

Application of geo-microbial prospecting method for finding oil and gas reservoirs

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Abstract Microbial prospecting of hydrocarbons is based on the detection of anomalous population of hydrocarbon oxidizing bacteria in the surface soils, indicates the presence of subsurface oil and gas accumulation. The technique is based on the seepage of light hydrocarbon gases such as C₁–C₄ from the oil and gas pools to the shallow surface that provide the suitable conditions for the development of highly specialized bacterial population. These bacteria utilize hydrocarbon gases as their only food source and are found enriched in the near surface soils above the hydrocarbon bearing structures. The methodology involves the collection of soil samples from the survey area, packing, preservation and storage of samples in pre-sterilized sample bags under aseptic and cold conditions till analysis and isolation and enumeration of hydrocarbon utilizing bacteria such as methane, ethane, propane, and butane oxidizers. The contour maps for the population density of hydrocarbon oxidizing bacteria are drawn and the data can be integrated with geological, geochemical, geophysical methods to evaluate the hydrocarbon prospect of an area and to prioritize the drilling locations thereby reducing the drilling risks and achieve higher success in petroleum exploration. Microbial Prospecting for Oil and Gas (MPOG) method success rate has been reported to be 90%. The paper presents details of microbial prospecting for oil and gas studies, excellent methodology, future development trends, scope, results of study area, case studies and advantages.

Keywords hydrocarbon utilizing bacteria, microbial prospecting, micro-seepage, petroleum exploration

1 Introduction

Microbial prospecting method for hydrocarbon research and exploration is based on the premise that the light gaseous hydrocarbons migrate upward from subsurface petroleum accumulations by diffusion and effusion, and are utilized by a variety of microorganisms present in the sub-soil ecosystem (Wagner et al., 2002; Rasheed et al., 2011). The methane, ethane, propane, and butane-oxidizing bacteria exclusively use these gases as 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. The microbial prospecting method helps to prioritize the drilling locations and to evaluate the hydrocarbon prospects of an area (Pareja, 1994). 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 the variety of hydrocarbon-induced chemical and mineralogical changes (Mani et al., 2011; Rasheed et al., 2013a). 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 methods, magnetic, electrical methods, radioactivity and satellite-based methods etc., (Schumacher and Abrams, 1996). Figure 1 shows a generalized model of hydrocarbon microseepage and hydrocarbon-induced effects on soils and sediments.

Geo-microbial prospecting method for hydrocarbons is well known in the realm of hydrocarbon research and

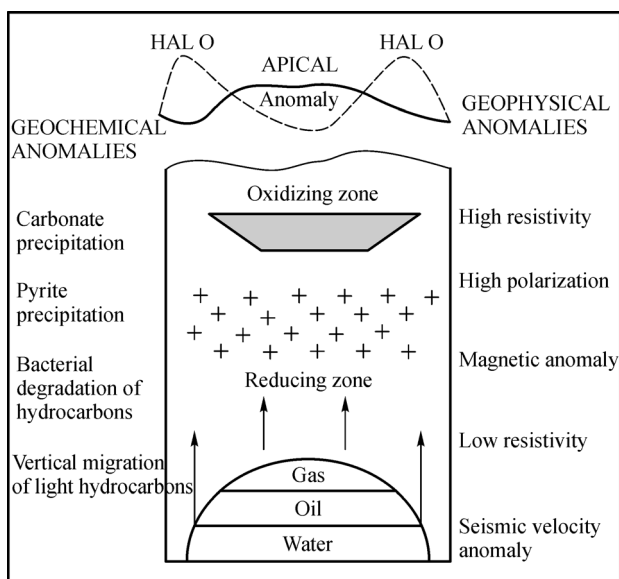


Fig. 1 Hydrocarbon micro-seepage model and hydrocarbon-induced effects on soils and sediments (after Schumacher and Abrams, 1996).

exploration. It is based on the premise that the light gaseous hydrocarbons migrate upward from subsurface petroleum accumulation by diffusion and are utilized by a variety of microorganisms present in the sub-soil ecosystem. The hydrocarbon oxidizing bacteria exclusively use these gaseous hydrocarbons as carbon source for their metabolic activities and growth. These bacteria are mostly found to be enriched in the shallow soils/sediments above hydrocarbon-bearing structures and different in hydrocarbon prospective area and non-prospective area. Bacteria are ubiquitous in the environment but will concentrate where a food source is available. The hydrocarbon microseepage of oil and gas mature source rocks and reservoirs provide this food source. The light hydrocarbon gases (C_1 – C_4) utilized by methane, ethane, propane and butane oxidizing bacteria are considered as indicator microbes owing to specificity of these gases to petroleum and the detection of anomalous population of hydrocarbon oxidizers in the surface soil or sediments helps to evaluate the prospect for hydrocarbon exploration (Rasheed et al., 2008, 2011).

