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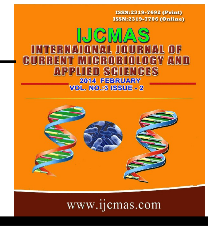

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Original Research Article

Evaluation of Petroleum Prospects using Geo-microbial prospecting method from Seabed Sediment Samples of Gulf of Mannar, Kerala-Konkan offshore Basin, India

M.A.Rasheed[1,2]*, M. Lakshmi[1], P. L. Srinivasa Rao[1,2], M. S. Kalpana[1], D. J. Patil[1] and A. M. Dayal[1]

[1]National Geophysical Research Institute (Council of Scientific and Industrial Research), Uppal Road, Hyderabad – 500 007, India
[2]Gujarat Energy Research and Management Institute (GERMI), Gandhinagar, Gujarat-382007, India
Corresponding author e-mail:

A B S T R A C T

With the increase in demand for petroleum products and diminishing indigenous production, it has become necessary to look for probable potential zones. Geo-microbial prospecting is one such unconventional technique that has emerged as one of the vital components of any exploratory program. It is based on the seepage of hydrocarbons from the subsurface reservoirs to the shallow surface environment which results in surface bacterial anomalies as indicator for oil and gas exploration. 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 C_1 - C_4 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. Surface geochemical studies include microbial prospecting method and hydrocarbon gases estimation was applied in the Kerala-Konkan Offshore Basin for evaluation of hydrocarbon resource potential of the Basin. The gas chromatographic analyses of adsorbed soil gases showed the presence of C_1 to C_5 hydrocarbons. The concentrations of adsorbed soil gases ranged for methane C_1 = 3 to 492 ppb, ethane C_2 = 4 to 326 ppb, propane C_3 = 2 to 296 ppb, butane nC_4 = 2 to 72 ppb, iC_5 = 4 to 94 ppb, nC_5 = 2 to 23 ppb and $\Sigma C2_+$ (C_2H_6, C_3H_8, i-C_4H_{10}, n-C_4H_{10}, i-C_5H_{12}, n-C_5H_{12}) = 3 to 837 ppb. The scatter plots between C_1 to C_4 components depict linear trend indicating that all the gases are derived from the same source. The bacterial count of propane oxidizing bacteria ranged from 0.2 x 10^2 to 6.5 x 10^4 cfu/g of soil. Two microbial blooms of high populations of the propane oxidizing bacterial were identified between (latitudes 7.6° to 8.0° N and longitudes 77.65° to 77.85° E) in the study area and appears to be more potential. Our study supports the Kerala-Konkan Offshore Basin to be promising in terms of its hydrocarbon prospects.

Keywords

Adsorbed soil gas; Hydrocarbons, Kerala-Konkan Offshore; Propane-oxidizing bacteria.

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. 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. 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. Microbial prospecting method can be integrated with geological, geophysical and other surface hydrocarbon prospecting techniques to prioritize the drilling locations and to evaluate the hydrocarbon prospects of an area. Microbial anomalies have been proved to be reliable indicators of oil and gas in the subsurface (Rasheed et al., 2008). The propane oxidizing bacteria is used as an important indicator in microbial prospecting for oil and gas exploration. 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, 2013). 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). The main objective of the study is to assess the hydrocarbon prospects of the Kerala- Konkan Offshore Basin based on the adsorbed soil gas analysis and microbial analysis to demarcate the anomalous hydrocarbon zones, for future exploration and exploitation activities.

Geological Setting

The Kerala-Konkan basin in the southern part of the western continental margin of India has engaged much interest because of its probable hydrocarbon potential. The basin lies to the south of Bombay offshore basin, the major hydrocarbon producer of India (Somen Mishra et al., 2010). The Kerala-Konkan Offshore Basin located at South of 16 degree N latitude. It forms the Southern part of the Western continental margin of India and extends from Goa in the North to Cape Comorin in the South. In the Westward, the Basin extends to the Arabian Abyssal plain. On the Eastern side it is bounded by peninsular shield, the Kerala-Konkan Basin situated South of East-West trending the Vengurla Arch and extends upto Cape Comorin in the South of Indian sub continent in the Western Offshore Basin (Murty and Ramamurty et al. 2005). The location map of the Kerala-Konkan Offshore Basin is shown in Figure 1. The Stratigraphic sequence is comprised of Mesozoic and Tertiary sediments. The Deccan Traps (Cretaceous – Early Paleocene) form the technical basement of the Tertiary Basin, The generalized Statrigraphic sequence is shown in Figure 2. The depositional models of the initial post-rift and late post-rift phases of Basin evolution, suggest that the Kerala-Konkan Basin holds promise for hydrocarbons.

