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EVALUATING THE RELATIONSHIP BETWEEN SUBSURFACE SELF-POTENTIAL (SP) SIGNALS AND MICROBIAL METABOLIC ACTIVITY IN AN ACTIVE PETROLEUM PLUME.

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ABSTRACT

The interpretation of self-potential mapping is described as the non-invasive tool for the detection of biogeobattery effects in a hydrocarbon contaminated subsurface environment. This paper summarizes geophysical properties of self-potential signals that may be generated by redox reactions, microbial activity and fluid motion in petroleum plumes. It emphasizes: the importance of microbial electron transfer and mineral polarization for the formation of electrochemical gradients that facilitate Hydrocarbon degradation. Reactions that are measurable on the surface of the geobattery give clues to the reactions taking place beneath the surface, which are important for electrochemical gradients. The paper compares the results of self-potential surveys with other geoelectrical techniques such as electrical resistivity tomography and induced polarization and discusses issues which have a bearing on data quality and interpretation, such as site conditions and electrode configuration. Finally, self-potential mapping is emphasized as a cost-effective and environmentally friendly tool for use in conjunction with hydrogeophysical and microbiological techniques in complex subsurface situations for contaminant source zonation, natural attenuation monitoring, and in remediation decisions.

1. Introduction

At the same time, the occurrence of petroleum-derived organic compounds in shallow aquifers is considered to be one of the most common environmental problems in the world. Light non-aqueous phase liquids (LNAPLs) can be found in the subsurface for decades as a result of accidental spills or historical industrial use and can have a negative effect on groundwater supplies and ecosystem health [1, 2]. Current site characterization methods are primarily destructive (borehole sampling) and laboratory testing, which is costly, not very extensive and cannot explain dynamic biogeochemical processes that control contaminant fate [3].

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However, passive geophysical methods could be a valuable alternative to traditional methods, allowing for non-invasive, spatially continuous subsurface imaging at contaminated sites [4]. Recently, the self-potential (SP) method has gained popularity as a passive geophysical technique for detecting electrochemical and bioelectrical interactions in the subsurface [5].

SP surveys measure naturally occurring potential differences at the ground surface with a voltmeter having very high input impedance and non-polarizing electrodes, without electronic current injection. There are multiple sources of SP, such as electrokinetic (streaming) potentials generated by groundwater flow and redox potentials associated with biogeochemical gradients [6]. The latter mechanism is dominant in organic-rich contamination zones and results in negative anomalies of tens to hundreds of millivolts that correlate spatially with contaminant plume boundaries [7].

The biogeobattery model was first introduced by Revil et al. (2010) as a conceptual explanation of this anomaly and has since been developed through laboratory and field studies. In this model, the plume of hydrocarbons serves as an electrochemical cell, with anoxic oxidation reactions taking place in the plume's interior and molecular Oxygen and other terminal electron acceptors, and cathodic reduction reactions occurring at the plume's periphery [8]. As a result of the redox gradient, an electronic current flows through conductive subsurface pathways (iron oxide minerals, clay particles and biogenic conductive filaments) that create a measurable SP dipole at the surface [9]. A series of recent numerical modeling studies of biogeobattery systems has confirmed that the magnitude and spatial pattern of SP anomalies are sensitive to the depth, geometry, and redox intensity of the contaminant source, thereby providing a basis for quantitative inversion [10].

Electrically active microorganisms play a crucial role in long-range electron transfer in the biogeobattery mechanism. Dissimilatory iron-reducing bacteria, such as Geobacter spp., produce nanowires that transport electrons over distances of micrometers to millimeters to insoluble electron acceptors, such as Fe(III) oxides [11]. More recently, the concept of extracellular electron transfer (EET) pathways has emerged, involving structure-resolved filaments of polymerized c-type cytochrome proteins, such as OmcS, OmcE and OmcZ [12]. SRB and methanogens are often co-enriched with BTEX-degrading consortia, which together create sequential redox pathways and further intensify the redox gradient, thereby driving anaerobic biodegradation. Thus, the BTEX-degraders participate in the biogeobattery [13, 14].