Microbial anomalies have been proved to be reliable indicators of oil and gas in the sub-surface in many parts of the world (Pareja, 1994; Tucker and Hitzman, 1994; Rasheed et al., 2012a, b, c). A direct and positive relationship between the microbial population and the hydrocarbon concentration in the soils has been observed in various producing reservoirs worldwide (Wagner et al., 2002). Phylogenetically diverse group of bacteria belonging to the genera of *Brevibacterium*, *Corynebacterium*, *Flavobacterium*, *Mycobacterium*, *Nocardia*, *Pseudomonas*, and *Rhodococcus* utilize the light hydrocarbons (Rasheed et al., 2013a, b). The success of Microbial

Prospection for Oil and Gas has been reported to be around 90% (Wagner et al., 2002). Microbial prospecting method involves isolation and enumeration of hydrocarbon oxidizing bacteria in sub-soil strata for the demarcation of hydrocarbon prospects. This technique has been successfully applied in both on land and offshore areas (Lakshmi et al., 2012). This method can be integrated with geological, geochemical and geophysical methods, thereby reducing the drilling risks and achieving higher success in petroleum exploration is thus a valuable and less expensive value addition exploration tool to evaluate the seismic prospects (Veena Prasanna et al., 2013). A disadvantage of the microbial prospecting method is that it cannot predict the depth of the reservoir, because it is a surface prospecting method and directly depends of the migration of light hydrocarbon gases from oil and gas reservoirs. Also this method cannot differentiate between biogenic and thermogenic gases.

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 (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 (Miqueletto et al., 2011).

The microbial surveys were first proposed and applied in U.S.S.R. It was reported that out of 20 microbial anomalies, 16 were proved by successful drilling. These

investigations initiated the interest of petroleum geochemists worldwide. Microbial prospecting surveys in USA had showed positive correlation of 85.7%. Microbial Prospecting survey has been widely used in Germany since 1961 and a total 17 oil and gas fields were identified and the success rate has been reported to be 90%. This method can be integrated with geological, geochemical, 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 (Rasheed et al., 2013a, b).

2 Methodology

2.1 Sampling techniques

The microbial surveys for prospecting of oil and gas involve collection of suitable samples from sub soil horizon and detection of specific micro flora in the sample. The sampling is important since the validity of the test results depend largely on the manner in which the samples are taken. There are two types of field surveys for collection of soil samples, one is reconnaissance pattern and the other is grid pattern. In reconnaissance surveys the samples were collected along the existing roads, usually in an interval of 3 to 5 km. This type of surveys is helpful in studying larger area or sedimentary basin, and helpful for preliminarily assessment of hydrocarbon resource potential of the study area. The grid surveys are designed based on the size of the survey area; usually the samples were collected in a close interval of 200 m × 200 m or 500 m × 500 m (~200–1,000 m). The grid surveys are mainly used for locating hydrocarbon microseepage and also used for probable oil or gas well drilling location point following correlation the results with other geo-scientific studies. The samples were sealed in the plastic bags with their sample number and Global Positioning System (GPS) locations marked. Disturbed or excavated areas, soils contaminated with hydrocarbon, chemicals or animal waste, swamps and areas under water shed or water logged areas were avoided for sampling. While collecting the samples, rocks, coarse materials, plant residues, and animal debris have been excluded. The soil samples were collected using hollow metal pipe by manual hammering. The soil samples of about 100 gm each were collected in pre-sterilized polythene bags under aseptic conditions from a depth of about 0.5 to 1 m, the samples were transported to the laboratory and stored at 2°C to 4°C till analysis.

2.2 Culture method

Isolation and enumeration of methane, ethane, propane and butane oxidizing bacteria for each sample need to be carried out using Standard Plate Count (SPC) or Most

Probable Number (MPN) or Turbidometric analysis. In 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 plated on to Mineral Salts Medium (MSM) petri plates containing 1.0 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.7 g of K_2HPO_4 , 0.54 g of KH_2PO_4 , 0.5 g of NH_4Cl , 0.2 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 4.0 mg of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 mg of H_3BO_4 , 0.2 mg of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 mg of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.06 mg of $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.03 mg of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.02 mg of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, and 0.01 mg of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ in 1000 mL of distilled water, at pH 7.0. These plates were placed in a glass desiccators, filled with desired hydrocarbon gas (methane/ethane/propane/butane with 99.99% purity) and zero air (purified atmospheric gas devoid of hydrocarbons) in a ratio of (1:1). For isolation of propane oxidizing bacteria, the desiccator was filled with propane gas and zero air. Similarly, for isolation of ethane, propane and butane oxidizing bacteria, the desiccators were filled with ethane, propane and butane gas with zero air respectively. These desiccators were kept in bacteriological incubators at $(35 \pm 2)^\circ\text{C}$ for 10 days. After, incubation, the developed bacterial colonies propane oxidizing bacteria were manually counted using colony counter and reported in colony forming unit (cfu/gm) of soil sample (Rasheed et al., 2008). Standard hydrocarbon bacterial cultures need to obtain form any culture collection centre, such as American type culture collection centre (ATCC) or Microbial type culture collection centre (MTCC). The standard bacterial cultures should be kept along with the test soil samples during the experiment.