Petroleum plays

The petroleum system wherein the source rock is mainly Paleocene to Eocene shales, the Karwar Formation (Eocene) has rich organic matter and the Kasargod Formation (Late Paleocene) has fair to good source potential. The Paleocene to Middle Eocene shales, have fairly high TOC content mainly of type II and III organic matter, and are thermally mature. The Shelfal Depressions and the Lakshadweep Depressions are considered to be kitchens for hydrocarbon generation. The reservoir rock is mainly Eocene to Middle-Miocene Carbonates and Paleocene to Middle Miocene Sandstones are the possible reservoirs of the Kerala-Konkan Basin. The synrift Paleocene sediments, deposited in supratidal to intertidal setup comprise coal shale, shale, siltstone and sandstone. The organic matter is dominantly terrestrial with maturation level at the early phase of oil window (Bhowmick, 2009). The geochemistry of the recent seabed sediments of deep waters reveals the presence of hydrogen rich organic matter of type II kerogen indicating marine origin. Higher hydrogen index values (average 306) and higher organic carbon contents (average 3.28 wt%) in the upper slope sediments indicates enhanced preservation under reducing conditions in the anoxic waters of deep area. Quantitative and qualitative composition of adsorbed gas, total scanning fluorescence data of sea bed core samples in the Kerala- Konkan deep sea area indicate liquid hydrocarbon prone area towards the identified and mapped prospects confirming the generation and presence of hydrocarbons (Kuldeep Chandra, 1999).

Materials and Methods

Field Work and Sample Collection

The Offshore geochemical survey was carried out using Coastal Research Vessel M. V. Sagar Purvi of National Institute of Ocean Technology (NIOT), Chennai. About 75 stations were covered for the collection of core samples using gravity corers (160/100 Kg) in the grid pattern with a sample interval of 14 x 14 km. The sample location map of the study area is shown in Fig. 3. A total of 31 sediment samples were collected using gravity corer, at approximately 30 cm below the top of the sediment were used for microbial analysis. The core sediment samples were packed in the doubly capped plastic containers. The top and bottom of the samples were marked and the Global Positioning System locations (Latitude and Longitude) were labeled. These sediment samples were frozen onboard and then transported to the laboratory at cryogenic conditions.

Isolation of propane oxidizing bacteria

Isolation and enumeration of propane oxidizing bacteria for each sample was carried out by Standard Plate Count (SPC) method. 1 g 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) (Ronald and Lawrence, 1996). These plates were placed in a glass desiccator, filled with propane 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. These desiccators were kept in bacteriological incubators at 20 ± 2^{o}C for 10 days. After incubation, the developed bacterial colonies of propane oxidizing bacteria were counted using colony counter and reported in colony forming units (cfu/g) of soil sample (Rasheed et al. 2008). Development of molecular biology methods for microbial prospecting for oil and gas by applying culture independent methods for microbial prospecting for oil and gas exploration (Dionisi et al. 2003; Fan Zhang et al., 2010; Kaster et al. 2009; Lysnes et al. 2009).

Analysis of light gaseous hydrocarbons

The light gaseous hydrocarbons were extracted from the soil samples using a gas extraction system (Horvitz, 1981). 1g of 63 micron wet sieved soil sample was used to extract light gaseous hydrocarbons after acid treatment in glass degasification apparatus and its subsequent analyses on Gas Chromatograph (GC) for all samples and Gas Chromatograph-Combustion-Isotope Ratio Mass Spectrometer (GC-C-IRMS) analysis for samples that had higher concentrations of hydrocarbons. During acid treatment, the dominant gas released was CO_2 and was trapped in KOH solution. The light gaseous hydrocarbons were collected by water displacement in a graduated tube fitted with rubber septa. The volume of desorbed gas was recorded and 500µl of desorbed gas sample was injected into Varian CP 3380 Gas Chromatograph fitted with Porapak Q column, equipped with flame ionization detector. The Gas Chromatograph was calibrated using external standards with known concentrations of methane, ethane, propane and n-butane. The quantitative estimation of light gaseous hydrocarbon constituents in each sample was made using peak area measurements and the correction for moisture content on wet basis was also applied. The hydrocarbon concentration values of individual hydrocarbons from methane through pentane are expressed in parts per billion (ppb) (Rasheed et al, 2008).

Results and Discussion

Microbiological study

The soil samples collected from the Gulf of Mannar, Kerala-Konkan Offshore Basin were analyzed for the presence of propane oxidizing bacteria using Standard Plate Count (SPC) method. The bacteria, which are able to utilize propane gas as a sole carbon source, are merely developed as bacterial colonies on the MSM plates. Positive control of known hydrocarbon oxidizing bacterial strain namely, *Rhodococcus rhodochrous* MTCC 291 were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh, which were inoculated onto MSM plates and incubated along with the test soil samples. The growth was observed in the positive control and in the test samples after incubation. The propane oxidizing bacteria in soil samples of the Kerala-Konkan Offshore Basin are given in Table 1. The bacterial count of propane oxidizing bacteria ranged from 0.2×10^2 to 6.5×10^4 cfu/g of soil. The arithmetic mean and the standard deviation values are 5.09×10^3 cfu/g and 1.51×10^4 cfu/g, respectively. Table 2 shows the statistical analysis of propane oxidizing bacteria in sediments of the Kerala-Konkan Offshore Basin.

