Microbial Techniques for Hydrocarbon Exploration

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Additional information is available at the end of the chapter

1. Introduction

Bacteria are ubiquitous in distribution and their exceptionally high adaptability to grow on different nutrient sources form the basis of microbial prospecting. Several investigators have used bacteria that degrade hydrocarbons, used as indicators for finding oil and gas reservoirs. Microbial prospecting method for hydrocarbons is a surface exploration method based on the premise that the light gaseous hydrocarbons namely methane (C_1), ethane (C_2), propane (C_3) and butane (C_4) migrate upward from subsurface petroleum accumulations by diffusion and effusion (Horvitz, 1939) and are utilized by a variety of microorganisms present in the sub-soil ecosystem. The methane, ethane, propane, and butane-oxidizing bacteria exclusively use these gases as a carbon source for their metabolic activities and growth. These bacteria are mostly found enriched in the shallow soils/sediments above hydrocarbon bearing structures and can differentiate between hydrocarbon prospective and non-prospective areas (Tucker and Hitzman, 1994). The detection of various groups of methane, ethane, propane or butane oxidizing bacteria, in the surface soils or sediments, helps to evaluate the prospects for hydrocarbon exploration. Microbial prospecting is a surface prospecting technique,, well known in the realm of hydrocarbon exploration researchers. Microbial anomalies have been proved to be reliable indicators of oil and gas in the sub-surface (Pareja, 1994). The direct and positive relationship between the microbial population and the hydrocarbon concentration in the soils have been observed in various producing reservoirs worldwide (Miller, 1976; Sealy 1974a, 1974b; Wagner et al., 2002). These light hydrocarbon are utilized by a phylogenetically diverse group of bacteria belonging to genera of *Brevibacterium, Corynebacterium, Flavobacterium, Mycobacterium, Nocardia, Pseudomonas, Rhodococcus* etc., (Perry and Williams, 1968; Vestal and Perry, 1971). The microbial prospecting method involves the collection of sub-soil samples from the study/survey area, packing of samples, preservation and storage of samples in pre-sterilized sample

bags under aseptic and cold conditions till analysis and followed by isolation and enumeration of hydrocarbon utilizing bacteria. The contour maps for population density of hydrocarbon oxidizing bacteria are drawn and the data is integrated with other geo-scientific and geophysical data to find the hydrocarbon prospectivity of the area.

The microbial survey was first proposed and applied in the U.S.S.R. Early use of bacterial soil flora as a means of detecting gas seepages was a development stemming from soil gas surveys performed in the U.S.S.R (Mogilevskii 1959). The initial microbial investigations of Mogilevskii and his associates in the field of petroleum prospecting incited the interest of petroleum geochemists worldwide. The microbial surveys were first proposed and applied in the U.S.S.R, (Mogilevski, 1940). It has been shown that out of 20 microbial anomalies, 16 were proved by successful drilling. These investigations initiated the interest of petroleum geochemists worldwide. Sealy (1974a) in USA, carried out microbial prospecting surveys and showed a positive correlation of 85.7%. Miller (1976) reported microbial survey carried out in the oil fields of the U.S.A, in which the microbial activity profile indicated a good contrast between oil fields and nearby dry area. Beghtel (1987) predicted hydrocarbon potential of 18 wildcat wells in the Kansas; out of which, 13 have proved to be commercial producers of oil and gas. As a result, microbial methods for detecting petroleum gas in the soil and waters have been tested in Europe, U.S.A and elsewhere. A sustained effort for the purpose of efficiently utilizing microbiological prospecting methods had been underway since 1938 including independent surveys, surveys coordinated with geochemical and geophysical operation, microbiological tests of both soils and sub surface waters and analysis for methane oxidizing as well as higher hydrocarbon oxidizing bacteria. However, oil and gas fields also build up micro-seepages at the sub-surface soils, and these micro-seepages are detectable using a variety of analytical techniques that have been developed in the past 70 years. Geochemists developed the basis for these new surface prospecting methods. The pioneers were Laubmeyer (1933) in Germany, Rosaire (1939) and Horvitz (1939) in the United States. Using methods such as extraction of adsorbed hydrocarbon gases from surface samples, they documented a correlation between higher hydrocarbon concentrations and oil and gas fields. At almost at the same time, the microbiologists Mogilewskii (1938, 1940) in the U.S.S.R. and Taggart (1941) and Blau (1942) in the United States described the use of hydrocarbon-oxidizing bacteria (HCO), when measured in surface soil samples, as an indicator for oil and gas fields in the deeper subsurface. In the 1950s and early 1960s, many relevant publications came from the United States (Updegraff et al., 1954; Davis, 1956). The U.S.S.R. (Bokova et al., 1947; Davis, 1967 and Sealy, 1974 a, b) have published reviews of this early work. Several microbiological methods for detecting the distribution and activity of HCO were developed, such as enumeration of cell content in soil samples, measuring gas-consumption rates and radioautography. The field trials conducted by Sealy (1974) in U.S.A., using microbiological techniques, showed that out of 89 locations tested in west Texas, wildcats predicated as productive and non-productive correlation of 54% and 92% respectively. Sealy (1974) reported positive prognosis for producers, 8 out of 11 and for dry holes 28 out of 31, having an overall correct prognosis of 85.7%. Miller (1976)