2.3 Plotting of bacterial anomaly

The results of hydrocarbon oxidizing bacterial concentration were plotted in terms of population density of aerial basis on the surveyed map using Arc GIS (Geographical Information System) or Golden Surfer Software's etc., 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.

3 Results and discussion

In the present study propane oxidizing bacteria was considered as microbial indicator. The propane oxidizing bacterial counts (POB) in the soil samples of Saurashtra, Gujarat are given in Table 1. The POB was found to vary from 0.6×10^2 to 6.86×10^5 cfu/gm of soil sample. The arithmetic mean and the standard deviation are found to be 3.93×10^4 and 6.37×10^4 cfu/gm, respectively. The percentage analysis of POB is shown in Table 2. Out of total 150

samples, 83.6% of samples show positive bacterial counts, whereas only 16.4% of samples show nil bacterial count (Fig. 2).

Table 1 Statistical analysis of propane oxidizing bacteria

Parameters	Propane oxidizing bacteria (POB)(cfu·gm ⁻¹)
Minimum count	0.6×10^2
Maximum count	6.86×10^5
Arithmetic mean	3.93×10^4
Standard deviation (σ)	6.37×10^4

Table 2 Percentage analysis of propane oxidizing bacteria.

Parameters	Propane oxidizing bacteria (POB)
Total No. of samples	150
Positive samples	83.6%
Nil samples	16.4%
Positive samples > Mean	26.7%
Positive samples > S.D	12.5%

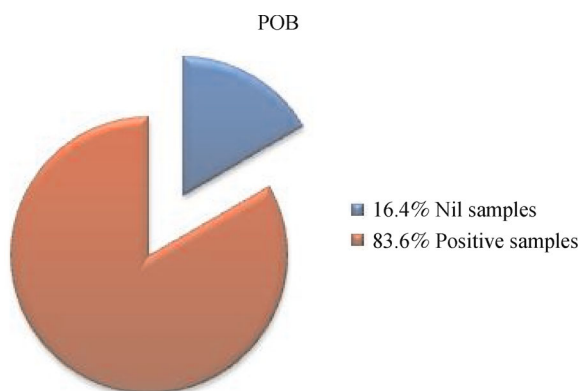


Fig. 2 Pie diagram showing the percentage of propane oxidizing bacteria (POB).

The propane oxidizing bacterial anomaly map (Fig. 3) shows anomalous concentration of propane oxidizing bacteria, in the study area. The samples are above standard deviation value are considered as anomalous concentration. In the present study, propane oxidizing bacterial count is found to a maximum of 10^4 to 10^5 cfu/gm of soil. The possibility of detecting oil or gas fields using microbial prospecting method depends on the hydrocarbon oxidizing bacterial count in soil or sediment samples that ranges between 10^3 to 10^6 cells/gm in soil-receiving hydrocarbon micro-seepage area and based on the ecological conditions (Wagner et al., 2002). In extreme areas, such as marshland, the methane oxidizing bacterial count reaches to 10^6

cfu/gm. The scientists from U.S.S.R have shown a preference for using propane oxidizing bacteria, because they found that these were more abundant in the soils. Propane oxidizers give a better bacterial profile for the assessment of the hydrocarbon prospects of the area. Some examples of propane oxidizing bacteria named as *Rhodococcus* sps., *Rhodococcus rhodochrous*, *Pseudomonas india*, *Brevibacterium* sps., *Mycobacterium propanicum*, *Mycobacterium rubrum*, *Mycobacterium flavum* etc. (Rasheed et al., 2012a, c).

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. It can be used for prospecting stratigraphic traps and work as an additional tool. Hydrocarbon micro seepage detection adds value to 2-D and 3-D seismic interpretation by identifying those features that are charged with geomicrobial anomalies. Sub-surface petroleum accumulations can be complex. Proper interpretation required integration of data with geological, geophysical and other available data. Indeed, it is a 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. This method can substantially reduce the exploration risks associated with trap integrity and hydrocarbon charge, especially in the hunt for much allusive subtle traps. It can give principal evidence on the occurrence of hydrocarbon anomalies in large areas. The subsequent seismic and geological investigations could thus be concentrated on favorable areas in those regions where structure data of the sub-surface already exists. As a result therefore, the seismic structural maps and microbial anomalies can be drawn independently and can be compared. It is well established that geochemical methods can provide direct evidence for the presence of petroleum accumulations and are relatively cheap and rapid. Today geological and geophysical exploration is undergoing extensive refinement to develop techniques that aimed at finding stratigraphic or non-structural accumulation which is elusive to these techniques.