The results of propane oxidizing bacteria are plotted on the surveyed map of the Kerala-Konkan Offshore Basin (Fig. 4) indicate two bacterial anomalies in the study area and appears to be more potential. In another the producing oil and gas fields of the Mehsana, Cambay Basin, Gujarat, and the Krishna Godavari Basin, the bacterial counts were found between 10^5 to 10^7 cfu/g of soil. The hydrocarbon-oxidizing bacteria ranged between 10^3 and 10^6 cfu/g in soil/sediment in receiving hydrocarbon micro-seepages (Rasheed et al. 2011; Wagner et al. 2002). The sediments from Offshore surveys also indicate low bacterial count in view of oxygen deficient conditions at sea bed. In the present study area of the Kerala-Konkan Offshore Basin, propane oxidizing bacteria ranged between 10^3 to 10^4 cfu/g of soil sample, which is significant and thereby substantiates the seepage of lighter hydrocarbon accumulations from oil and gas reservoirs. Geo-microbial prospecting studies suggest that hydrocarbon micro-seepage of subsurface origin is present in the Kerala-Konkan Offshore study area and indicate that the area has positive prospects for petroleum exploration.

Evaluation of adsorbed soil gas data

The gas chromatographic analysis of seabed sediment samples and the magnitude of each of the organic constituents (C_1 to C_5) were measure and are expressed in part per billion of the soil gas mixture. The number of samples, ranges of values, and statistical values for each analyzed soil gas constituent are summarized in Tables 3 and 4. The concentrations of adsorbed soil gases ranged for methane C_1 (3 to 492 ppb), ethane C_2 (4 to 326 ppb), propane C_3 (2 to 296 ppb), butane nC_4 (2 to 72 ppb), iC_5 (4 to 94 ppb), nC_5 (2 to 23 ppb) and $\Sigma C2_+$ (C_2H_6, C_3H_8, i-C_4H_{10}, n-C_4H_{10}, i-C_5H_{12}, n-C_5H_{12}) (3 to 837 ppb). The Pearson correlation coefficients of various light hydrocarbon components are presented in Table 5; from which, it is clear that excellent correlation exists among methane, ethane, propane, butanes and pentanes. The cross-plots (Fig. 5) between C_1, C_2, C_3, nC_4 and ΣC_{2+} show excellent correlation (r = > 0.9) indicating that i) these hydrocarbons are genetically related; ii) are not affected by secondary alteration during their migration from subsurface to subsequent adsorption on to the surface soil and iii) might have been generated from a thermogenic source because of the presence of nC_3, nC_4 and nC_5 components (Kalpana et al. 2010). Fig. 5 illustrates the scatter plots of C_1–C_2 and C_1–ΣC_{2+} which follows a linear trend. The linear correlation between all the gas species suggests that they all originate from the same source/origin and migrate under the same condition (Madhavi et al. 2009). Most of the methane and ΣC_{2+} components indicate their origin from a single source. High correlation of C_1 with ΣC_{2+} indicates that the C_1 in the samples must have been derived from thermogenic source (Kalpana et al. 2010). The light hydrocarbon ratios are generally used to discriminate biogenic hydrocarbons from thermogenic hydrocarbons and can also predict the oil/gas potential of the Basin. Histograms

Table.1 Concentration of Propane Oxidizing Bacteria (POB) in sediment samples of Kerala – Konkan Offshore Basin, Gulf of Mannar

Sample no.	Longitude	Latitude	Depth(m)	POB (cfu/gm)*
KK/01	76.8750	7.6250	102	0
KK/02	76.8750	7.5017	116	0
KK/03	76.9842	8.0019	55	200
KK/04	76.9856	7.7514	80	5500
KK/05	76.9856	7.5025	98	8800
KK/06	77.1250	8.0033	52	0
KK/07	77.1250	7.7500	62	2500
KK/08	77.1250	7.6250	78	57500
KK/09	77.2583	8.0011	44	0
KK/10	77.2522	7.8750	50	2800
KK/11	77.2517	7.7506	55	0
KK/12	77.2503	7.6250	74	500
KK/13	77.3750	8.0006	42	2600
KK/14	77.3750	7.8750	48	0
KK/15	77.3750	7.7506	53	0
KK/16	77.3750	7.6250	72	0
KK/17	77.5022	8.0033	34	200
KK/18	77.5019	7.8750	45	300
KK/19	77.5019	7.7500	56	4000
KK/20	77.6250	8.0014	28	2200
KK/21	77.6250	7.8750	42	300
KK/22	77.6250	7.7522	53	3800
KK/23	77.6250	7.6250	67	0
KK/24	77.7519	8.0017	27	65000
KK/25	77.7519	7.7517	50	0
KK/26	77.8750	8.0019	32	1600
KK/27	77.8750	7.8750	42	200
KK/28	77.8750	7.7514	47	0
KK/29	77.8750	7.6250	65	0
KK/30	78.0019	8.0019	37	0
KK/31	78.0006	7.7594	41	0

*POB: Propane oxidizing bacteria;
cfu/gm: colony forming unit per gram of sediment sample.

Table.2 Statistical analysis of propane oxidizing bacteria in sediments of Kerala-Konkan Offshore Basin

Parameter	Propane oxidizing bacteria (cfu/gm)*
Minimum	0.2×10^2
Maximum	6.50×10^4
Arithmetic mean	5.0×10^3
Standard deviation	1.51×10^4

*cfu/gm: colony forming unit per gram of sediment sample

Table.3 Results of Gas Chromatographic analyses of light gaseous hydrocarbons (ppb) from sediment samples of Kerala-Konkan Offshore Basin.