reported microbial survey of a number of oil fields in the U.S.A. some of which were primarily stratigraphic and showed that microbial activity profiles indicated a good contrast between oil fields and nearby dry areas. Beghtel et al., (1987) described a new Microbial Oil Survey Technique, named MOST, which uses the higher butanol resistance of butane-oxidizing bacteria to detect hydrocarbon micro-seepages. Microbial anomalies have been proved to be reliable indicators of oil and gas in the subsurface (Pareja et al., 1994). Hydrocarbon micro-seepage detection adds value to 2-D and 3-D seismic by identifying those features that are charged with hydrocarbons (Schumacher, 1997). There is a direct positive relationship between the increased hydrocarbon concentrations and increased hydrocarbon indicating microbial populations. This relationship is easily measurable and distinctly reproducible. Microbial anomalies can also used for development of field and reservoir characterization studies (Hitzman et al 1999, Hitzman 1994, Schumacher et al 1997).

Microbial Prospecting survey has been widely used in Germany since 1961 and a total of 17 oil and gas fields were identified. The success rate of Microbial Prospecting for Oil and Gas (MPOG) method has been reported to be 90%. This method can be integrated with geological, geochemical and geophysical methods to evaluate the hydrocarbon prospect of an area and to prioritize the drilling locations; thereby, reducing drilling risks and achieving higher success in petroleum exploration (Wagner et al., 2002).

1.1. Oxidation reduction zones

Bacteria and other microbes play a profound role in the oxidation of migrating hydrocarbons. Their activities are directly or indirectly responsible for many of the diverse surface manifestations of petroleum seepage. These activities, coupled with long-term migration of hydrocarbons, lead to the development of near-surface oxidation-reduction zones that favor the formation of this variety of hydrocarbon-induced chemical and mineralogical changes. This seep-induced alteration is highly complex and its varied surface expressions have led to the development of an equally varied number of geochemical exploration techniques. Some detect hydrocarbons directly in surface and seafloor samples, others detect seep-related microbial activity, and still others measure the secondary effects of hydrocarbon-induced alteration.

The activities of hydrocarbon oxidizing bacteria cause the development of near-surface oxidation –reduction zones and the alteration of soils and sediments above the reservoirs. These changes form the basis for other surface exploration techniques, such as soil carbonate, magnetic, electrical, radioactivity and satellite-based methods (Richers et al., 1982; Schumacher, 1996).

2. Indicators of microbial prospecting

Methane, ethane, propane and butane oxidizing bacteria have been used by various researchers as indicator microbes for prospecting of oil and gas.

3. Methane oxidizing bacteria

Methane oxidizing bacteria were the first type of bacteria studied to identify the location of petroleum accumulations. The presence of methane oxidizing bacteria in the soil, in the absence of cellulose oxidizing bacteria, has been interpreted as indicating the presence of methane exhalation from the subsurface (Tedesco, 1995). Mogilewskii (1938) described the possibility of using methane-oxidizing bacteria for gas exploration. Methane oxidizing bacteria were found in the petroleum prospecting operations of the Soviet Union (Kartsev et al., 1959). Methane oxidizing bacteria have been isolated from soil as a means of prospecting from natural gas and/or oil deposits (Brisbane and Ladd, 1965). The methane oxidizing bacteria are usually predominant over gas fields as the gas reservoirs are commonly dominated by methane. Microbial Prospection for Oil and Gas (MPOG) method establish the separate activities of methane-oxidizing bacteria as gas indicators and those bacteria that oxidize only ethane and higher hydrocarbons as oil indicators, it is possible to differentiate between oil fields with and without free gas cap, and gas fields (Wagner et al., 2002). Methane oxidizing bacteria have been deployed as one of the indicator microbes (Jain et al., 1991). Thermogenic processes produce methane and substantial amounts of other saturated hydrocarbons by irreversible reaction of residual organic matter or kerogen (Klusman, 1993). Whittenbury et al., (1970) reported the isolation of more than 100 strains of methane-oxidizing bacteria. Hanson and Wattenberg (1991) and Hanson and Hanson (1996) gave overviews of the ecology of methylotrophic bacteria and their role in the methane cycle. The methane oxidizing bacteria (Methylotrophs) are *Methylococcus, Methylomonas, Methylobacter, Methylocyctis, Methylosinus, Methylobacterium* etc., (Hanson and Hanson 1996). However, methane oxidizing bacteria are known to be poor indicators in petroleum prospecting because methane can occur in the absence of petroleum deposits and moreover, some methane oxidizing bacteria are unable to oxidize other aliphatic hydrocarbons. Detection of ethane and longer chain hydrocarbon oxidizing bacteria on the other hand provides presumptive evidence for a hydrocarbon seep and an underlying petroleum reservoir (Davis, 1967). The principal advantage of using methane-oxidizing bacteria for petroleum prospecting is the preponderance of methane in petroleum gas and it is the most mobile of petroleum hydrocarbons and this cannot be ignored.