3.1 Integration with geological and geophysical studies

The microbial prospecting studies were integrated with geological and geophysical studies in the Saurashtra, Gujarat. The integrated maps of hydrocarbon utilizing bacteria over sediment thickness in the Jamnagar study area showed that the bacterial anomalies were observed in areas with high Mesozoic sediment thickness > 2,000 m (Fig. 4). The integrated map of sediment resistivity with hydrocarbon utilizing bacteria showed that bacterial anomalies were observed in the high sediment resistivity areas (Fig. 5). Hydrocarbon fluids are an exception,

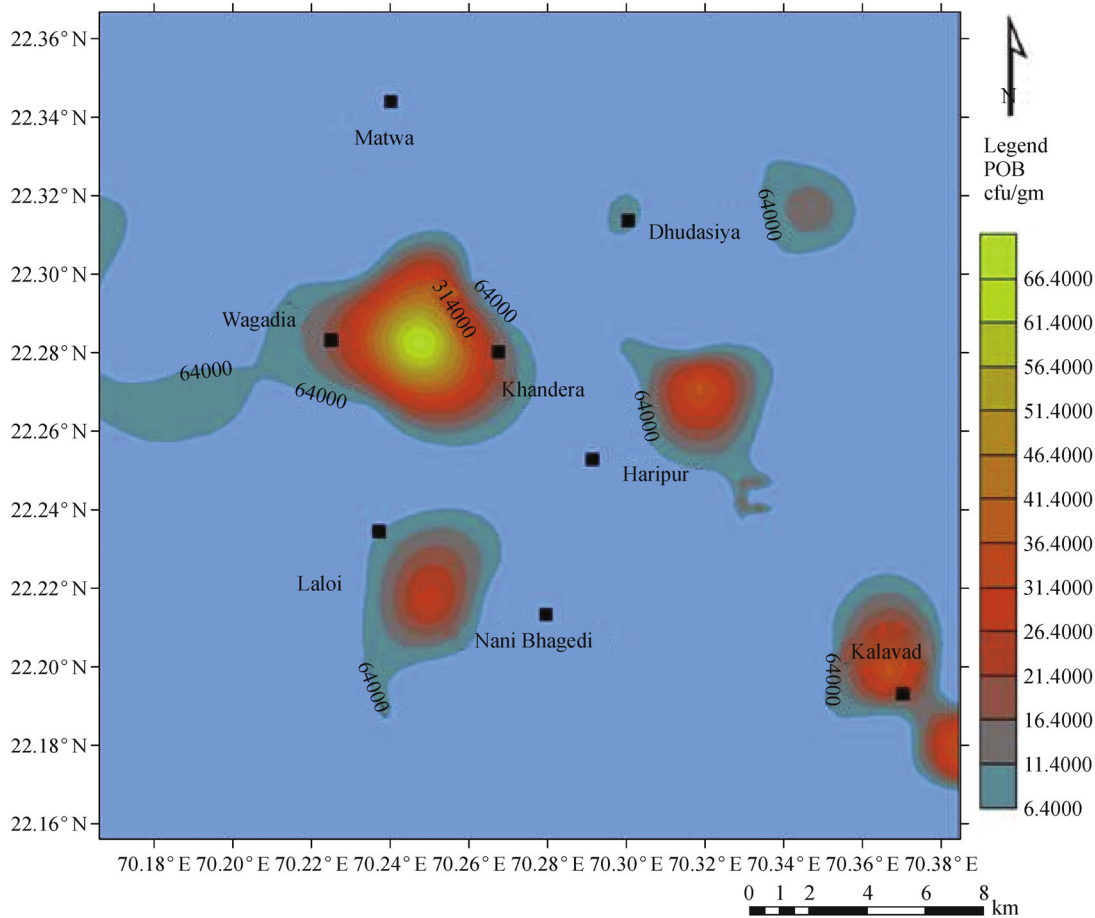


Fig. 3 Propane oxidizing bacterial anomaly map of study area, Gujarat.

because they are almost infinitely resistive. When a formation is porous and contains salty water, the overall resistivity will be low. When the formation contains hydrocarbon, its resistivity will be high. High resistivity values may indicate a hydrocarbon bearing formation. The lineaments in the study area were integrated with the hydrocarbon utilizing bacterial anomalies, and showed good correlation, as these bacterial anomalies coincide with the lineaments (Fig. 6). High values for hydrocarbon oxidizing bacterial anomalies were seen around the lineaments. Thus microbial anomalies coincide with lineaments, which probably serve as preferential conduits for the microseepage of hydrocarbons.

