# Engineered Anaerobic Bio-Oxidation Systems for Petroleum Hydrocarbon Residual Source Zones with Soluble Sulfate Application

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Traditional methods of remediating petroleum hydrocarbons (PHCs) and other biologically oxidizable contaminants have historically focused on strategies employing volatilization (i.e., in situ air sparging and soil vapor extraction) and/or aerobic biodegradation of the water-soluble fraction of residual mass. There are some settings where these technologies are hampered by long remediation timeframes and escalated costs, generating a renewed interest in exploring other innovative and alternative petroleum-specific remediation techniques to bring sites to closure more quickly.

One technique that continues to gain traction is the engineered application of anaerobic bio-oxidation (ABOx), where soluble electron acceptors other than oxygen are applied to PHC release sites to stimulate biodegradation. The basis for this lies in the degradation of PHCs by ABOx pathways, which have been reported in the literature for well over 30 years with the initial focus on PHC releases in marine environments (Atlas, 1981) and more recently in soil systems (Leahy and Colwell, 1990; Chapelle, 1999). A review of biogeochemical data from multiple PHC release sites demonstrates that groundwater conditions are predominantly anaerobic; based on the availability of background sulfate concentrations in these systems and favorable reaction yield (i.e., mass of PHC degraded per mass of sulfate used), ABOx via sulfate reduction represents the dominant terminal electron accepting process (TEAP) accounting for approximately 70% on average of the natural biodegradation capacity (Wiedemeier et al., 1999).

Moving beyond natural attenuation studies, the engineered application of non-oxygen electron acceptors, such as sulfate, to enhance the degradation of PHCs under anaerobic conditions is a more recent development (Lunardini and Dickey, 2003). Most PHC plumes that persist following source removal are usually the result of residual mass present within a "smear zone" located at or near the water table. Therefore, a viable remedial strategy must be capable of addressing both the dissolved phase PHC's and residual source mass. Through effective bio-oxidation of the water soluble fraction and through expedited "weathering" of residual source mass, ABOx has been found to be effective as a polishing remedial approach in achieving groundwater compliance concentrations.

In engineered applications, sulfate has a number of advantages including high solubility, prolific populations of naturally occurring microbes capable of utilizing sulfate, and persistence in the subsurface once applied. This combination gives sulfate-driven ABOx the potential to provide more efficient use of electron acceptor, and thus decrease remedial timeframes compared to intrinsic natural attenuation and to lower overall remediation costs when compared to more active remedial systems. With a solid body of supporting science behind engineered ABOx and increasing number of field implementations, the stage is set to review the current practices regarding successful remediation of PHCs, and closure of related sites with this technology.

The following sections explore the advantages of using sulfate-based ABOx processes, related geochemical considerations, observations from field implementation, and finally how this approach may be positioned in the spectrum of conventional PHC remediation techniques.

## ABOx Using Sulfate

Oxidation is the dominant intrinsic degradation process for PHCs, whether under aerobic or anaerobic conditions. This involves the transfer of electrons from the hydrocarbon (oxidizing it) to an electron acceptor. The most familiar electron acceptor (oxidizing agent) is oxygen; however, any process that removes electrons from other substances via an electron-accepting process results in oxidation.

Aerobic bioremediation requires that adequate oxygen be available for the metabolic processes to occur. However, oxygen is readily consumed in the subsurface with the kinetics of utilization seeming to be instantaneous when compared to the relatively slow movement of groundwater. In the presence of a degradable source of organics there will be numerous mechanisms

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that can scavenge oxygen and prevent it from being available for PHC biodegradation. Since the rate of oxygen recharge under natural conditions is not usually adequate to meet the demand, petroleum-impacted zones are typically anaerobic.

Anaerobic metabolism of PHCs still involves their oxidation, but is supported by alternate TEAPs such as nitrate reduction, ferric iron reduction, sulfate reduction, and methanogenesis depending on the availability of electron acceptors compared to the availability of degradable organics.