Sample no.	**C1**	**C2**	**C3**	**iC4**	**nC4**	**iC5**	**nC5**	**∑C2+**
KK/01	15	0	0	0	0	0	0	0
KK/02	26	0	0	0	0	0	0	0
KK/03	67	25	20	3	0	0	0	48
KK/04	50	22	15	0	0	0	0	38
KK/05	10	0	0	0	0	0	0	0
KK/06	18	0	0	0	0	0	0	0
KK/07	8	0	0	0	0	0	0	0
KK/08	30	12	12	2	4	0	0	30
KK/09	7	0	0	0	0	0	0	0
KK/10	6	0	0	0	0	0	0	0
KK/11	61	15	9	0	0	0	0	24
KK/12	15	3	0	0	0	0	0	3
KK/13	5	0	0	0	0	0	0	0
KK/14	6	0	0	0	0	0	0	0
KK/15	81	31	23	3	4	0	0	61
KK/16	30	6	0	0	0	0	0	6
KK/17	3	0	0	0	0	0	0	0
KK/18	10	4	4	0	0	0	0	8
KK/19	70	23	15	0	0	0	0	38
KK/20	6	0	0	0	0	0	0	0
KK/21	27	0	0	0	0	0	0	0
KK/22	31	7	3	0	0	0	0	10
KK/23	26	8	7	0	0	0	0	14
KK/24	111	46	43	10	13	2	2	115
KK/25	492	326	296	72	94	23	23	834
KK/26	12	0	0	0	0	0	0	0
KK/27	32	11	8	0	0	0	0	20
KK/28	48	16	10	0	0	0	0	26
KK/29	39	16	12	0	0	0	0	28
KK/30	36	11	7	0	0	0	0	18
KK/31	243	105	75	13	15	0	0	208

Table.4 Statistical concentrations (ppb) of adsorbed soil gases in soil samples

	C1	C2	C3	iC4	nC4	iC5	nC5	ΣC2+
Maximum	492	326	296	72	94	23	23	834
Minimum	3	4	2	4	4	4	4	3
Arithmetic Mean	64	31	26	5	7	1	1	72
Standard Deviation	93	59	53	12	17	4	4	151

Table.5 Pearson correlation coefficients of adsorbed light hydrocarbons.

	C1	C2	C3	C4	ΣC2+
C1	1.00				
C2	0.97	1.00			
C3	0.89	0.94	1.00		
C4	0.37	0.39	0.44	1.00	
ΣC2+	0.94	0.98	0.96	0.56	1.00

Fig.1 Kerala-Konkan Offshore Basin map showing the study area, Source: Directorate General of Hydrocarbons (DGH), India

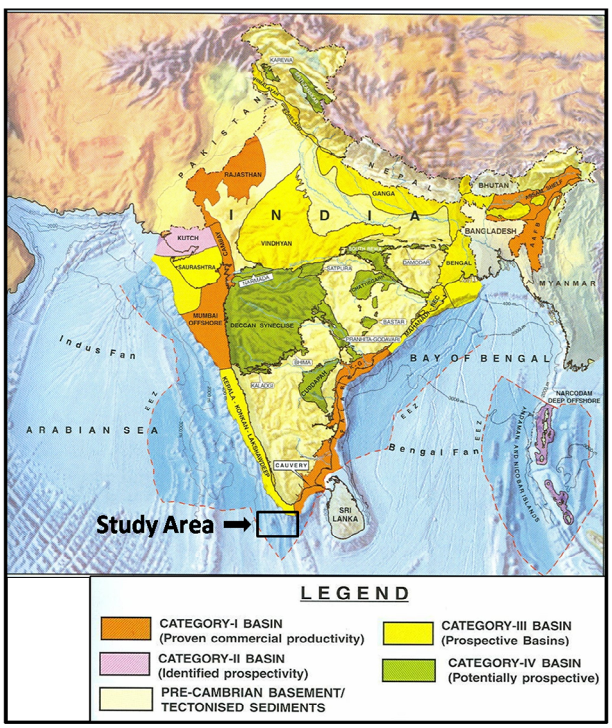


Fig.2 Generalized statigraphy sequence of Kerala-Konkan Offshroe Basin, Source: Directorate General of Hydrocarbons (DGH), India