Figure 7 shows the integrated map of gravity with hydrocarbon utilizing bacteria. Geophysical studies have shown that thick Mesozoic sediments (about 2 km) occur in the NW of the Saurashtra Peninsula around Jamnagar. The Deccan Traps are in general 1.5 to 3 km thick in the south, but are thinner in the NW, possibly allowing the migration of hydrocarbons over the Mesozoic sediment thickness, as the concentration of microbial anomalies correlates well with sediment thickness. The northern part of the Saurashtra peninsula holds significantly thick

Mesozoic sediments; the regions of low gravity around Jamnagar are associated with thick Mesozoic sediments. Integrated geophysical studies in the Saurashtra Peninsula have indicated that thick Mesozoic sedimentary successions are present in two areas, one around Jamnagar in the NW, and another near Dwarka to the west below the Deccan Traps. These can be considered for future hydrocarbon exploration (Harinarayana, 2008). Geological and magneto-telluric surveys in the Saurashtra Peninsula have shown the presence of thick Mesozoic sedimentary sequences in the western part, which led to the drilling of the *Lodhika-1* well near Rajkot (Fig. 8). The generalized lithostratigraphy of the *Lodhika-1* well is shown in Fig. 9 (after Singh et al., 1997), and confirms the presence of a Mesozoic section beneath the Deccan Traps (Harinarayana, 2008).

3.2 Case studies

3.2.1 Keitz oil field in Germany

Microbial prospecting method for oil and gas (MPOG) was applied in Kietz region, Germany (Fig. 10), where a total

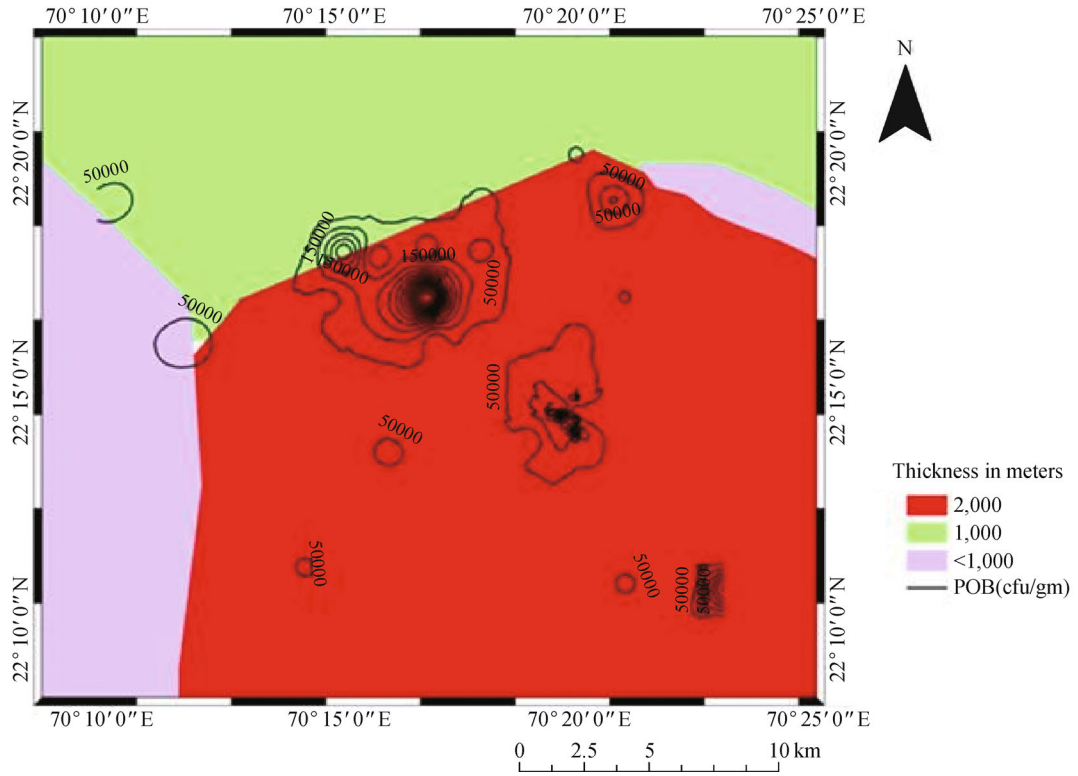


Fig. 4 Integrated map of Mesozoic sediment thickness with hydrocarbon utilizing bacteria

