Biogeophysical signatures of microbial natural gas accumulation

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Natural gas is produced by subsurface thermogenic and biogenic processes. Although the contribution of microbial processes in the formation of methane gas accounts for more than 20% of the global natural gas resources, microbial contribution to natural gas accumulation is rarely considered in geophysical exploration. Thus, a laboratory study has been undertaken to investigate the possibility of monitoring and detecting the microbial formation of methane by electrical potential (EP) technique. Distinct EP observed from acetoclasitc and hydrotropic methanogenesis suggests that the detection and monitoring of biogenic formation of methane is possible by employing biogeophysical techniques.

Keywords: Biophysical techniques, electrical potential, methanogenesis, natural gas.

A few decades ago, applications of electrical geophysical techniques on near surface were restricted to delineate buried ore body and archeological structures, and exploration of groundwater[1–5]. In the last decade, these methods have found new applications in monitoring and detecting contaminant plume in groundwater, estimation of hydraulic properties of aquifers, monitoring the recovery of hydrocarbons from extraction wells as well as microbial-induced physico-chemical alterations on the subsurface[6–9]. In recent years, biogeophysics, a new research sub-discipline in near surface geophysics has emerged from the fusion of three pristine research disciplines, namely microbiology, geochemistry and geophysics; this is capable of monitoring and detecting biofilm growth, and microbial-induced alterations in physical and chemical properties of geological media[10–12]. Both laboratory and field studies have confirmed that the geophysical techniques can be used for monitoring biodegradation of contaminants, and biomineralization of heavy metals in natural and engineered systems[12]. Amongst these methods, self-potential (SP) is considered as the most probable candidate for monitoring biogeochemical processes as it is sensitive to microbial induced redox and geochemical processes. However, mechanistic understanding of SP signals associated with microbe-mediated environments is still incomplete as these signals originate from various biogeochemical processes[13,14]. Recent laboratory studies suggest that the electrical potentials (EPs) generated due to chemical interaction between silver–silver chloride (Ag–AgCl) electrodes and by-products of microbial reduction of contaminants, and the redox fronts generated by biogeochemical processes are responsible for the generation of large EPs, which may not necessarily represent the true SP[15,16]. Thus, a general term ‘EP’ instead of SP is used throughout the text hereafter. EP signals are sensitive to alterations in both the surface chemistry of electrodes and physico-chemical alterations in media by micro-organisms[14]. Some laboratory studies conducted in the past few years suggest that the dramatic change in EP of the order of a few hundreds (–425 to –750 mV) is an indicator of microbial sulphate reduction. Application of biogeophysics in the detection of microbial sulphate reduction has been well established in the past couple of years; however, its application in the detection and monitoring of biogas generation has not yet been reported. This study is an attempt to detect and monitor microbial natural gas accumulation by EP measurements[14,15,17].

The present study is motivated by the capability of the SP method to sense the redox processes[18,19], where electron transfer from a terminal electron donor (TED) to a terminal electron acceptor (TEA) is bridged by micro-organisms through bionanowires (pili)[13,20,21]. Pili are hair-like conductive structures emanating from the bacterial cell which catalyse the redox processes by transferring electron from reducing to oxidizing environment via various pathways[22]. It is an established fact that the micro-organisms extract energy via redox processes for their growth and proliferation in organics-rich environment, and interestingly, EP is also generated by the gradient of redox potentials developed by various processes both in abiotic and biotic environments, indicating the possibility of application of EP as a monitoring tool for microbial processes. A biogeophysical study is conducted to understand the mechanism of microbial formation of methane gas. Russian microbiologists Sergei Winogradsky has used a column in 1880 to create a natural aquatic ecosystem for studying complex microbial communities in the laboratory; this is called the Winogradsky column after him[23,24]. A Winogradsky column is deemed to create a natural ecosystem in a laboratory experiment representing the occurrence of major biogeochemical cycling of carbon (C), nitrogen (N) and sulphur (S). A vast variety of aerobic and anaerobic bacteria grow in this column[16,23,25,26]. The column is packed with soil or mud from virtually any source and water from the same or a different source. Supplemental C, N and S are added to these natural components for facilitating growth of aerobic and anaerobic bacteria[23]. This column illustrates how different microorganisms perform their interdependent roles. Thus, Winogradsky column is a complete and self-contained recycling system, and is driven by energy from light and organic and inorganic matters present in the medium.