3.1. Microbial oxidation of methane

Microbial methane oxidation starts with an activation of methane by methane-oxygenase (MMO), this enzyme is a mono-oxygenase because only one atom of the dioxygen molecule is inserted into the methane molecule to produce methanol; and leads, in the presence of oxygen, to methanol and then to formaldehyde. Formaldehyde can be directly assimilated to produce biomass or oxidized to CO_2 for the generation of energy (Leadbetter and Forster 1958). Because of the high specialization of these bacteria, methane oxidizers can be isolated from all other bacteria. The successful detection of these specialists indicates methane occurrence in soil samples (Wagner et al., 2002).

4. Ethane, propane and butane oxidizing bacteria

The Soviets were first to use microbial prospecting method. They established that certain bacteria specifically consume ethane, propane, or butane, but not methane.   These hydrocarbons are assumed to be from petroleum migrating from depth and are not associated with generation in the soil (Tedesco 1995).   The isolation and enumeration of specific C_{2+} alkane-oxidizing bacteria have been used as indirect petroleum prospecting method (Davis, 1967, Wagner et al., 2002). The detection of bacteria that oxidize *n*-alkanes having chain lengths of 2 to 8 carbon atoms, without any adaptation period, indicates the existence of short-chain hydrocarbons in the investigated soil samples, and thus can indicate the presence of oil accumulations in the subsurface. In those areas in which both short-chain hydrocarbons and methane are detected in this manner, one can assume a thermogenic gas with significant quantities of short-chain hydrocarbons, depending on the intensity of the signals (Wagner et al., 2002). The short-chain hydrocarbons ethane, propane, and butane can be used by a large number of bacteria (*Mycobacteria, Flavobacteria, Nocardia*, and *Pseudomonas*). The microbial anomalies have proven to be reliable indicators of oil and gas in the subsurface (Beghtel et al, 1987; Lopez et al., 1993; Tucker and Hitzman, 1994). The microbial prospecting method has been used to prioritize the drilling locations and to evaluate the hydrocarbon prospects of an area (Pareja, 1994)**,** thus reducing risks and achieving higher success ratio in petroleum exploration.

4.1. Microbial oxidation of ethane, propane and n-butane

The microbial oxidation of ethane, propane and n-butane proceeds stepwise to alcohols, then to aldehydes and finally to acetate, which can be assimilated into cell material (Figure 2). The number of species of bacteria capable of using such hydrocarbons in this process increases with the expansion of the chain length of the alkanes. The degradation of the alkanes occurs by terminal oxidation by means of mono-oxygenase and by β-oxidation to acetyl-CoA, which is the initial substance in several biochemical reactions (Atlas, 1984).

5. Halo and apical anomalies

Distinct and definite petroleum gas seepage could be readily identified by geochemical means such as gas chromatographic analysis. There is a possibility that chemically detectable petroleum gases will be absent in the soil receiving micro seepages, due to microbial oxidation. Idealized model of bacterial effect on adsorbed soil gaseous hydrocarbons showed negative signal (Halo anomaly) for adsorbed soil gas and positive signal (Apical anomaly) for microbial activity. (Richers, 1985; Tedesco, 1995; Schumacher, 1996).   The 'halo' theory may be reconciled on the basis of microbiology. It is observed that bacteria present over the petroleum accumulation would consume the petroleum gases adsorbed to the soil surface in this higher concentration zone thus, markedly decreasing the concentration of gas directly over soil receiving micro seepages, but the