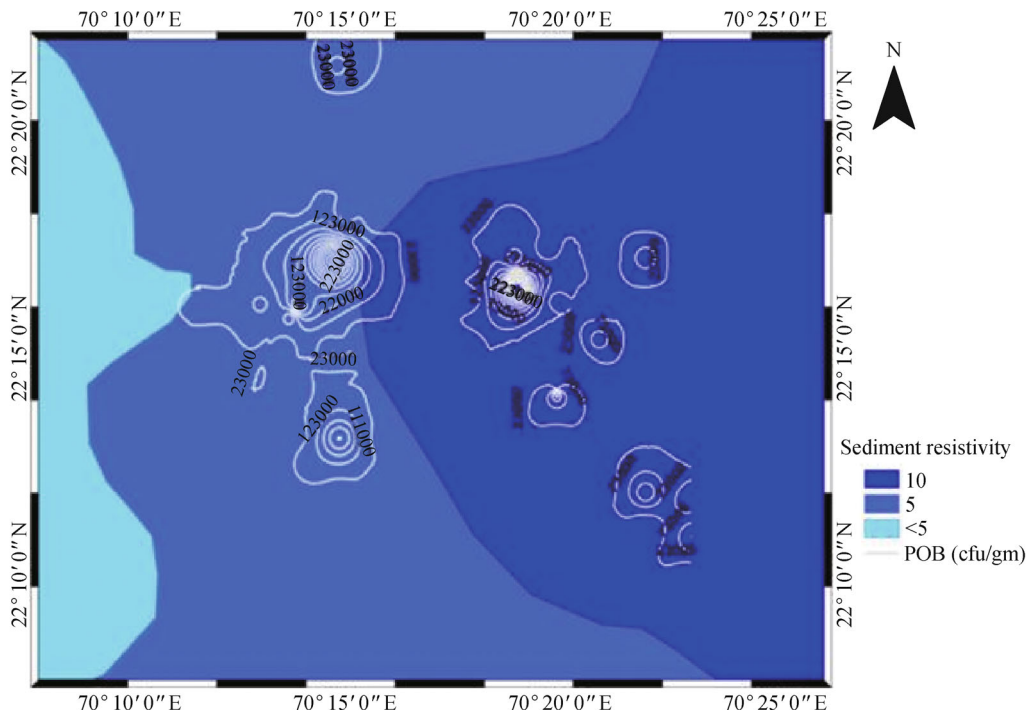


Fig. 5 Integrated map of sediment resistivity with hydrocarbon utilizing bacteria.

numbers of 197 soil samples were collected over an area of 120 km² and analyzed microbiologically. The hydrocarbon

oxidizing bacterial values showed distinct microbial anomaly and based on the results of microbial prospecting

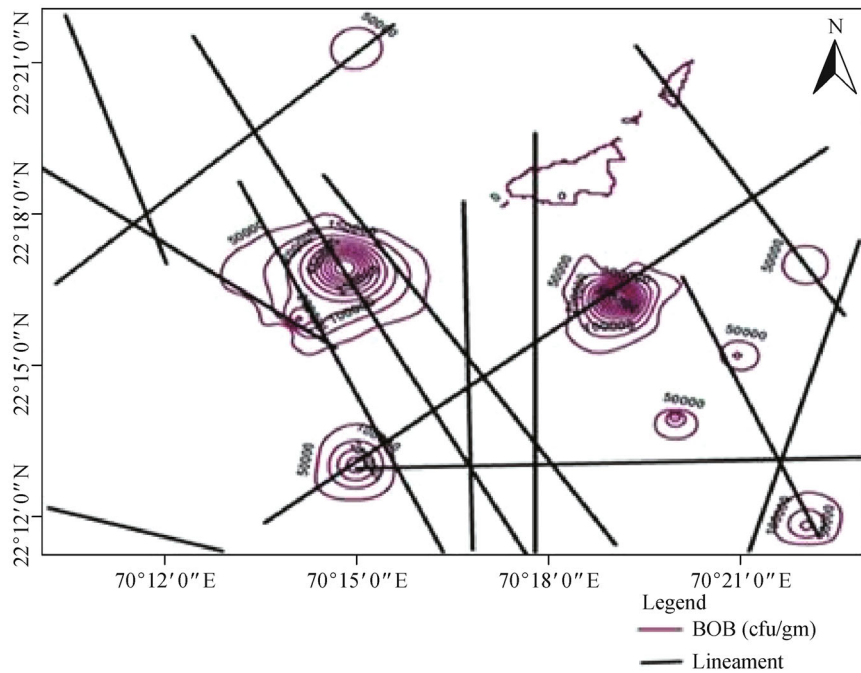


Fig. 6 Integrated map of lineament with hydrocarbon utilizing bacteria.