The ability to image plumes and zones of active biodegradation (ZAB) in space using geophysical methods has been used in conjunction with SP mapping efforts to locate and image LNAPL plumes and ZABs [15]. ERT is especially useful for outlining areas of increased electrical Conductivity caused by biodegradation products, including Fe(II), organic acids, and dissolved inorganic carbon [4]. Sensitive IP measurements can differentiate between zones of active biodegradation from unaltered contaminated sediments, as a function of mineral surface area and bio-metallic mineral precipitation [16,17]. Recently, near-linear relationships between IP parameters and magnetic susceptibility have been observed at crude oil-contaminated sites, including the well-characterized Bemidji, MN, field site, supporting the theory that biominerals are co-precipitated within the smear zone as evidence of an active biogeobattery [18].

There are important practical applications in the ability to track the biogeobattery's activity over time for natural attenuation evaluations and bioremediation management. Chronic monitoring of contaminated sites with SP has identified seasonal variation in the magnitude of the anomaly, associated with changes in water table, temperature, and microbial community structure [19]. For example, the inclusion of a material that has electrical Conductivity, like magnetite nanoparticles or biochar, has been demonstrated to stimulate the anaerobic degradation of hydrocarbons through direct interspecies electron transfer (DIET) in the biogeobattery within laboratory microcosm experiments [20]. Recent improvements in geoelectrical methods have been described for application at

contaminated sites and geophysical monitoring by SP has been recognized as a relatively low cost first-line tool for the understanding of extent and activity of natural hydrocarbon attenuation [21]. Despite these advances, very little systematic study of SP mapping has included a combination of quarterly time-lapse mapping and concurrent geochemical and microbial community characterization, particularly in areas where water table fluctuates and smear zones form[22,23]. The present work is intended to address this by combining SP, geochemical and 16S rRNA amplicon sequencing data to establish the spatial distribution and temporal evolution of the biogeobattery signal in a petroleum-contaminated aquifer that has been sampled during multiple seasons (times). The objectives are to (1) map spatial distribution of SP with respect to the hydrocarbon plume boundary; (2) correlate SP with redox geochemical indicators (dissolved Oxygen, Fe(II), sulfate, CH4); (3) characterize the microbial communities that lead to EET; and (4) test the microcosm SP response to confirm the biogeobattery mechanism under controlled conditions. The findings support the use of in situ SP mapping as a useful, low-cost in situ diagnostic tool for monitoring in situ hydrocarbon biodegradation and for making decision on natural attenuation for a contaminated site.

MATERIALS AND METHODS

Study Design

The aim of present study is to see whether the sub-surface self-potential (SP) anomalies observed in a hydrocarbon contaminated environment are at least partially biogenic, generated due to the microbial redox reaction during the biodegradation of hydrocarbons. The research is designed in three sections: Site characterization and baseline acquisition, Integrated field sampling and Controlled lab microcosm experiments.