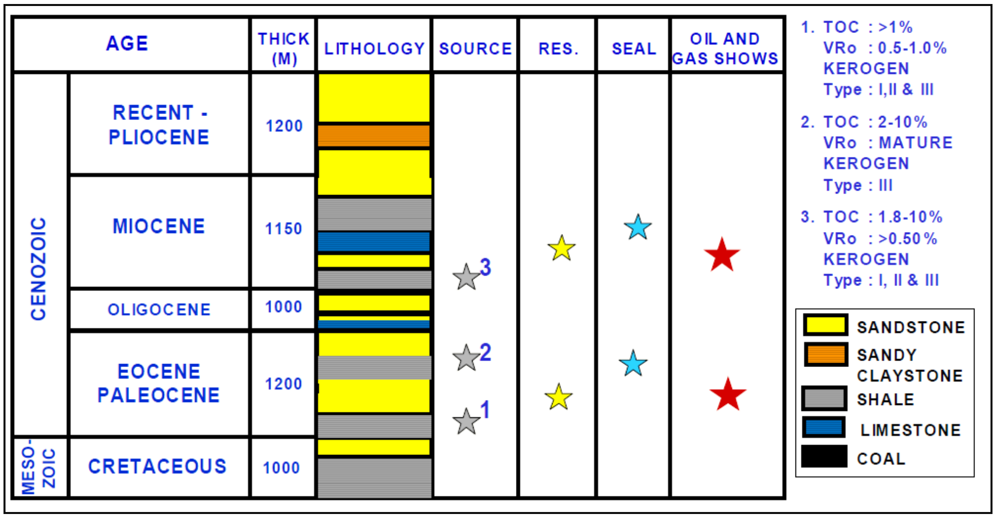


Fig.3 Seabed sediment sample location map of Gulf of Mannar, Kerala-Konkan offshore Basin

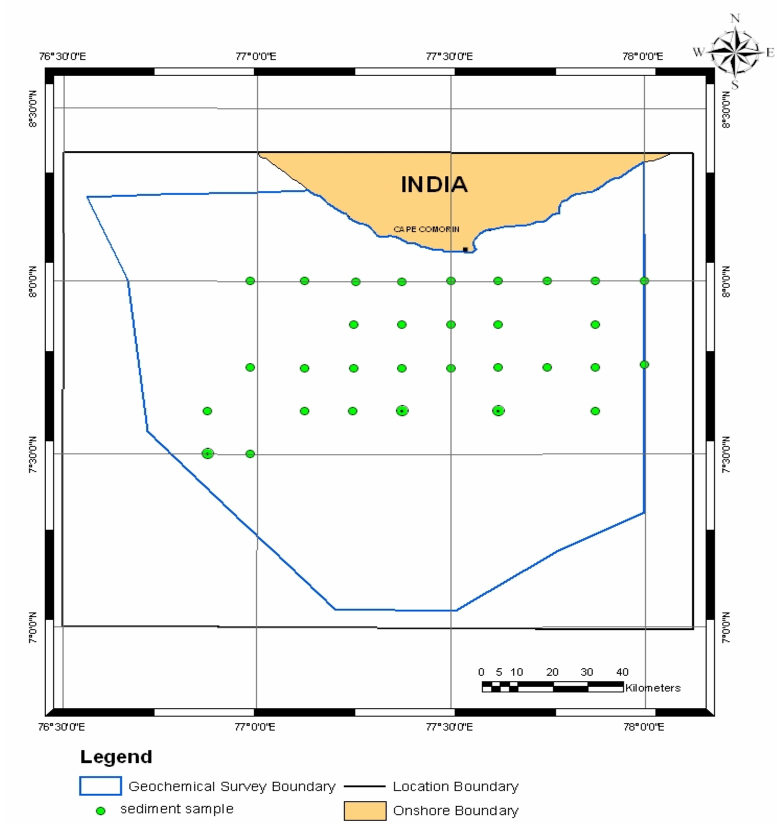


Fig.4 Concentration distribution map of Propane oxidizing bacteria (POB) in Seabed samples for Gulf of Mannar, Kerala-Konkan offshore basin.

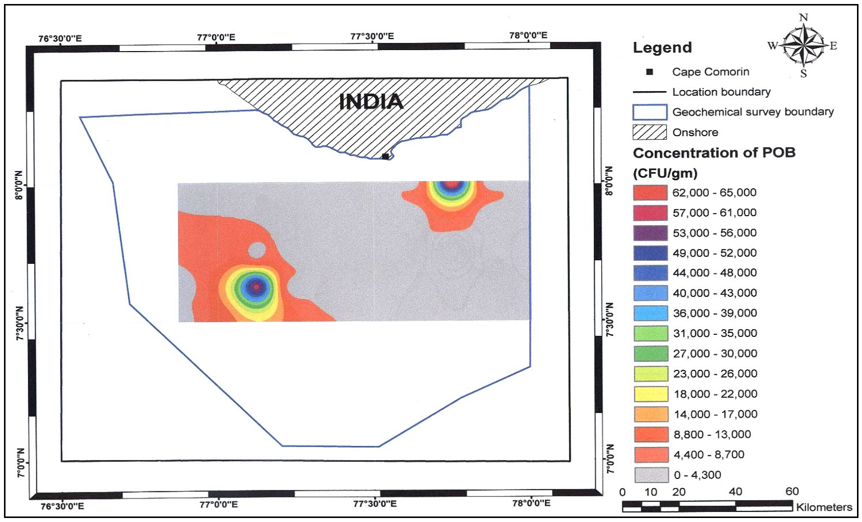


Fig.5 Cross plots of adsorbed light hydrocarbon gases in the study area.