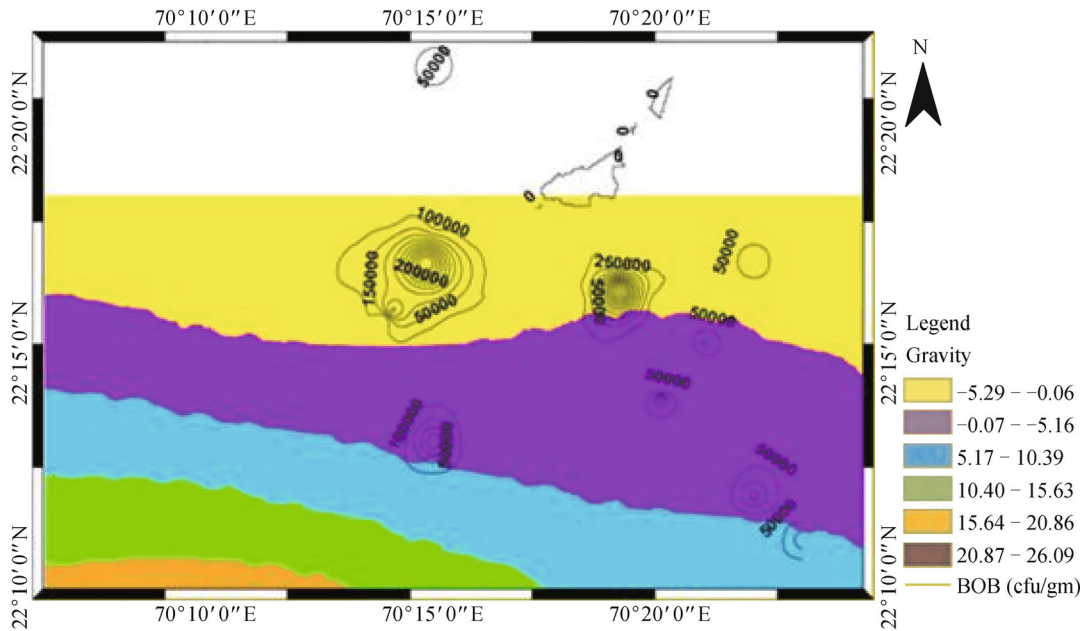


Fig. 7 Integrated map of gravity with hydrocarbon utilizing bacteria.

two wells were drilled, which resulted in oil two producing wells namely KiSe 2 and KiSe 5 in the anomaly “A” region (high concentration of hydrocarbon oxidizing bacteria), whereas two dry wells were observed in inconclusive zone, where the hydrocarbon oxidizing bacterial concentration is low (Wagner et al., 2002).

3.2.2 Conglomerate field in Montague County, Texas

Microbial surveys using butane oxidizing bacteria was carried out at Park Springs (Conglomerate) field in Montague County, Texas during 1995–1996. Microbial oil survey Technique (MOST) survey displayed strongly

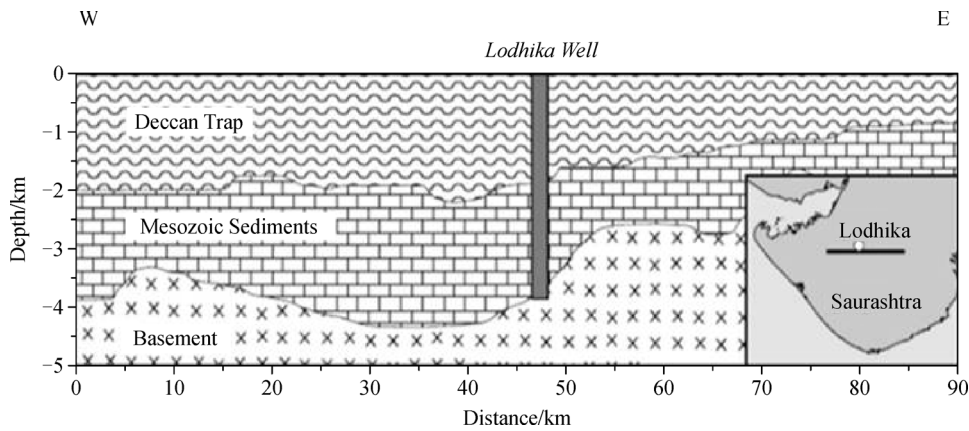


Fig. 8 An East-West Profile across the Saurashtra Peninsula showing the occurrence of a relatively thick sedimentary succession towards the western Part of the basin (after Harinarayana, 2008).

Age	Formation	Depth /m	Litho-log	Lithology
Upper Cretaceous Paleocene	Deccan Trap	0 - 1,200	Basalt, weathered basalt with amygdales in places traversed by calcite veins.	
	Wadhwan	1,400 - 1,600	Light grey to brown sandstone with clay alterations	
Upper Jurassic to Lower Cretaceous	Dhrangadhra	1,600 - 2,200	Grey, brownish grey sandstone, hard and compact with dark grey to brownish grey claystone, in places carbonaceous.	
		2,200 - 2,800	Dominantly polymictic conglomerate with red claystone. Brown sandstone with alterations of siltstone/claystone.	
	Basalt/dolerite.	2,800 - 3,000		
Jurassic	Lodhika	3,000 - 3,200	Basalt with red claystone.	
		3,200 - 3,500	Tuff.	