EPs associated with microbial sulphate reduction and methanogenesis have been monitored to study the possi-

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bility of non-invasive monitoring of natural gas formation. It has been reported that more than 20% of natural gas reservoir is produced by microbial activities[27]. Milkov[28] has suggested that the global microbial natural gas resource base is secondary, and is derived from existing hydrocarbon reservoirs and organic-rich rocks such as coals and organic-rich shales. Thus, the combined contribution of microbial processes in primary, secondary and mixed processes may be higher than that reported by various researchers[28,29]. This implies that the microbial contribution to the natural gas accumulation is underestimated and therefore, there is a need to study the role of microbial processes in natural gas accumulation.

The EP method in conjunction with supportive liquid phase and solid phase analysis of soil and water samples for estimation of metal concentrations, bacterial populations and biominerals has been employed to understand the microbial formation of natural gas. Bacterial population was estimated by the most probable number (MPN) test, which is based on tenfold dilution theory[30,31]. Soil samples were examined for the presence of bionanowires or pili, microbes and biominerals by scanning electron microscopy (SEM). Biogenic gases carbon dioxide (CO_2), hydrogen sulphide (H_2S) and methane (CH_4) evolved in the column were monitored by connecting the landfill gas analyser to the headspace of the column; these gases are used as the indirect indicators of methanogenesis and sulphate reduction (Figure 1).

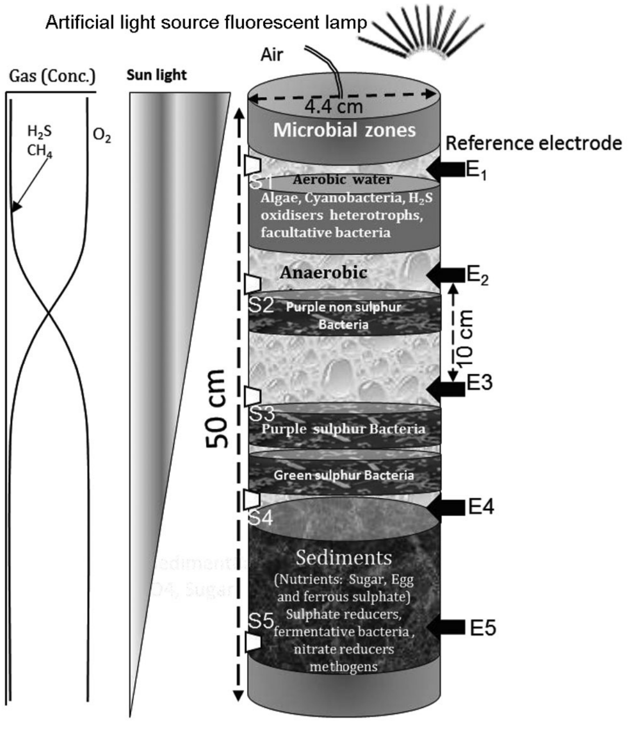

Figure 1. Experimental set-up of Winogradsky column, electrode configuration, sampling ports, anticipated microbial activities and gradient of evolved biogenic gases.

A Perspex column having diameter 4.4 cm and height 50 cm was used for construction of Winogradsky column in a controlled laboratory environment at Queen's University, Belfast, UK. One-fourth of the column was filled with soil, source of sulphate, sugar as carbon source and egg as the source of vitamins, and the remaining three-fourths of the column was filled with Lagan river water collected from a depth of 0.5–1.0 m (Figure 1). The column was illuminated by a fluorescent lamp for allowing the growth of bacteria that extract energy from light. Five Ag–AgCl electrodes, namely E1–E5 were installed in the column at equal spacing of 10 cm. Reference electrode (E1) was installed in aerobic zone and all the EPs were monitored twice a day with reference to it. Water samples were collected through the sample ports (S1–S5) with the help of micro-syringe (Figure 1). Both sample and electrode ports were closed with rubber stoppers and sealed with silicone gel for preventing the intrusion of air into the column.