bacteria would not be stimulated to utilize the lower 'edge' concentrations. In soil-gas analysis, the edge or halo concentrations would show little difference from soil gas over the soil receiving microseepages. However, in soil analysis (gas desorption) samples from the halo region would actually have a higher concentration of hydrocarbons (Rosaire et al, 1940) since bacteria have not been stimulated to develop on hydrocarbons adsorbed to soil in this lower emanation area. The consumption of vertical migrating light hydrocarbons by bacteria will result in varying degrees of depletion of the hydrocarbons in the free soil gas and in hydrocarbons adsorbed soil gas (Richers 1985, Klusman 1993). The light hydrocarbons micro seepage is preferentially consumed over the area of highest seepage, resulting in a high rate of bacterial activity. The bacteria present over the petroleum accumulation would consume petroleum gases, whereas the edges will show high concentration of gases as not being utilized by bacteria where the microbial activity is very low. Thus geochemical technique of quantitative or qualitative adsorbed soil gas analysis may have limitations in place where high soil microbial activity exists resulting in nearly complete utilization of soil gases by microbes. The light hydrocarbon distribution will show a low signal directly over the source, resulting in a halo anomaly. The ratio of bacterial activity to hydrocarbon concentration will then exhibit an apical anomaly. The microbial indicators are therefore target specific, associated directly over the oil pool, where microbes flourish utilizing upcoming hydrocarbon gases. The Halo anomaly theory for hydrocarbon exploration is reconciled on the basis of microbiology, and has significant importance in hydrocarbon exploration (Horvitz, 1981; Jillman, 1987; Baum, 1994).

6. Sample collection method

The geo-microbial surveys of prospecting involve collection of suitable samples from sub soil horizon and detection of specific micro flora in the samples. Sampling is important since the validity of the test results depend largely upon the manner in which the samples are taken. The samples are collected using a hollow metal pipe by manual hammering. The soil samples of about 100 gm each were collected in pre-sterilized whirl-pack bags under aseptic conditions from a depth of about 0.5 to 1m (Davis 1967), and preferably on a grid pattern with a spacing of 200m. The samples were immediately stored at 2 to 4°C and subsequently transported to the laboratory and are stored cryogenic conditions till analysis. Soil samples should not be stored for long time, and samples should be analyzed as early as possible after collection.. Sampling should not be done in disturbed or excavated areas, soils contaminated with hydrocarbons, chemicals or animal wastes, swamps and areas under water shed. During collection of soil samples, rocks, coarse materials, plant residues, and animal debris have to be excluded.

7. Isolation of hydrocarbon oxidizing bacteria

The specific bacterial populations are measured by standard microbiological screening techniques for hydrocarbon-indicating microorganisms. A selective growth media is used

which cultures only microorganisms capable of utilizing light hydrocarbons. Various methods such as Bacterial pellicle formation, Gas uptake, soil dilution and plating, soil sprinkled plating, clumped soil plating and Radio autography have been used by various researchers to determine the quantitative abundance of hydrocarbon oxidizing bacteria or oxidation of gaseous hydrocarbons. However gas uptake and soil dilution and plating methods have been widely used. Other techniques can determine the presence of living bacterial cells in soil samples. The determination of the number of colony-forming units (CFUs) in solid feeding media and the most-probable-number (MPN) procedure in nutrient solutions can be applied.

Isolation and enumeration of methane oxidizing bacteria and (C2+) ethane/propane/butane oxidizing bacteria for each sample is usually carried out by Standard Plate Count (SPC) method. 1 gm of soil sample was suspended in 9 ml of pre-sterilized water for the preparation of decimal dilutions (10^{-1} to 10^{-5}). A 0.1 ml aliquot of each dilution was placed on to Mineral Salts Medium (MSM) petri plates containing 1.0 g of $MgSO_4.7H_2O$, 0.7 g of K_2HPO_4, 0.54 g of KH_2PO_4, 0.5 g of NH_4Cl, 0.2 g of $CaCl_2.2H_2O$, 4.0 mg of $FeSO_4.7H_2O$, 0.3 mg of H_3BO_4, 0.2 mg of $CoCl_2.6H_2O$, 0.1 mg of $ZnSO_4.7H_2O$, 0.06 mg of $Na_2MoO_4.2H_2O$, 0.03 mg of $MnCl_2.4H_2O$, 0.02 mg of $NiCl_2.6H_2O$, and 0.01 mg of $CuCl_2.2H_2O$ in 1000 mL of distilled water, at pH 7.0. These plates were placed in a glass desiccator, filled with the desired hydrocarbon gas (methane/ethane/propane with 99.99 % purity) and zero air (purified atmospheric gas devoid of hydrocarbons) in a ratio of (1:1). For isolation of methane oxidizing bacteria, the desiccator was filled with methane gas and zero air. Similarly, for isolation of ethane, propane and butane oxidizing bacteria, the desiccators were filled with either ethane/propane/butane gas with zero air respectively, whereas for isolation of n-pentane or n-hexane oxidizing bacteria, these plates were placed in glass desiccators containing air saturated with n-pentane or n-hexane vapor respectively (Rasheed, 2011). These desiccators were kept in bacteriological incubators at 35 ± 2°C for 10 days. After incubation, the developed bacterial colonies of methane, ethane, propane and butane oxidizing bacteria were manually counted using colony counter and reported in colony forming unit per gram (cfu/gm) of soil sample (Wittenbury et al., 1970; Rasheed et al. 2008).