Fig. 9 Generalized lithostratigraphy of the Lodhika-1 well (after Singh et al., 1997).

consistent grouping of high microbial values aligned with the geophysical features as defined by the 3-D seismic

data. Figure 11 shows microbial signatures after the seismic survey. The contour values are concentrations of

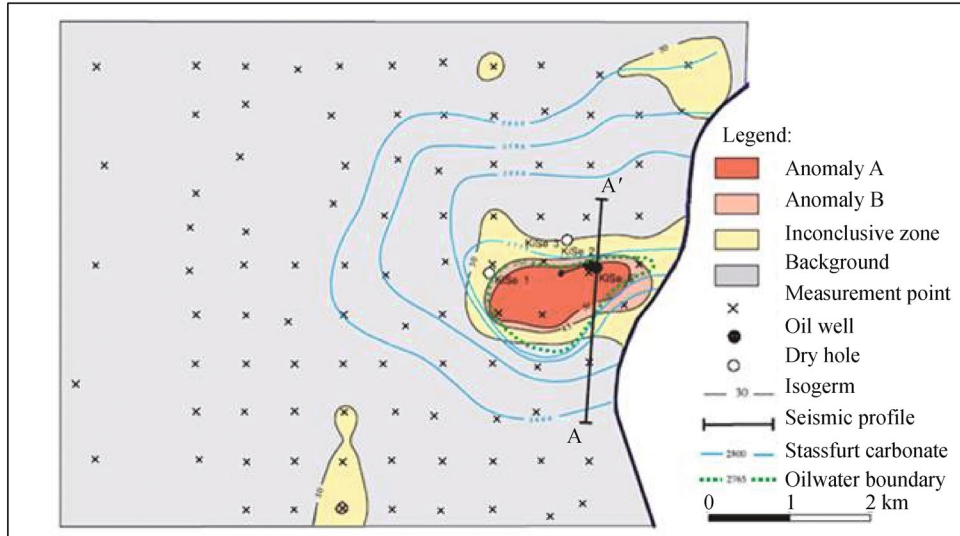


Fig. 10 Map of Kietz Oil field detected by microbial prospecting techniques (After Wagner et al., 2002).

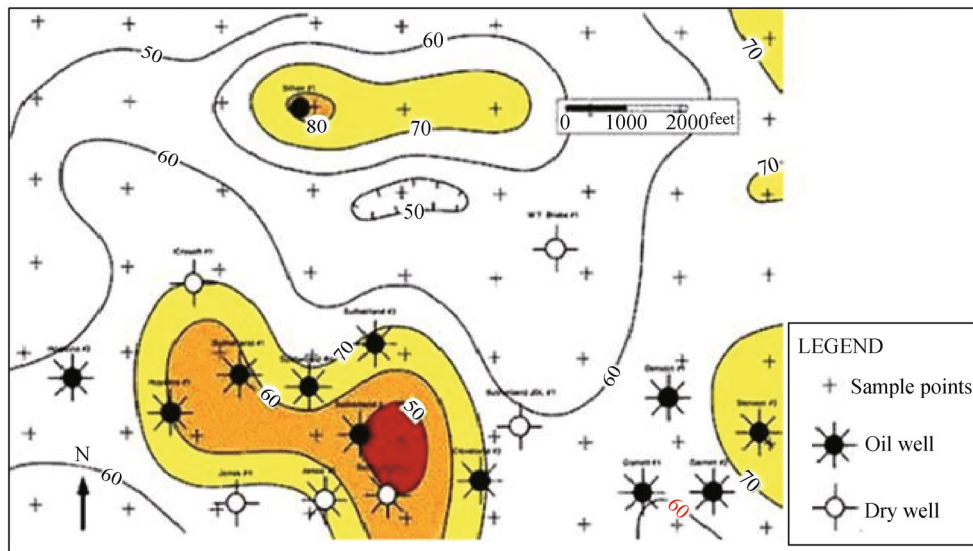


Fig. 11 Results of drilled wells in microbial anomalies using microbial surveys for oil technique (MOST) (modified after Schumacher, 2003).

butane oxidizing bacteria. Red, orange and yellow are the anomalous hydrocarbon-indicating microbial signatures.

4 Advantages of microbial prospecting

Microbial prospecting method has increasingly becoming a reliable and dependable tool in hydrocarbon research and exploration. Since the drilling operations are costly, it is essential to use appropriate and efficient exploratory methods. In order to cut down the drilling cost proper integration with geological and geophysical data is necessary. This way can contribute to the successful high

grading of exploration and prospects and helps in risk reduction. 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 the risks and achieve higher success ratio in petroleum exploration. The success of Microbial Prospection for Oil and Gas has been reported to be around 90% (Wagner et al., 2002). Microbial prospecting method has certain advantages over the adsorbed soil gas survey. There is a possibility that chemically detectable petroleum gases will be deficient where high soil microbial activity exists. 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. 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. This method can give principal evidence on the occurrence of hydrocarbon anomalies in large areas. The subsequent seismic and geological investigations could thus be concentrated on favorable areas in those regions where structure data of the sub-surface already exists. Application of Microbial Oil Survey Techniques (MOST) method as a reservoir characterization tool expands a surface geochemistry technique from exploration into the production industry. The detailed microbial evaluation of these producing fields have identified microbial patterns related to hydrocarbon withdrawal and reservoir heterogeneities (Tucker and Hitzman, 1994).

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