EPs monitored over a period of 10 months show dramatic change (–425 mV) on day 131 and these large variations in the EP are characterized by elevated concentrations of H_2S and CO_2, and depleted concentrations of O_2 in the experiment column (Figure 2). Large variations in EP signals are generated by chemical reaction between hydrogen sulphide and Ag–AgCl electrodes, resulting in their conversion into Ag–Ag_2S electrodes[15,16]. Depleted

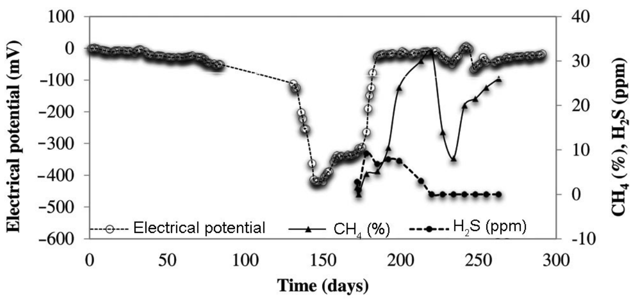

Figure 2. After completion of microbial sulphate reduction on day 171 onwards, plots of concentration of biogenic gases and electrical potential (EP) versus time indicate the presence of acetoclastic and hydrotropic methanogenesis and variations in EP in these processes.

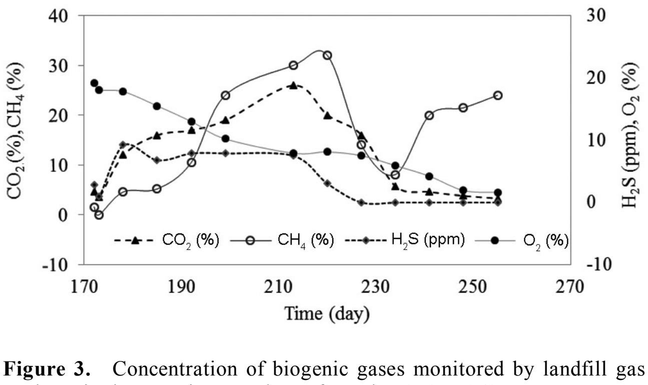

Figure 3. Concentration of biogenic gases monitored by landfill gas analyser in the experiment column from day 171 to 263.

concentration of O_2 indicates that the environment within the column favours the growth of anaerobic bacteria, which is indirectly confirmed by the deposition of iron sulphide (FeS) on the inner walls of the column, resulting in blackening of the column. The presence of sulphate reducing bacteria (SRB) is further confirmed and enumerated by conducting an MPN test on water samples. This test confirms the presence of high bacterial density of SRB (>11,000 MPN/ml). Usually, in natural systems, microbial sulphate reduction occurs before methanogenesis, which is the last step of degradation of carbon substrate[16,32]. Biogenic gases evolved in the Winogradsky column were monitored from day 172 to 263 by the landfill gas analyser before and after methanogenesis begins (Figure 3). It has been observed that the production of H_2S gas increases with increase in production of CO_2 and decrease in production of O_2 (Figure 3).

Observed concentrations of CH_4 and CO_2 after completion of sulphate reduction suggest that the column is dominated by acetoclastic methanogenesis followed by hydrotropic methanogenesis. Acetoclastic methogens utilize acetate as a carbon substrate formed by decomposition of organic matter by fermentative bacteria[33]. The activity of acetoclastic methogens can be described by chemical reactions

$$C_6H_{12}O_2 \xrightarrow{\text{Fermentation}} 6CH_3COOH, \quad (1)$$

$$CH_3COOH \xrightarrow{\text{Acetoclastic methanogenesis}} CO_2 + CH_4. \quad (2)$$

From eq. (2), it is evident that the concentrations of CO_2 and CH_4 increase simultaneously at the time of acetoclastic methanogenesis. A very good fit ($R^2 = 0.8676$) between the concentrations of CO_2 and CH_4 suggests that acetoclastic methanogenesis occurred after microbial sulphate reduction (Figure 4). Variance (R^2) is the square of standard deviation (σ) and is a measure of goodness of fit between the two datasets. EP ranging from –25 to 30 mV has been observed associated with acetoclastic methanogenesis. Hydrotropic methanogenesis is a metabolic process where microorganisms use the simplest form of organics, CO_2 as TEA and H_2 or H_2O as TED to produce methane gas. This implies that hydrotropic methogens reduce carbon dioxide into methane gas. The activity of hydrotropic methanogenesis is described by the reaction

$$CO_2 + 4H_2 \xrightarrow{\text{Hydrotropic methanogenesis}} CH_4 + 2H_2O. \quad (3)$$

Equation (3) shows that CO_2 is utilized completely in hydrotropic methanogenesis. This is well supported by the analysis of concentrations of CO_2 and CH_4 measured on day 230 onwards. The concentration of CH_4 increases with decrease in concentration of CO_2, which is an indirect indicator of occurrence of hydrotropic methanogenesis (Figure 5). A linear fit between these two gases ($R^2 = 0.9036$) supports the activity of hydrotropic methogens in accordance with the eq. (3). EPs associated with hydrotropic methanogenesis are observed in the range of –50 to –26 mV.