When the natural supply of electron acceptors is insufficient to biodegrade the water-soluble PHC fraction dissolving or desorbing from residual source mass, sulfate enhancement offers advantages in supporting the PHC remediation via the ABOx process. The applicability of ABOx to the water soluble hydrocarbon classes found in fuels through sulfate-reduction has been well established in the literature. In comparison to oxygen, which is susceptible to numerous non-target scavenging reactions (Deutsch, 1997), the removal of sulfate in groundwater systems is predominantly through biologically-mediated sulfate reduction, with the exception of some high-solubility precipitates that can form (Atlas and Bartha, 1998). Further, sulfate reducing bacteria are prolific and capable of metabolizing broad classes of PHCs (Chapelle 2001). As a remediation reagent, high solubility, low-cost sulfate salts are commercially available at the industrial scale.

Table 1 provides a comparative summary of various electron acceptors that are applicable to PHC degradation, from the perspective of (1) dissolved electron acceptor concentrations that can be achieved, (2) the stoichiometric relationship of benzene mass mineralized per unit mass of each electron acceptor, and (3) the corresponding biodegradation yield that could theoretically be realized based on the achievable dissolved electron acceptor concentrations. As shown on Table 1, the elevated feasible concentrations that can be achieved with sulfate results in an oxidizing payload that is orders of magnitude larger than oxygen-based systems, with only a secondary maximum contaminant level (MCL) to be aware of in terms of residual sulfate concentrations. The ultimate biodegradation potential of each electron acceptor strategy in field-scale engineered systems will be controlled by the effective concentration achieved in groundwater, which will likely be less than those maximum values presented in Table 1, as well as the pace of electron acceptor loading (i.e., continuously operating air sparge well vs. periodic pulsed sulfate injections).

## Geochemical Considerations

With sulfate serving as the electron acceptor, the mineralization of benzene as a specific PHC of interest at most fuel release sites is given by Equation 1 at neutral pH:

$$\text{(benzene) } C_6H_6 + 3.75\ SO_4^{2-} + 3H_2O \rightarrow 1.875\ H_2S + 1.875\ HS^- + 6HCO_3^- + 0.375\ H^+ \quad (1)$$

This chemistry reflects the fact that the utilization of sulfate to support the ABOx process can have a variety of geochemical secondary effects that must be understood to best implement the technology. If not adequately understood and accounted for, these geochemical factors can limit the effectiveness of the technology. A number of specific considerations stemming from the sulfate-supported

**Table 1**
**Comparison of Various Electron Acceptors in Their Potential to Degrade Benzene (adapted from Cunningham et al. 2001)**

| Electron Acceptor | Effective Maximum Concentration in Water (mg/L) | Reaction Yield: Benzene Mass Degraded Per Unit Mass of Electron Acceptor | Potential Maximum Benzene Degraded (mg/L) | Potential Complications |
|---|---|---|---|---|
| Oxygen (ambient air source) | 9–10 | 0.33 | 3.0–3.3 | • Limited solubility<br>• Numerous non-target scavengers<br>• Potential aquifer clogging through biofouling and precipitation of iron |
| Oxygen (pure) | 60–70 | 0.33 | 19.8–23.1 | |
| Nitrate | 670,000 ($NaNO_3$)<br>$1.1 \times 10^5$ ($Mg(NO_3)_2$) | 0.21 | 140,000<br>220,000 | • Primary MCL of 10 mg/L $NO_3$-N (45 mg/L $NO_3$) |
| Iron (III) | 0–1 | 0.024 | 0–0.024 | • Not practical to inject—very low solubility at neutral aquifer pH |
| Sulfate | 48,000 ($Na_2SO_4$)<br>140,000 ($MgSO_4$) | 0.22 | 9000<br>25,000 | • Secondary MCL for sulfate—250 mg/L<br>• Hydrogen sulfide produced as a byproduct; rarely documented as an issue in the field |



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ABOx biogeochemistry, as well as the intrinsic characteristics of the reagents, are discussed further below.