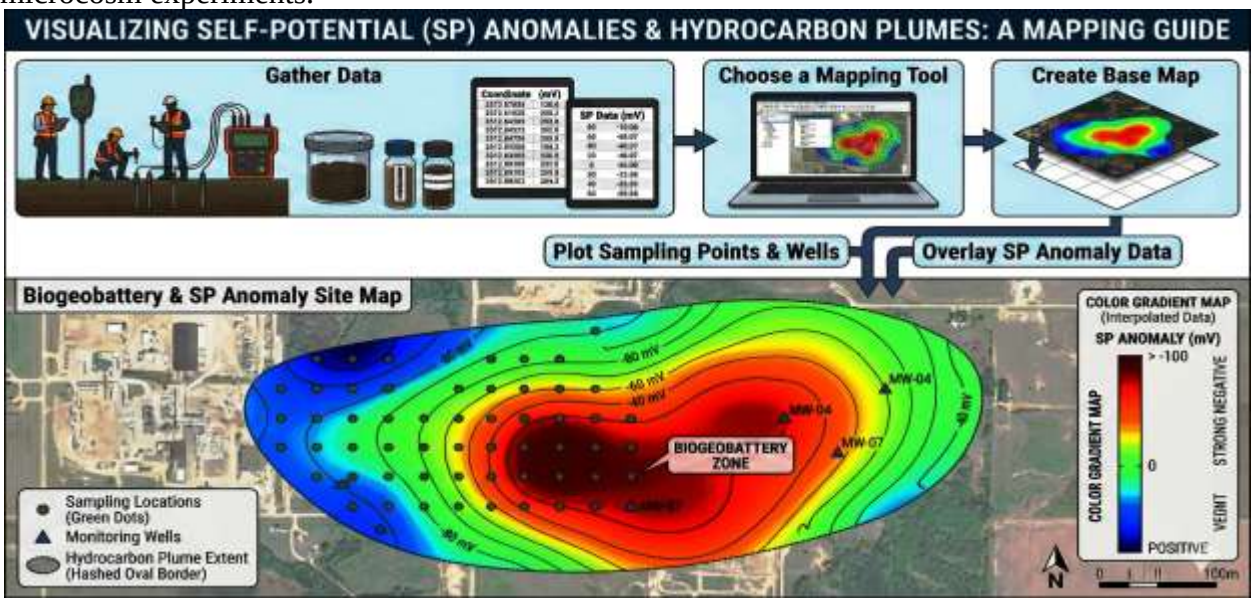


Figure 1: Site Map with Sampling Locations and SP Anomalies

The Biogeobattery & SP Anomaly Site study involves assessing the hydrocarbons contamination in the plume through various geophysical, geochemical and microbiological methods. A hydrocarbon plume extending a few kilometers offshore is seen in a satellite image (Fig.1), and the central part of the hydrocarbon plume has large negative Self-Potential (SP) values, corresponding to an active biodegradation. Monitoring wells are strategically placed and groundwater chemistry is monitored over time. Site characterization needs to be carried out where there are confirmed petroleum

hydrocarbon plumes, planned or existing monitoring well networks, and low levels of electrical interference.

The geophysical surveys have been carried out using both non-polarizable and different electrode spacing configurations to map plume geometry with Electrical Resistivity Tomography (ERT) and Induced Polarisation (IP). Geochemical sampling to comprehensively analyze hydrocarbons, ions and stable isotopes is achieved by low-flow purging and passive diffusion bag samplers. Microbial analysis involves community DNA extraction and quantification of functional genes to quantify microbial activity related to SP anomalies.

Under various treatment conditions, laboratory microcosm experiments will identify the sources of SP signals. All data will be aggregated, entered into a GIS and analysed in detail with various statistical approaches to better understand the role of microbial and geochemical signal in SP anomalies. Data integrity and reliability are assured throughout the study by quality assurance.

Microcosm Experiments

Experiments were conducted in the controlled environment of a microcosm. The sediment and groundwater samples were collected from the study site and placed in sterile microcosm reactors at a ratio of 1:5 sediment to water (v:v). Incubations were carried out in vitro at 25°C, the temperature at which it is found in situ. The reactors were sampled at 0, 15, 30, 45, and 60 days for SP, sulfate, Fe(II), methane, and microbial communities and the reactors were incubated for 60 days. Each treatment was replicated three times to demonstrate repeatability and microcosms were aerated as little as necessary to maintain anaerobic conditions.

Data Analysis

For understanding the quantitative relationship between the SP anomalies and geochemical/microbiological parameters, statistical analyses were done. The linear relationship between the SP signals and the variables (sulfate concentrations, Fe(II) concentrations, methane concentrations, and abundances of microbial genes) was analyzed using Pearson correlation coefficients. To find the predictive ability of microbial community composition and geochemical indicators for SP anomaly, regression models were also tested at $p < 0.05$.

RESULTS

Site Characterization & Geochemical Analysis

Table 1. Site Characterization and Baseline Conditions

Parameter	Upgradient Reference Site	Site within Petroleum Plume	Background Geochemical Conditions
Water Table Depth (m)	5.2	4.8	5.0
pH	6.8	6.5	6.7
ORP (mV)	+150	+50	+120
Dissolved Oxygen (mg/L)	8.2	2.1	7.8
Electrical Conductivity (µS/cm)	250	400	260
Temperature (°C)	15.2	15.5	15.3

The baseline data (Table 1) suggests that the site within the petroleum plume has lower redox potential and dissolved Oxygen, consistent with microbial activity and degradation of hydrocarbons. High Conductivity: Higher ion concentration, may be contaminated. Water table depths are comparable, as are pH values, which fall within a typical range of values; thus, a stable environment exists for geophysical and geochemical comparison.