8. Molecular biology techniques

Currently, molecular biology techniques has achieved great development in studies of soil samples. Development of molecular biology methods for microbial prospecting for oil and gas by applying culture independent techniques will improve the accuracy rate of microbial prospecting for oil and gas exploration (Fan Zhang et al., 2010). The most-probable-number (MPN) procedure has traditionally been applied to determine the numbers of colony forming units (CFUs) in soil samples. The real-time polymerase chain reaction (RT-PCR) is now being widely used to detect and quantify various target microorganisms without experimental cultivation (Dionisi et al. 2003; Skovhus et al. 2004; He et al. 2007). Molecular techniques related with 16S ribosomal DNA (RNA) have been proven effective as a basis for understanding the

microbial diversity in environmental communities. The cloning and sequencing of 16S rDNA is sufficient for the identification of the microorganisms present in a given habitat and for the discovery of previously unknown diversity (Hugenholtz et al. 1998). These techniques were also applied to investigate microbial communities in the formation water of the produced water of oil fields (Kaster et al. 2009; Lysnes et al. 2009). Characterization of microbial communities involved in short-chain alkane metabolism, namely methane, ethane and propane, in soil samples from a petroliferous soils through clone libraries of the 16S rRNA gene of the Domains Bacteria and Archaea and the catabolic gene coding for the soluble di-iron monooxygenase (SDIMO) enzyme alpha subunit. Further studies on the occurrence and diversity of SDIMO genes in soil, as well as the improvement of primer sets to be applied in real-time PCR, are necessary in order to overcome the obstacle of the low abundance of catabolic genes in natural environments and enable their quantification from complex genetic backgrounds (Paula, et al., 2011). Immunological or DNA probes for gaseous hydrocarbon utilizing bacteria; Researchers are on for developing immunological probe or DNA probe which will rapidly identify the specific hydrocarbon oxidizers from the survey area. Immunological probes are based on the technique of preparing monoclonal antibodies as probes. In DNA probes, a selective small piece of DNA either from plasmid or from chromosomal segment serves as a probe. This segment is coded with the gene sequence, responsible for specific hydrocarbon gases oxidation. DNA hybridization with homologous strain gives the detection of hydrocarbon oxidizers. Detection of Biomarkers – Diploterol; The hapanoid diploterol is a known product of various aerobic bacteria that is particularly abundant in methanotrophic bacteria. The methanotrophs are in fact the source of diploterol is indicated by the compound light isotopic composition or around -60 per mil. Moreover 12C enriched hydrocarbon derivatives of diploterol were found in other studies of sediments in seep environments. This finding makes it a promising developmental technique for bio-prospecting of hydrocarbons. The above methods are the future thrust areas in bio-processing and will enhance the efficacy of the technique.

9. Plotting of bacterial anomaly

The results of hydrocarbon oxidizers population are plotted in terms of population density of aerial basis on the surveyed map using Arc GIS (Geographical Information System) or Golden Surfer Software's. A statistical approach has been followed and standard deviation value is taken as a background value for the demarcation of anomalous zones. The results of hydrocarbon oxidizing bacterial population are plotted on the surveyed map.

10. Evaluation of bacterial anomalaies

The samples showing bacterial population less than the background values indicate negative prospects, while the value above the standard deviation value gives the anomalies concentration of these gaseous hydrocarbon oxidizers. If the results of investigations are negatives; the completeness and the accuracy of the observations must be determined beforehand because the negative results may be due to various defects in the methods of field and laboratory work, such as insufficient sample taken at the location, inadequate reproducibility of bacterial cultures and keeping the samples for a

long time. Bacterial anomalies detected in a soil survey may be classified as focal or continuous anomalies according to number of features depending upon a degree of localization of positive points. The anomalies are superimposed on prospect map or other geological or geophysical inputs for integration and interpretation. Classification of microbial anomalies according to the predominating types of indicator microorganism is also essential.

