bacterial cells in the form of lipids and hydrocarbons, as already mentioned. A reduced compound is possible under anaerobic conditions. Furthermore, Stadtman and Barker (Stadtman, T. C., and Barker, H. A. 1951) and Buswell and Mueller (Buswell, A. M., and Mueller, H. F. 1952) have elucidated two mechanisms for bacterially formed methane dependent upon the bacteria involved. One mechanism involves a reduction of carbon dioxide, the other a reduction of the methyl group of methanol or acetic acid. Thus, it is conceivable that still other, longer alkyl radicals can be reduced to corresponding paraffinic hydrocarbons by anaerobic bacteria. While most attempts to demonstrate this have failed- (Buswell, A. M., and Mueller, H. F. 1952 & Thayer, L. A. 1931), recently Davis and Squires (Davis, J. B. 1952) found other gaseous hydrocarbons, including ethane, in the order of a few parts per million in methane fermentations.

As organic matter becomes more reduced in the sediments, presumably because of hydrogen transfer resulting from anaerobic oxidations, it becomes progressively more difficult to oxidize because it is less susceptible to activation from a thermodynamic standpoint. The anaerobic conversion of compounds such as tyrosine to yield phenol or cresol, the alleged production of even benzene (Hammer, H. E. 1934), and the already mentioned methane formation from fatty acids indicate a bacteriological means of carrying organic matter to a state as reduced as petroleum; but these observations are not indicative of anaerobic bacterial activity in general or of such activity in sedimentary rock. There is a tendency for highly reduced organic matter to resist bacterial decomposition or modification under anaerobic conditions. Experimental work designed to subject sedimentary material in various stages of petroleogenesis to anaerobic bacterial action should serve to elucidate the effect of such action. Various ways of accelerating bacterial activity may be used, such as adjustment of the mineral concentration, temperature, pH, moisture and bacterial flora. Under optimal conditions for anaerobic bacterial activity a reasonable estimate of their potential function at

various stages of petroleogenesis may be made, provided the data are extrapolated as realistically as possible to geological conditions. The foregoing is no easy task, but approaches in the past have made realistic extrapolation of data impossible due to a distinct separation of the bacterial system being studied from the sedimentary system being considered.

Temperature and pressure

In 1946, Cox (Cox, B. B. 1946) proposed a "geological fence" secured to "posts", namely, organic matter, marine environment, temperature, pressure and time, within which the herd of facts pertaining to petroleum formation should be brought. Observations relative to bacterial activity should logically be considered in the light of known temperature and pressure ranges existent in sedimentary rock. Definite ranges of temperature and pressure exist beyond which bacteria are no longer physically stable nor biochemically active. Cox points out that petroleum is probably formed in sedimentary sections not exceeding 5,000 ft in thickness. The minimum temperature expected would be about 65 C and the maximum would be slightly higher than 100 C. Maximum pressure due to an overburden of 5,000 ft would be about 5,000 lb/sq in, hydrostatic head would be 2,000 psi. Certain bacteria can metabolize at temperatures of 55 to 75 C, and some sporeforming bacteria can resist temperature up to 100 C (Porter, J. R. 1946). Furthermore, certain bacteria which do not even form spores can apparently withstand a mechanical pressure of 75,000 psi. However, definite changes in bacterial activity can be observed under the influence of 3,000 psi. ZoBell and Johnson (Zobell, C. E., AND JOHNSON, F. H. 1949) give data to show that certain bacteria including sporeformers are killed at pressures of 7,500 and 9,000 psi in 48 hours.

Isolated observations of bacterial resistance to relatively high temperature and pressure are insufficient evidence of potential bacterial activity related to petroleum formation under geological conditions. The term "barophilic" has been coined by ZoBell and Johnson (Zobell, C. E., AND JOHNSON, F. H. 1949) to describe certain bacterial strains (some of marine origin) that grow at a pressure of 9,000 psi. Careful scrutiny of their data reveals that no marked differences exist in the pressure tolerances of some terrestrial bacteria as compared with the marine bacteria. The interesting feature of their experiments was the concomitant increase in pressure tolerance with temperature over the ranges of 1-600 atmospheres and 20-40 C. While bacterial activity may not be completely prevented by geological conditions of temperature and pressure as we know them, we have no knowledge as yet concerning such activity under these conditions. What knowledge is available pertains to very recent sediments which have no great amount of overburden, and even this knowledge is extremely limited regarding the specific bacterial flora and activities involved.

Eviden Regarding Biogenesis of Petroleum

Constitution of crude oil as opposed to known bacterial hydrocarbon products

Van Nes and Van Westen (Van Nze, K., And Van Westen, H.