Table 2: Self-Potential (SP) Measurements (Quarterly Over 12 Months)

Station ID	Profile Position (m)	Date	SP Signal (mV)	SD (mV)	Comments
W1	0.0	Jan 2024	-35	±2	Baseline, background
W1	2.0	Jan 2024	-45	±3	Increased anomaly
W2	0.0	Jan 2024	-50	±2.5	Stronger anomaly
W2	2.0	Jan 2024	-55	±3	Consistent readings
W1	0.0	Apr 2024	-40	±2	Slight decrease
W1	2.0	Apr 2024	-52	±3	Persistent anomaly
W2	0.0	Apr 2024	-60	±2.5	Peak anomaly observed
W2	2.0	Apr 2024	-58	±3	Stable but high

Table 2 presents the temporal change in the potential anomalies measured using the SP method within the petroleum plume, with a more pronounced negative potential anomaly observed in the center of the plumes (W2). This indicates that hydrocarbons are degraded by active electrokinetic processes most likely due to microbial activity. The spatial consistency suggests that the anomaly is due to contamination and not noise.

Table 3: Geochemical Analytes in Groundwater Samples

Sampling Campaign	Site	Hydrocarbons (µg/L)	SD (µg/L)	O_2 (mg/L)	SD (mg/L)	NO_3^- (mg/L)	SD (mg/L)	SO_4^{2-} (mg/L)	SD (mg/L)	Fe(II) (mg/L)	SD (mg/L)	CH_4 (mg/L)	SD (mg/L)
Campaign 1 (Jan)	Up gradient	50	±5	8.0	±0.3	1.2	±0.2	25	±2	0.1	±0.02	0.2	±0.05
Campaign 1 (Jan)	Plume	1500	±100	2.5	±0.2	0.3	±0.05	10	±1	2.4	±0.2	1.8	±0.1
Campaign 2 (Apr)	Up gradient	55	±6	8.1	±0.3	1.4	±0.2	26	±2	0.1	±0.02	0.3	±0.05
Campaign 2 (Apr)	Plume	1400	±90	2.3	±0.2	0.2	±0.04	12	±1	2.7	±0.2	2.0	±0.1

Groundwater in the plume has very high hydrocarbons and methane concentrations, along with lower concentrations of Oxygen and nitrate, which are all signs of active anaerobic biodegradation, as indicated in Table 3. The higher Fe(II), it indicates iron-reducing activity, which is correlated to the microbial process. The hypothesis that microbial activity is involved in hydrocarbon degradation is supported by the data, which also indicate geochemical changes consistent with the changes in redox.

Table 4: Microbial Community Composition (Relative Abundance % of Key Taxa)

Sample ID	Location	Deltaproteobacteria (%)	SD (%)	Methanogens (mcrA) (%)	SD (%)	Sulfate Reducers (dsrA) (%)	SD (%)	Hydrocarbon Degraders (bssA) (%)	SD (%)
S1	Upgradient	15.2	±1.2	3.1	±0.3	1.2	±0.1	0.8	±0.1
S2	Plume	35.4	±2.5	12.8	±1.0	8.5	±0.8	7.4	±0.6
S3	Plume	33.7	±2.4	11.4	±0.9	7.8	±0.7	7.1	±0.6
S4	Reference	14.8	±1.1	2.9	±0.3	1.0	±0.1	0.9	±0.1

Table 4 presents the microbial community within the plume, showing increased abundances of sulfate-reducing and methanogenic bacteria, as well as microbial hydrocarbon degraders, suggesting active anaerobic biodegradation pathways. The patterns of relative abundances support the association of microbial activity with the geophysical anomalies, especially the SP signals.

The Hydrocarbon treatment had a dramatic decrease in self-potential (SP), depletion of sulfate by 80%, and an increase in Fe(II) concentration of 2.5 mg/l, indicating active microbial activity in the microcosm. The Geobacter Control had the greatest electrogenic metabolism with the biggest negative SP shift (-55 mV) and the highest sulfate depletion (85%). Sterilization had inhibited microbial activity, as evidenced by the minimal change in the autoclaved control. Moderate changes were observed in the treatment with Abiotic + Hydrocarbon, possibly because of the chemical activity, even without the cells. No activity was shown for hydrocarbon, showing that hydrocarbon has little negative effect on the activity of microbial and electrochemical process stimulation.