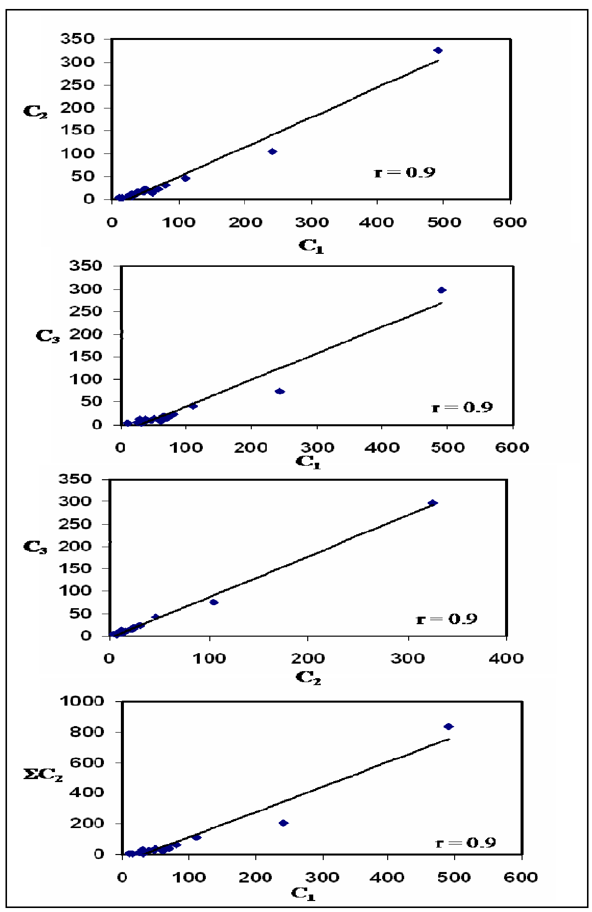


Fig.6 Histograms of C1 and ΣC_{2+} hydrocarbon concentration (ppb) in the sediment samples

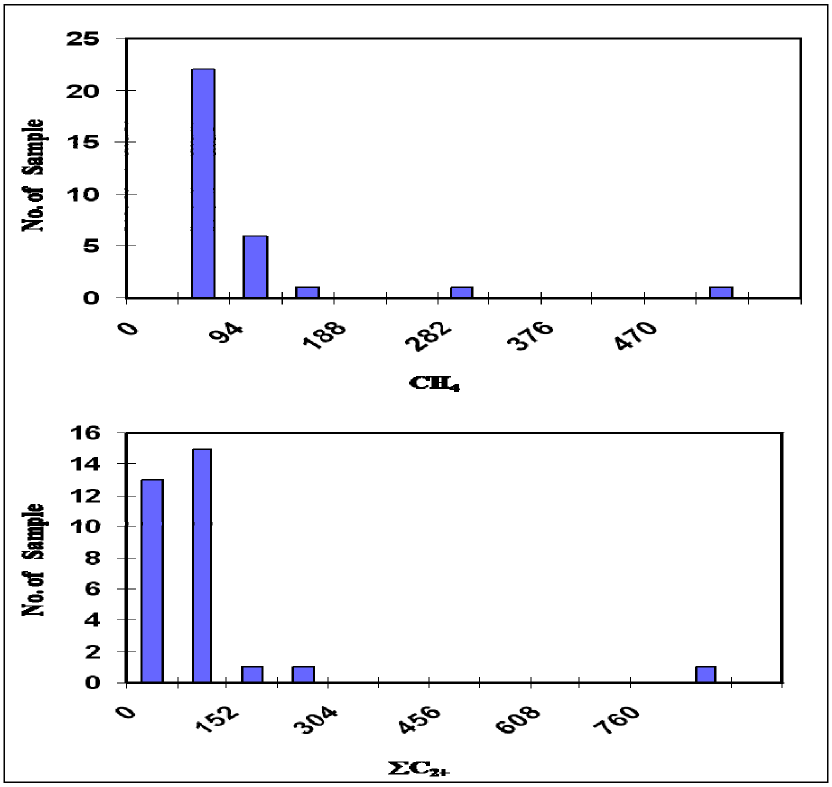


Fig.7 Histogram for C3/C1*1000 in sediment samples of Kerala-Konkan Basin

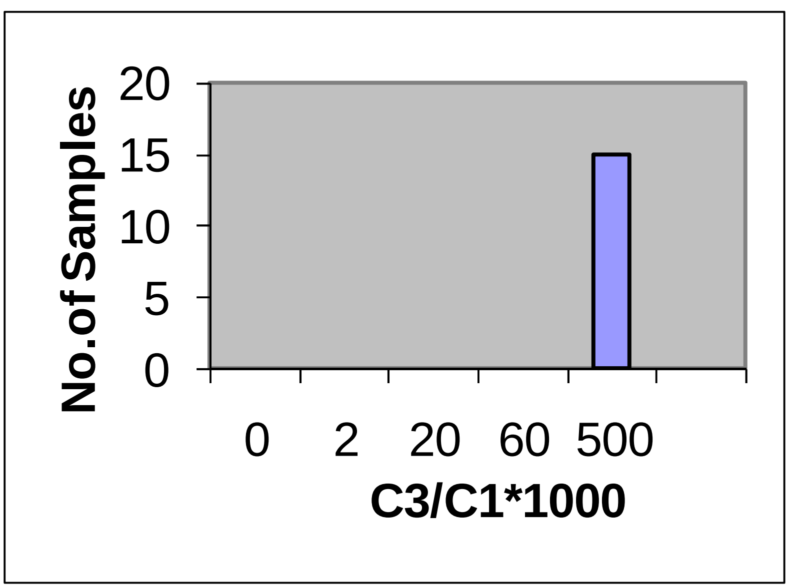


Fig.8 Concentration distribution map of Methane in Seabed sample for Gulf of Mannar, Kerala-Konkan Offshore Basin