The bacterial populations of SRB, nitrate reducing bacteria (NRB) and iron reducing bacteria (IRB) in water samples were estimated by the MPN test. Very high bacterial densities of SRB (>11,000), NRB (>1100) and IRB (>1100) confirm the occurrence of major microbial activities in the Winogradsky column[16] (Table 1). Photomicrographs obtained from both soil and biofilm collected from the experiment column confirm the presence of a variety of microbial communities (Figure 6). The presence of methogens has been confirmed indirectly by measuring concentration of CH_4 as soon as the microbial sulphate reduction is completed (Figures 2 and 3). No H_2S gas was observed to be formed in the column from day 221 onwards. This implies that sulphate (SO_4^{2-}) is completely reduced into H_2S and the environment in the column favours the growth of methogens. The metal analysis of water sample shows high concentration of Ca, Na, K, Mg, Cr and silica (Figure 7). Very high concentration of Na (549.6 ppm) indicates saline environment in the column similar to the sea. These metals may be utilized in biomineralization processes and the study of these processes is not within the scope of this communication.

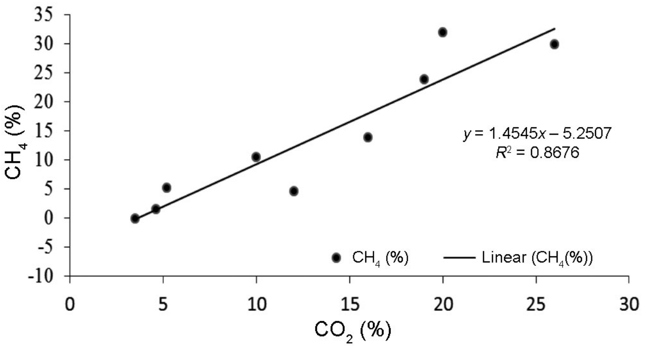

Figure 4. Concentration of methane and carbon dioxide increases simultaneously just after completion of microbial sulphate reduction implying the activity of anticipated acetoclastic methanogen.

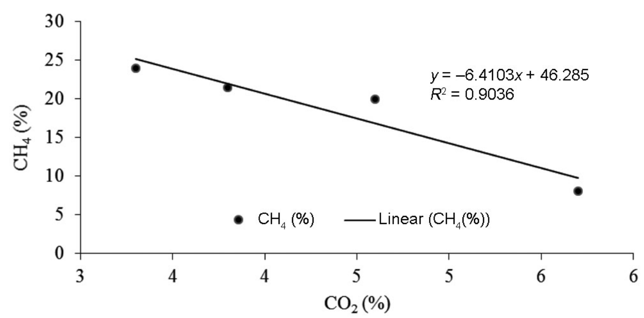

Figure 5. An excellent fit between the concentration of methane and carbon dioxide gases suggests carbon dioxide gas may be reduced into methane by hydrotropic methogens.

Table 1. Enumeration of sulphate reducing bacteria (SRB), nitrate reducing bacteria (NRB) and iron reducing bacteria (IRB) population in water samples by most probable number (MPN) test shows high densities of sulphate, nitrate and iron reducers and confirms their presence

	MPN test	
Microbial activity	Dilution level	MPN/ml
Sulphate reducing bacteria	10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}	> 11,000
Nitrate reducing bacteria	10^{-1}, 10^{-2}, 10^{-3}	> 1100
Iron reducing bacteria	10^{-1}, 10^{-2}, 10^{-3}	> 1100