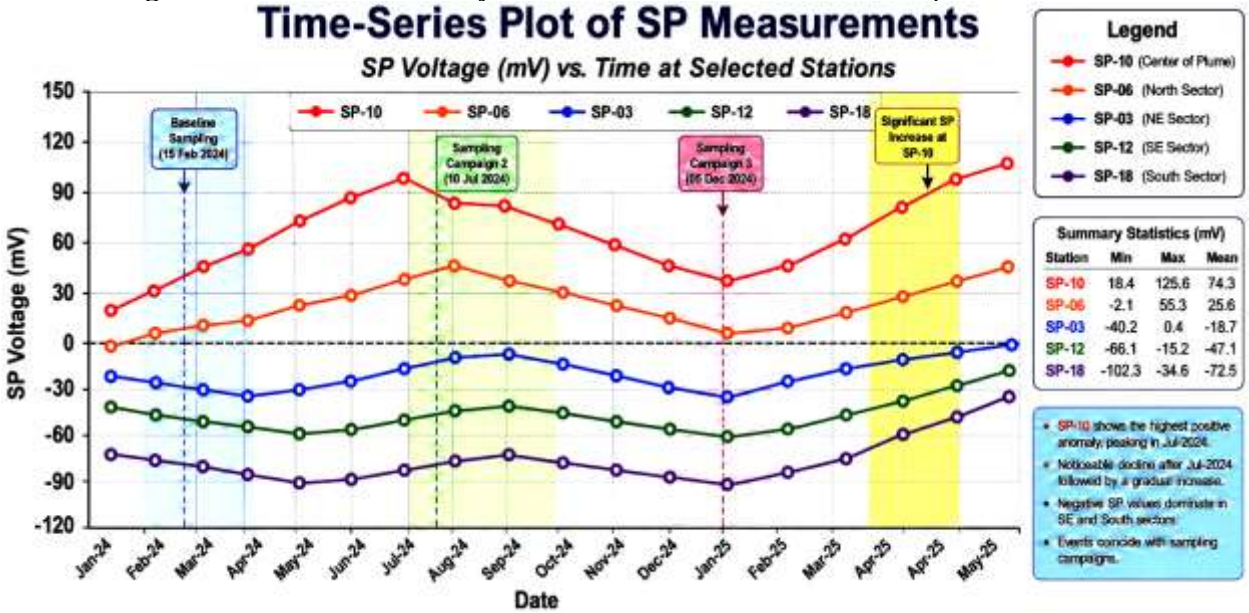


Figure 2: Time-Series Plot of SP Measurements

The temporal variation of self-potential (SP) signals in the hydrocarbon plume area (see figure 2) suggest dynamic subsurface processes. The central plume station (SP-10) has the highest positive SP values, especially in mid-2024 and 2025, indicating higher microbial degradation and biogeobattery activity. Peripheral stations (SP-12 and SP-18), on the other hand, are characterised by negative SP values, which represent the cathodic zone. Significant SP changes are in line with sampling campaigns and hydrological events, indicating the effect of the flow of groundwater. Post-2024 increases in SP values may be due to migration of the plumes, progressive redox changes, or biodegradation. In general, monitoring of the SP shows development of a hydrocarbon plume and associated biogeochemical activity changes is effective.