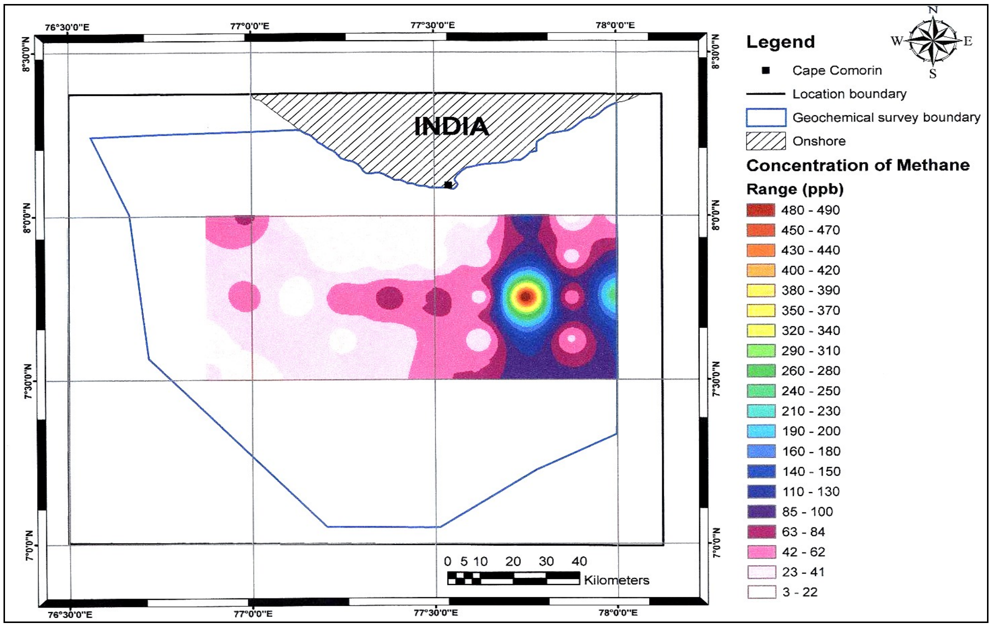


Fig.9 Concentration distribution map of ∑C2+ in Seabed sample from Gulf of Mannar, Kerala-Konkan Offshore Basin.

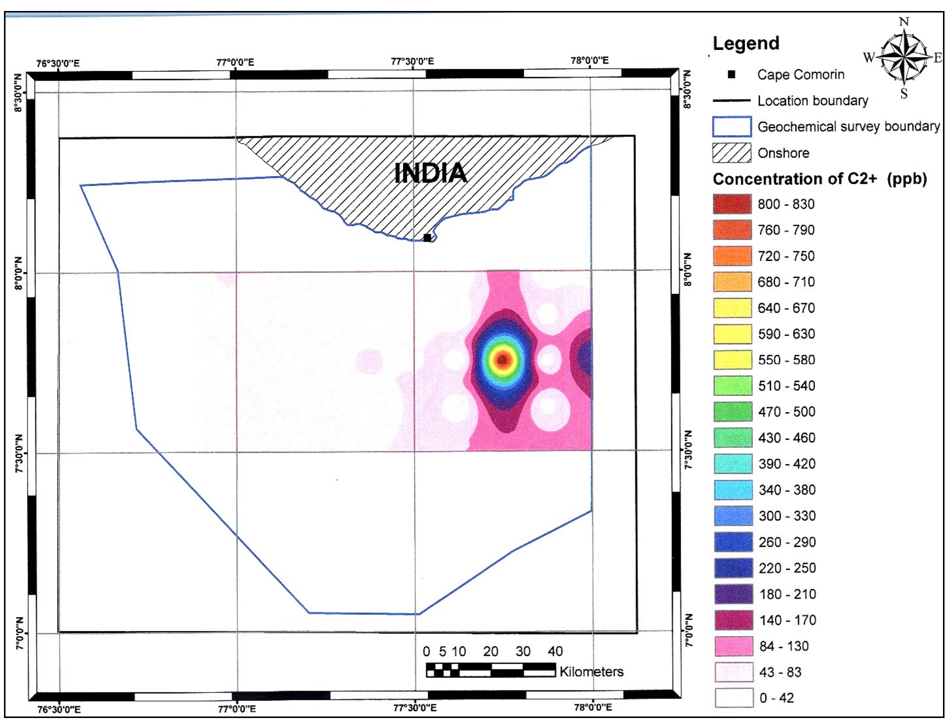
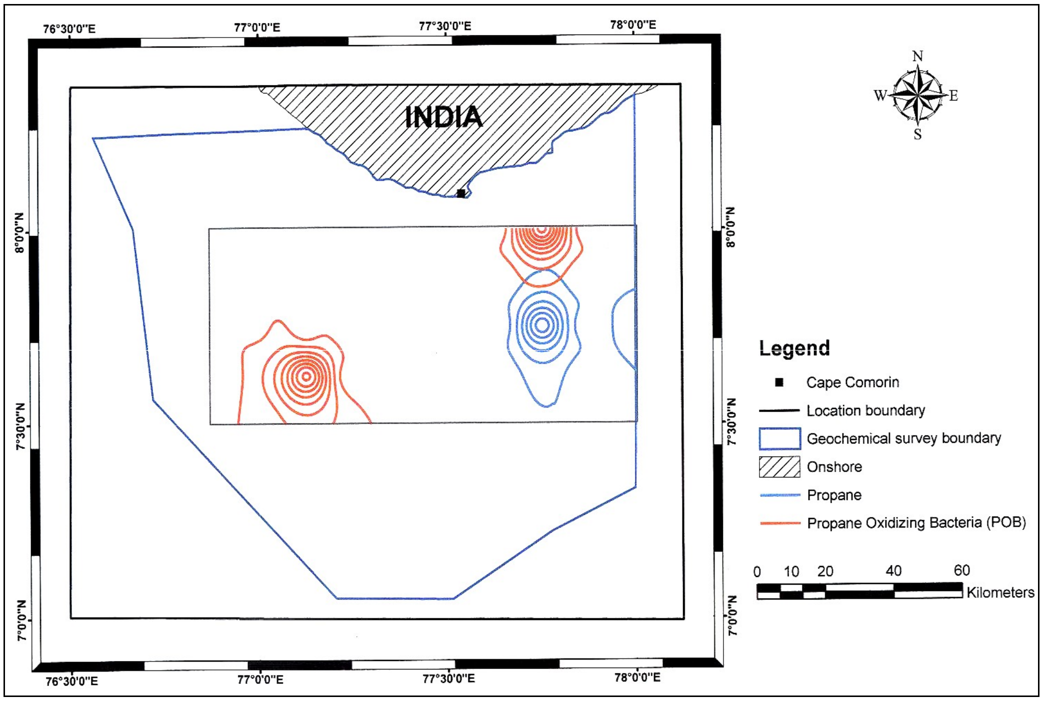