11. Case histories

We have presented some of the case histories of microbial surveys carried out in various sedimentary Basins of the Indian subcontinent by National Geophysical Research Institute (NGRI-CSIR), Hyderabad, India. A microbial survey was carried out in the established regions of the Kadi Kalol oil and gas fields of the Mehsana Block, Cambay basin, where the hydrocarbon utilizing bacteria ranged between 10^6 and 10^7 cfu/gm of soil (Table 1). In the other well-known areas, such as the Ponnamanda and Tatipaka gas fields of the Krishna Godavari basin, the hydrocarbon utilizing bacterial counts of these two areas were found to be 10^5 cfu/gm of soil, indicating the adaptation of microbes to utilize hydrocarbon seepage and possible presence of hydrocarbon deposits. The Oil and Natural Gas Commission (ONGC) of India has reported the presence of recoverable deposits of petroleum in these two areas. In the oil fields of Mehsana, and KG Basin, it is found that the number of hydrocarbon utilizing bacteria from a petroliferous area is in the range between 10^5 and 10^7 cfu/gm of soil. In the Jaisalmer gas fields the number of bacteria per gram of soil was always greater than 10^4 cfu/gm of soil. In the established oil and gas fields of Mehsana, Cambay basin, Jaisalmer basin and Krishna Godavari basin, soil samples showed bacterial growth $\geq 10^4$ account for 92%, 80% and 90% respectively.

The bacterial counts of these established oil and gas fields were ranged between 10^3 and 10^7 cfu/gm, while in the exploratory area, the soil samples showed bacterial growth $\geq 10^4$, indicating that oil or gas exist in the latter area, which was eventually found to be correct after drilling operations (Rasheed et al, 2011) . The possibility of discovering oil or gas reservoirs by the microbiological method is emphasized by the fact that the count of hydrocarbon-oxidizing bacteria in soil or sediment samples ranged between 10^3 and 10^6 cfu/gm in soil/sediment receiving hydrocarbon micro-seepages, depending on the ecological conditions (Wagner et al, 2002).

Sampling area	Total no. of samples	Hydrocarbon utilizing bacteria (cfu/gm) of soil
Mehsana (oil/gas fields)	135	$10^6 - 10^7$
Jaisalmer (Gas fields)	100	$10^4 - 10^5$
Krishna Godavari Basin (Gas fields)	150	10^5
Shri Ganga Nagar Block, Rajasthan (Oil field)	150	10^5

**Table 1.** Hydrocarbon utilizing bacterial count of various established oil and gas fields in India.

11.1. Bikaner-Nagaur basin

A geo-microbial survey was carried out in the Shri Ganga Nagar Block of Bikaner-Nagaur basin, Rajasthan to investigate the prospects for hydrocarbon exploration. The propane oxidizing bacterial counts in the study area of the Shri Ganga Nagar Block were found to be ranged between 1.0×10^2 and 3.84×10^5 cfu/gm. The bacterial concentrations are plotted on the surveyed map using Arc GIS software. The bacterial concentration distribution maps of hydrocarbon utilizing bacteria show distinct anomalies in the studied area. The hydrocarbon oxidizing bacterial count ranged between 10^5 and 10^6 cfu/gm of soil, which is significant and thereby substantiate the seepage of lighter hydrocarbon accumulations from the subsurface petroleum reservoirs. The map of propane utilizing bacteria of the study area showed distinct microbial anomalies, which confirm the seepage of light hydrocarbons from the subsurface oil/gas reservoirs. The microbial results showed high propane oxidizing bacterial population in the studied area of the Bikaner Nagaur Basin, indicating positive prospects for hydrocarbon exploration. The GAIL (Gas Authority of India Limited) has reported the presence of recoverable deposits of petroleum in this area. (Figure 1).

11.2. Mehsana block, Cambay basin

Microbial prospecting studies were carried out in known petroliferous Mehsana Block of North Cambay Basin, India. A set of 135 sub-soil samples collected, were analyzed for indicator hydrocarbon oxidizing bacteria. The bacterial concentration map showed anomalous zones of propane oxidizing bacterial (Figs. 4).It is observed that the bacterial anomalies are found away from the oil and gas wells. The hydrocarbon microseepage is dependent upon pressurized reservoirs driving the light hydrocarbon microseepage upward. The pattern of reduced microbial counts adjacent to producing wells has been a commonly observed phenomenon for older producing fields. Over some well-drained gas reservoirs, the microbial values have been found to even be anomalously low. The phenomenon of apparent microseepage over the shutdown producing fields is thought to be due to a change in the drive mechanism controlling microseepage. When a well is brought into production, the drive mechanism changes from vertical, buoyancy driven force to horizontal gas streaming to the pressure sinks created around producing wells. When this occurs, microseepage greatly decreases or stops and microbial populations at the surface decline rapidly.