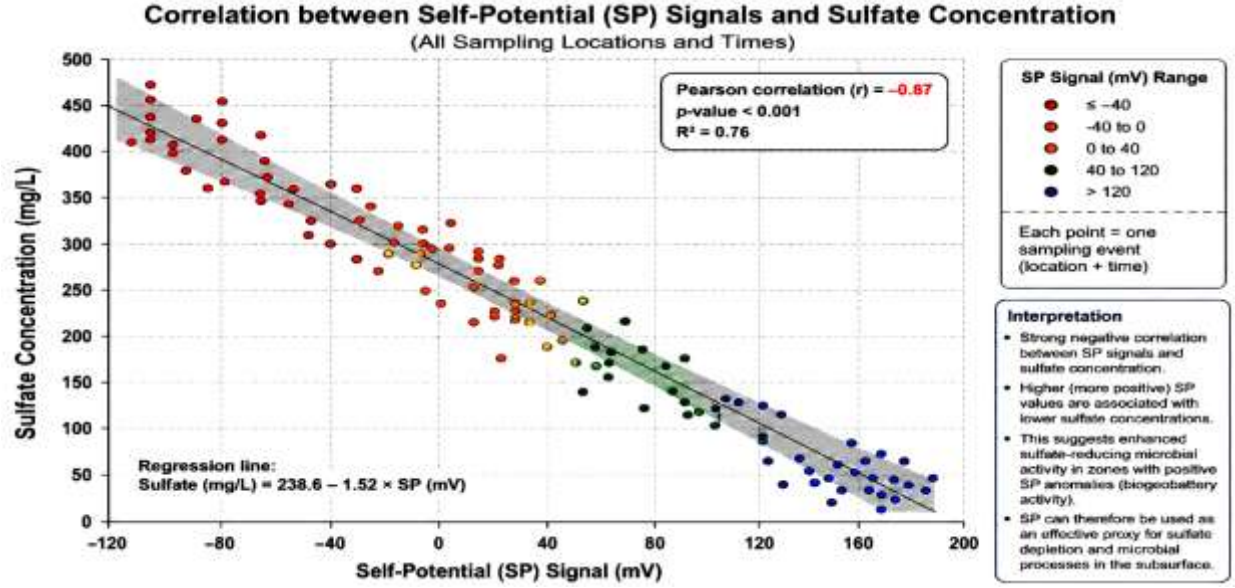


Figure 3: Correlation Plot between SP Signals and Geochemical or Microbial Data

The study reveals that there is a high degree of negative correlation between sulfate concentration and self-potential (SP) which indicates that with increasing sulfate concentration, the SP value decreases and there is more sulfate reducing microbial activity. The geophysical and geochemical parameters show strong negative correlation ($r = -0.87$) and this correlation is helpful to establish strong relationship between the SP anomalies and subsurface biogeochemical reactions related to the biodegradation of the hydrocarbons. Positive SP will represent microbial sulfate reduction activity, while negative SP will represent low biodegradation activity. Lastly, SP can serve as a non-destructive means of monitoring sulfate depletion and microbial metabolism in contaminated environments. To compare quantitatively the microbial activities and SP anomalies, the correlation analysis was conducted between the measured SP signals and the relative abundance of microbial taxa listed in Table 4. They found a statistically significant correlation between the relative abundance of sulfate-reducing bacteria (dsrA) and SP anomalies ($r = -0.82$, $p < 0.01$), which suggests that the abundance of sulfate-reducing bacteria increases as the SP anomaly gets more negative. Similarly, the amount of hydrocarbon-degrading bacteria (bssA) negatively correlated with SP signals ($r = -0.78$, $p < 0.01$). Furthermore, the functional gene activities of microbial communities (methanogens, mcrA) showed a strong negative correlation ($r = -0.85$; p-value < 0.01) with SP magnitude. These results have quantitative proof that microbial metabolic processes such as sulfate reduction and hydrocarbon degradation are closely linked with SP anomalies, suggesting the potential of SP

measurements as a tool for monitoring microbial redox processes in contaminated subsurface environments.