Fig.10 Composite anomaly Propane and Propane oxidizing bacteria (POB) in Seabed sample for Gulf of Mannar, Kerala-Konkan Offshore Basin



and probability plots the molecular composition of gases desorbed from 31 sediment samples are shown in Fig. 6 shows the histograms for methane, ethane, propane, butane and ΣC_{2+} hydrocarbon concentration in the soils reported as ppb against the number of samples. Evaluation of adsorbed soil gas data comprises of graphical representation and statistical analysis to identify the anomalous population of data from background samples. Initially, the histograms and probability diagrams of C_1 and ΣC_{2+} (C_2+C_3+iC_4+nC_4+iC_5+nC_5) are plotted to identify different populations of samples in a data set and to identify anomalous populations. Subsequently, C_1 to C_5 data is interpreted using hydrocarbon cross plots and their correlation coefficients are excellent statistical tools for numerically determining how well the grouped hydrocarbon pairings correlate to each other and also help in determining their sources. The light hydrocarbon ratios are used to predict the oil/gas potential of the Basin. The classification of compositional ratio of C_3/C_1 x 1000 given by Jones and Drozd (1983) predicts that the C_3/C_1 x1000 ratio between 60–500 fall in the oil zone, 20–60 in gas-condensate zone and 2–20 in the dry gas zone. The C_3/C_1 x 1000 ratios obtained for 17 samples having C_1 and C_3 show that almost all the samples fall in the oil zone (except one sample). Fig. 7 is indicative of the zone of occurrence of the accumulated hydrocarbons; here, the samples fall in oil and oil-gas zone. The concentration distribution maps of C_1, C_2, C_3, C_4 C_5 and ΣC_{2+} in the sediment samples of the Kerala-Konkan Offshore Basin are prepared with the help of Geographical Information System (GIS) to demarcate the potential areas with high yields. The concentration distribution maps of C_1 and ΣC_{2+} shown in Figs. 8 and 9 respectively are characterized by samples in the study area.

Integration with Adsorbed soil gas studies

Integrated anomaly plots between adsorbed propane (C_3) and Propane oxidizing bacteria (POB) (Fig. 10) shows inverse trend in the anomaly between in the study area. Composite anomalies for light gaseous hydrocarbons and hydrocarbon oxidizers anomalies are adjacent and follow the natural model depicting 'Halo' pattern (Rasheed et al.., 2008). The bacteria can survive even at low concentration of hydrocarbons of about 10^{-6} vol. % or higher and significantly reach a bacterial count of 10^3 to 10^6 cells per gram of soils or sediments (Wagner et al. 2002). Adsorbed soil gas and microbial intensities indicate that the Kerala Konkan Offshore Basin study area is considered for future hydrocarbons research and exploration.

Analyses of sediment samples of the Kerala-Konkan Offshore Basin show high concentrations of C_1-C_5 hydrocarbons and propane oxidizing bacteria. The presence of significant concentrations of ΣC_{2+} hydrocarbons provides additional evidence of seepage induced anomalies. The adsorbed light hydrocarbons gas and microbial analyses of the sediment samples from part of the Kerala-Konkan Offshore Basin indicate that the area between Latitude 7.6º N to 8.0º N and Longitude 77.65º E to 77.85º E appears to have good potential. Our study supports the Kerala-Konkan Offshore Basin to be promising in terms of its hydrocarbon prospects and need detail investigation with other geo-scientific methods.

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