This change in drive mechanism and microbial population densities can be used to define reservoir drainage direction, radius, and heterogeneities around existing wells in producing fields (Tucker and Hitzman, 1994). In undrilled areas this phenomenon will not occur and there will be a direct relationship between high microbial populations, micro seepage, and potential reservoirs. Anomalous hydrocarbon microseeps are identified by observing bacterial population concentrations and distribution patterns within a survey area. There is a direct and positive relationship between the light hydrocarbon concentrations in the soils and these microbial populations. Surface contamination of produced oil and changing soil types do not affect these light hydrocarbons reflected in the microbial population distributions.
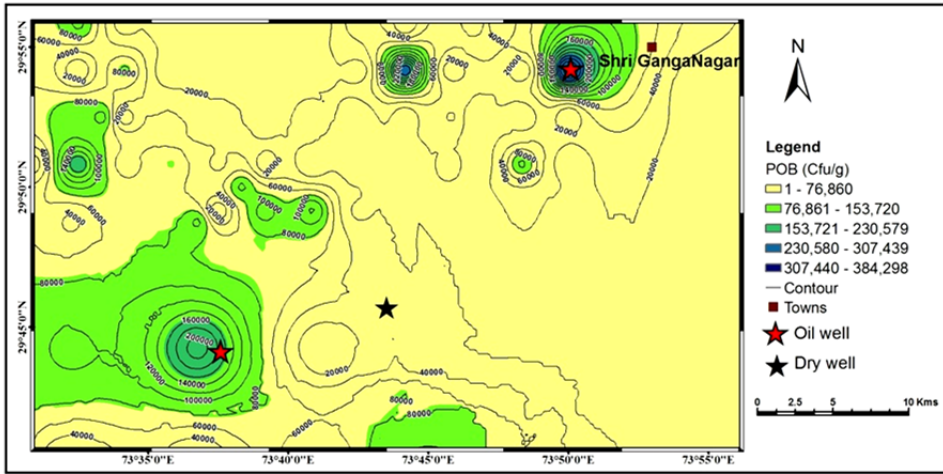
Figure 1. Results of microbial prospection studies in Shri Ganga Nagar Block, Rajasthan Basin, India.
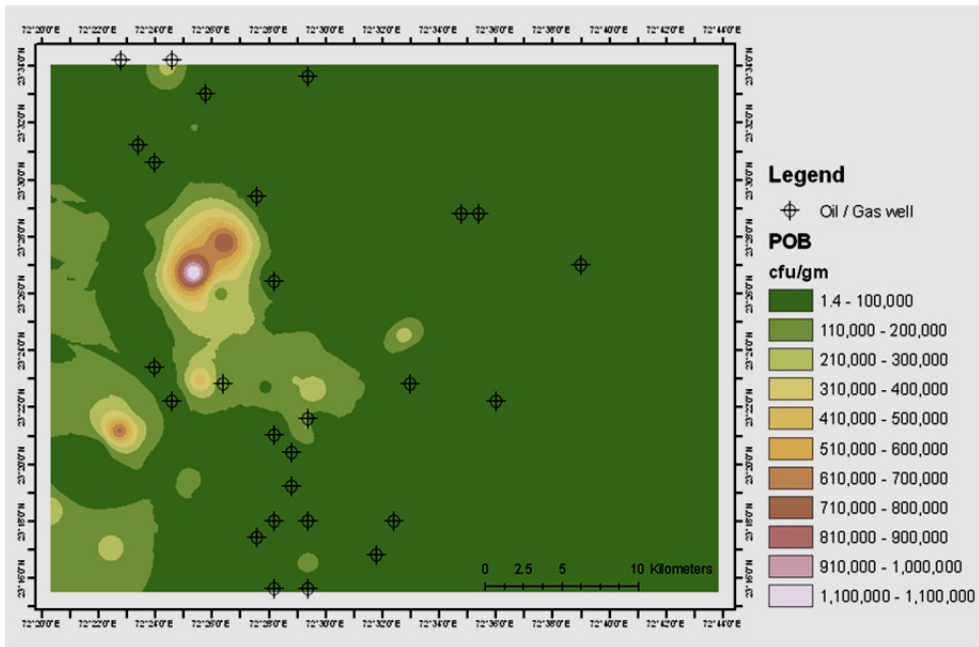
Figure 2. Map of Microbial survey using propane oxidizing bacteria (POB) in producing oil and gas field of Mehsana, Cambay Basin, Gujarat, India.