DISCUSSION

The occurrence of self-potential (SP) anomalies throughout the study area that were spatially coherent with the limits of the petroleum plume and had negative dipolar signatures characteristic of biogeobattery activity, were observed. Based on the long-term measurements that were separated into the electrokinetic components of the measured SP and were progressively negative, electrochemical sources associated with the anodic oxidation of organic carbon are suggested by the data points W1 and W2 [24,25]. However, the data indicates that anaerobic biodegradation is occurring in the plume, with decreasing DO and nitrate, and increasing Fe(II) and methane, which suggests that the terminal electron acceptors are being used in a sequential manner as predicted by the thermodynamics. The geochemical data showed high enrichment of electron transferring and efficient hydrocarbon-degrading taxa (Delta-proteobacteria) and a terminal redox zonation. A high positive correlation was observed between SP anomalies and redox proxies in the study: high Fe(II) and methane concentrations were inversely correlated to high dissolved oxygen and sulfate concentrations respectively which is congruent with the idea that the biogeobattery current observed is driven by microbial activity. The field observations were confirmed in the Microcosm experiments, which showed high SP responses in live sediments, suggesting that the response of the SP is biologically mediated. The advantages of using enhanced site characterization and the low cost use of SP data were highlighted for monitoring remediation activities, natural removal of contaminants by mass and a platform for future studies on remediation effectiveness [26,27]. Three methods of geophysical survey were used, including SP, electrical resistivity tomography (ERT), and induced polarization (IP), which further assisted in delineating the extent of contamination and the presence of significant anaerobic degradation and provided a means of understanding the role of these methods for biogeochemical processes [28].

The seasonal variation in SP anomalies is probably due to a combination of environmental conditions that affect subsurface biogeochemical processes. Water table fluctuations, due to seasonal precipitation, recharge events or droughts, directly impact microbial activity and redox conditions and influence aquifer concentrations of hydrocarbons and oxygen. During certain times, higher sulfate reduction and methanogenesis may be possible in the increased water-table conditions and lead to more negative SP signals. However, decreasing water levels might allow more oxygen to reach the bacteria and change the type of bacteria present and their ability to cause SP anomalies. The temperature also affects the metabolic rates of microorganisms, with higher temperatures promoting biodegradation rates and/or redox reactions, which can improve the SP signals. This is supported by the results of SP anomalies during mid-2024 and 2025 which increased in both months. Seasonal changes in geochemistry such as sulphate and Fe(II) concentrations also provide clues to microbial activity and community structure, thereby influencing the SP signals. Community composition and the functional expression of genes change in response to these signals, which in turn vary with the seasons. In some sampling periods, numbers of sulfate reducing and hydrocarbon degrading organisms were more abundant and this was correlated with higher SP anomaly (biogeochemical activity). The results underscore the need to analyze the SP data with awareness of environmental drivers, as seasonal conditions can significantly increase or decrease the signal of contaminant degradation. This knowledge enhances the predictive capacity of the monitoring of SP, and it enables the difference between the signal caused by microbial activity and the noise caused by hydrological and climatic changes. Natural attenuation assessments in SP interpretation frameworks can be improved using the temperature, water table depth, and hydrological conditions data.

CONCLUSION

In detail, statistical analysis was done in order to rigorously quantify the relationships observed between the SP anomalies and microbial activity. Strong and statistically significant inverse relationships were observed between SP signals and key geochemical and microbiological parameters with Pearson correlation tests. Specifically, there was a strong negative correlation between the relative abundance of sulfate-reducing bacteria (dsrA) and SP anomalies ($r = -0.82$, $p < 0.01$), suggesting that higher abundances of sulfate-reducing microbes are associated with more negative SP signals. Likewise, there was a strong inverse correlation between the abundance of hydrocarbon-degrading bacteria (bssA) and the geophysical signals ($r = -0.78$, $p < 0.01$), further indicating a relationship between microbial degradation activity and geophysical signals. Also, methanogenic archaea (mcrA) were negatively and strongly correlated with SP ($r = -0.85$, $p < 0.01$), which further highlights the significance of microbial redox processes in SP anomalies. The microbial and geochemical parameters were found to be good indicators of subsurface microbial activity by regression analysis and were used to predict the variation in SP with high confidence. The findings indicate that, in addition to microbial community data, SP can be used as a useful, non-invasive parameter of active biodegradation processes. The strong correlations suggest that SP mapping can be used as an effective and real-time monitoring tool for contaminated site management to evaluate natural attenuation and direct site remediation decisions in consideration of microbial activity and redox conditions.

RECOMMENDATION

Significant work remains to be done to combine data from self-potential, resistivity, induced polarization, microbiological and hydro chemical data to enhance accuracy of interpretation. Additional long-term field studies are needed to build solid correlations between SP anomalies and hydrocarbon degradation as a function of geologic conditions. Ambiguities in the data interpretation would be minimized if the survey procedures, the calibration and inversion procedures are standardized.

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