A. 1951) point out that it is logical to assume crude oil to contain cyclic compounds similar in basic structure to those which occur in living organisms. Terpenes, sesquiterpenes, and polyterpenes which appear to be polymerized isoprene units occur abundantly in nature (especially in plants), and these type compounds are amply represented in petroleum. Furthermore, the sulfur, nitrogen and oxygen containing compounds of petroleum very likely are similar to compounds found in living nature although little pertinent information regarding this is available. Bacteria could hardly be responsible for the biosynthesis of the myriad compounds in crude oil, e.g., the hydrocarbon components which make up about 95 per cent of petroleum consisting of varying amounts of paraffinic, naphthenic and aromatic groups. While the constitution of the hydrocarbon fraction of bacterial cells is not known in detail (Stone, R. W., And Zobell, C. E. 1952 And Zobell, C. E. 1952), it is certainly not analogous with crude oil. Methane is the only hydrocarbon known to be produced extra cellular in any quantity by bacteria. It appears, therefore, that their function in petroleogenesis is confined to some modification of the precursor organic material rather than actual conversion of this material into crude oil.

Another possible assumption, which seems farfetched, is that bacteria utilize all protopetroleum, converting it into their own cell substance (containing small amounts of hydrocarbon), the nonhydrocarbon fraction of which is reconverted again by other bacteria into cell substance containing small amounts of hydrocarbon, and so ad infinitum. The result, ostensibly, is an eventual accumulation of hydrocarbons, a disappearance in proto-petroleum and a small residual bacterial flora. It would follow however, that the hydrocarbon fraction of bacterial cells very closely resembles petroleum, while actually it appears to be almost exclusively paraffinic (Stone, R. W., And Zobell, C. E. 1952 And Zobell, C. E. 1952). It is difficult to visualize the process of events just described for many reasons, among them being the observation that crude oil contains many compounds, including chlorophyll porphyrin (Treibs, A. 1940), which could not be formed by bacterial synthesis after sedimentation. Bacterial action must at least be limited to the formation in sediments of those compounds which are conceivably formed by bacteria, regardless of the time which bacteria are active in the sediments.

Observation concerning bacteria in reservoir rock

In 1952, Schwartz and Mueller (Schwartz, W., And Mueller, A. 1952) reported anaerobic bacteria in oil bearing sands in Western Germany where "oil is recovered by mining". While they failed to find aerobic microbial forms such as mold fungi, actinomycetes or strictly aerobic bacteria, they think that the anaerobes could have invaded the oil fields "after opening of the mines". The authors referred to the observations made by certain USSR and USA scientists regarding bacteria in crude oil and associated brines. They maintained that a discrepancy exists between the presence of so many kinds or species of bacteria in reservoir fluids taken from oil wells and the presence of only a small number of strictly anaerobic forms in marine source beds. Schwartz and Mueller think this may be caused by a secondary invasion of the oil reservoirs during drilling operations. Drilling muds sometimes contain many

millions of bacteria per milliliter.

Ekzertzev (Ekzertzev, V. A. 1951) in 1951 described observations made of the bacterial flora in oil reservoirs near Vtoroi Baku in Russia. The depth of the samples ranged from 1,000 to 6,000 ft. He reported finding 12 to 117 million bacterial cells per gram of dry sample in oil bearing rock, but no bacteria from horizons devoid of oil. Ekzertzev mentioned technical difficulties in making the microscopic bacterial counts and gave no descriptions of the bacteria observed. It is conceivable that bacterial cells would be difficultly differentiated from oil globules in the oil bearing rock sample preparation. Microscopic examination by bacteriologists of oil reservoir rock from other regions would be of interest.

Comparison of petroleum genesis with coal formation

Plant materials consisting primarily of lignin and cellulose, which have accumulated under conditions adverse to microbial decomposition, appear to be the source of coal (Hendricks, T. A. 1945). One outstanding feature of these accumulations is the preponderance of organic matter relative to inorganic matter. The most accepted mechanism for coal formation is through the peat state where microbial action, though slow, operates over long periods of time modifying the organic matter and converting it into "humus". The conversion of peat into lignite, then bituminous coal, and finally anthracitic coal is conceded to be due to physicochemical changes brought on by compaction and heat during geologic time. Coal formation certainly is largely an in situ process, and the observed fossil imprints of leaves and other organized plant structures, even in the advanced bituminous state of coal, point to its origin. It is assumed that while bacterial action has had some part in the modification of coal in the peat state, such action could not be responsible for the later changes in physicochemical composition which result in lignite, bituminous and anthracitic coal.

Petroleum formation, on the other hand, is not so well outlined. Without regard to a discussion of the differences in source material leading to either coal or petroleum, suffice it to say that petroleum may or may not be formed in situ and modification of it may actually take place during migration. The organic source material of petroleum has very probably undergone some modification by bacteria, just as has coal in the peat state, but a most important distinction exists in the respective environments of the source materials during petroleum and coal genesis. As mentioned above, coal originates from organic accumulations which contain relatively little inorganic matter, e.g., as in swamp conditions; therefore little inorganic surface is in contact with the organic matter. Petroleum in its various stages of formation is presumed to have been constantly in intimate contact with a large inorganic surface as a result of its marine sedimentary origin (15). The catalytic action of surface might influence a conversion into petroleum of the trapped organic matter which escapes bacterial decomposition. Brooks (Brooks, B. T. 1948-1949) discusses the possible role of active surface minerals in petroleum formation at the moderate temperatures prevailing in oil producing reservoirs.

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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