11.3. Advantages

i.   Geo-Microbial prospecting method is well known in the realm of hydrocarbon exploration. Microbial prospecting method can be integrated with geological, geophysical and other surface hydrocarbon prospecting techniques. Microbiological methods have potential as a hydrocarbon exploration tool, development and extension of older fields.   The microbial prospecting method is mainly used to prioritize the drilling locations and to evaluate the hydrocarbon prospects of an area (Pareja, 1994; Tucker and Hitzman, 1994 and Schumacher, 2000) thus reducing risks and achieving higher success ratio in petroleum exploration.

ii. One of the main advantages of the microbial prospecting method is, this method can be used for identification for prospective oil and gas in areas where no geophysical data is available, or where such investigation is difficult.

iii. Microbial anomalies have been proved to be reliable indicators of oil and gas in the subsurface (Pareja 1994). Hydrocarbon micro seepage detection adds value to 2-D and 3-D seismic by identifying those features that are charged with geomicrobial anomalies and sub-surface petroleum accumulations can be complex; on proper integration with geological and geophysical data, can contribute to the success of exploration and helps in risk reduction of dry wells. Since the drilling operations are costly, it is essential to use appropriate and efficient exploratory methods, either singly or in combination, in order to cut down the drilling cost of dry holes as well as wild cats with unprofitable recovery. Microbiological prospecting methodology is a valuable and less expensive value addition exploration tool to evaluate the valuable seismic prospects. This method can substantially reduce the exploration risks associated with trap integrity and hydrocarbon charge, especially in the hunt for much elusive subtle traps.

iv. The method can also be used independently and basically no geological or seismic data is required to carry out microbial prospecting surveys. In areas that have not yet been investigated geophysically, this technique can be applied as wildcat prospecting tool. This method can give principal evidence on the occurrence of hydrocarbon anomalies in large areas. The subsequent seismic and geological investigations can thus be, concentrated on favorable areas in those regions where structure data of the sub-surface already exists. This approach likewise does not require any knowledge of the position of the structures. As a result therefore, the seismic structure maps and microbial anomalies, which have been drawn up independently from one another, can be compared and contrasted.

v.   Distinct and definite petroleum gas seepage could be readily identified by geochemical means such as gas chromatographic analysis. There is a possibility that chemically detectable petroleum gases will be deficient in the soil receiving micro-seepages, due to microbial oxidation. The consumption of vertical migrating light hydrocarbons by bacteria will result in varying degrees of depletion of the hydrocarbons in the free soil gas and in hydrocarbons adsorbed soil gas (Richers 1985, Klusman 1993). The light hydrocarbons micro seepage is preferentially consumed over the area of highest seepage, resulting in a high rate of bacterial activity. The bacteria present over the

petroleum accumulation would consume petroleum gases, and the edges will show high concentration of gases as not being utilized by bacteria where the microbial activity is very low. Thus geochemical technique of quantitative or qualitative adsorbed soil gas analysis may have limitations in place where high soil microbial activity exists resulting in partial or complete utilization of soil gases by microbes. The light hydrocarbon distribution will show a low directly over the source, resulting in a halo anomaly. The ratio of bacterial activity to hydrocarbon concentration will then exhibit an apical anomaly. The microbial indicators are therefore target specific, associated directly over the oil pool microbes flourish utilizing upcoming hydrocarbon gases. The Halo anomaly theory for hydrocarbon exploration is reconciled on the basis of microbiology, and has significant importance in hydrocarbon exploration (Horvitz, 1981; Jillman, 1987; Baum, 1994).

vi. Indeed, it is good supplementary tool for hydrocarbon prospecting and on proper integration with geological and geophysical data, can contribute to the success of exploration and helps in risk reduction of dry wells.

vii. According to the authors, microbial prospecting studies have to be taken up with the adsorbed soil gas analysis. As distinct and definite petroleum gas seepage could be readily identified by geochemical means such as Adsorbed soil gas analysis. There is a possibility that chemically detectable petroleum gases will be deficient in the soil receiving micro seepages, due to microbial oxidation. Thus geochemical technique of quantitative or qualitative adsorbed soil gas analysis may have limitations, where high soil microbial activity exists resulting in partially or nearly complete utilization of soil gases by microbes. Therefore, it is crucial to perform microbial prospecting studies along with the adsorbed soil gas analysis.

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