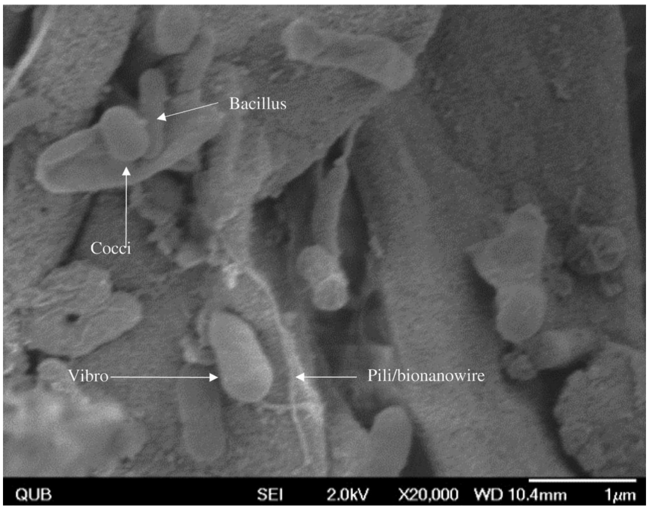

Figure 6. Photomicrograph of soil sample collected after completion of an experiment indicates the presence of complex microbial communities and bionanowires.

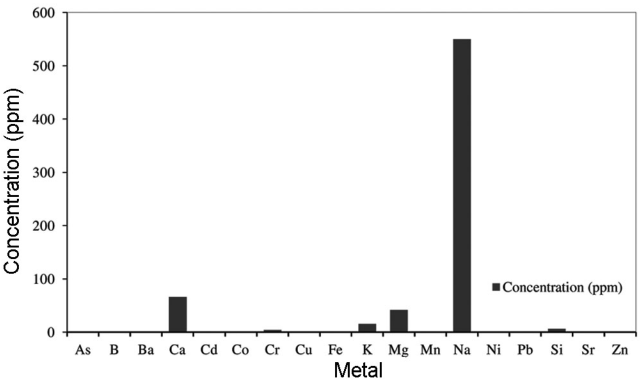

Figure 7. Metal analysis of water sample conducted after 1.42 years shows high concentration (ppm) of sodium (549.6), calcium (66.08), magnesium (42), potassium (15.55), chromium (4.131) and silica (6.225).

Results obtained from this study suggest that hydrotropic methanogenesis occurs after the complete reduction of sulphate into H_2S and formation of FeS (black precipitate). Although acetoclastic methanogenesis is confirmed by biogenic gases evolved in the column after microbial sulphate reduction, such activities may occur even before sulphate reduction, as acetate may be formed by facultative fermentative bacteria. However, it is difficult to draw any firm conclusion about the occurrence of acetoclastic methanogenesis prior to microbial sulphate reduction because biogenic gases evolved in the column could not be monitored until day 171 due to unavailability of gas analyser. This study suggests that both acetoclastic and hydrotropic methanogenesis may occur simultaneously. From the results obtained from this experiment, it is concluded that the processes involved in the microbial formation of natural gas are observable in biogeophysics.

The following conclusions are drawn from the above analysis:

- The Winogradsky column represents a natural system similar to the lakes and oceans in controlled laboratory experiments and the results obtained in the present study suggest that EPs associated with microbial accumulation of CH_4 are measurable by electrical geophysical methods. This study warrants the possibility of application of biogeophysical monitoring and detection of natural gas reservoir on field scales.
- Since the microbial contribution in natural gas accumulation has not yet been studied on large scale, this study encourages application of biogeophysical techniques in exploration of natural gas as a cost-effective and powerful tool in future. This may also be helpful in monitoring performance of biogas plants.
- EPs ranging from –25 to 30 mV and from –50 to –26 mV are the geophysical characteristics of acetoclastic and hydrotropic methanogenesis respectively.
- A well-targetted biogeophysical experimental study in conjunction with supportive microbiological and geochemical studies is required for better understanding of natural gas accumulation in water–sediment environment, and secondary production of natural gas from the existing hydrocarbon reservoir and in rocks rich in organics.
- Although some of the geochemical analyses could not be performed due to limitation of experimental design and unavailability of resources, this study shows that EP can be used to monitor natural gas production both in engineered and natural systems. Additionally, EP data may be used for modelling of subsurface geochemical species altered by methogens similar to sulphate and nitrate reduction processes[34,35].

- The study also suggests that the laboratory column and tank experiments are less time-consuming and cost-effective alternatives for understanding the mechanisms of biogeochemical processes occurring in marine environment, electron transfer from marine sediments to sea surface in microbe-mediated processes, studying ecosystems and biogeochemical cycles of S, N, Fe, C and other chemical elements[5,36,37].

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