### Sulfide Production

Sulfate reduction produces reactive sulfide (i.e., $H_2S$ and $HS^-$ from Equation 1). If not controlled, the accumulation of reactive sulfide can eventually become inhibitory to sulfate-reducing bacteria. In situ, sulfide inhibition has been reported to reduce the rate of sulfate reduction by approximately one-third at concentrations between 8.5 and 320 milligrams per liter (mg/L) (Roychoudhury and McCormick, 2006). In general, the specific concentration range at which hydrogen sulfide will be inhibitory is likely tied to the site-specific microbial community and geochemical conditions.

Despite this fact, there are a number of natural mechanisms that help suppress sulfide accumulation, which primarily relates to the natural availability of iron in aquifer minerals. Naturally occurring iron will help support sulfide control in two ways: (1) through direct oxidation/reduction reactions between iron in the aquifer soils and the sulfide, where sulfide is oxidized (primarily to elemental sulfur) and the iron is reduced to ferrous form ($Fe^{2+}$), and (2) through the formation and precipitation of iron sulfide precipitates by the reaction of sulfide with available ferrous iron. Several of the mechanisms serving as sulfide sinks are shown below in Equations 2 through 4:

$$4\ Fe(OH)_3\ (s) + 3\ H_2S + 3\ HS^- \rightarrow 2\ S^0\ (s) + 4\ FeS\ (s) + 9\ H_2O + 3\ OH^- \quad (2)$$

$$4\ Fe(OH)_3\ (s) + H_2S + HS^- \rightarrow 2\ S^0\ (s) + 4\ Fe^{2+} + 3\ H_2O + 9\ OH^- \quad (3)$$

$$4\ Fe(OH)_3\ (s) + 1.28\ H_2S + 1.28\ HS^- + 3.48\ HCO_3^- \rightarrow 2\ S^0\ (s) + 0.52\ FeS\ (s) + 3.43\ FeCO_3\ (s) + 7.28\ H_2O + 4.72\ OH^- \quad (4)$$

Ultimately, the ability to suppress sulfide accumulation will be a balance between the rate of its production and the rate of its reactions. Based on the natural abundance of iron in the subsurface, and assuming a reasonable level of control on sulfate dosing, the accumulation of sulfide will be limited at most sites; observed sulfide concentrations within the reactive zone have been generally less than 1 to 10 mg/L, with some sites maintaining non-detected sulfide concentrations through intrinsic control (i.e., Equations 1 through 4) following repeat sulfate applications.

In addition, sulfide oxidation in the presence of oxygen can be very rapid, with half-lives in the order of minutes to a few hours (Deutsch, 1997). So, while iron-related reactions generally represent the dominant mechanism for sulfide control within the biologically active zone, outside of that zone, the presence of oxygen (or nitrate) add another mechanism that will limit its persistence (Korom et al., 2005).

### Increases in Alkalinity and Total Dissolved Solids (TDS)

As indicated in Equation 1 describing the anaerobic oxidation of benzene, the anaerobic metabolism of PHC yields alkalinity in the form of bicarbonate. This bicarbonate will neutralize acidity in the aquifer, building up alkalinity and likely raising the pH of the groundwater system over time. These effects can support the precipitation of calcite (calcium carbonate), which if not managed can lead to permanent reductions in aquifer permeability.

An uncontrolled rise in alkalinity and aquifer pH can also inhibit the activity of sulfate-reducing bacteria. The optimal pH range for sulfate-reducing bacteria is generally between 7.5 and 8, with inhibition effects observed at values outside of the 5.5 to 9 range (Hao et al.,1996). Potential pH excursions (i.e., greater than 9) have been observed at ABOx sites with limited ambient buffering capacity. As with sulfide accumulation, short-term pH excursions will be a balance between the rate of alkalinity production and the rate of its neutralization.

In addition to the above, the introduction of soluble sulfate salts will result in a build-up of TDS and increased ionic strength within the reactive zone. The increase in TDS can cause density-driven migration of injection solutions downward, which should be considered in the delivery design, and can also act as a stressor to the microbial community, reducing overall treatment efficiency.

As with the considerations revolving around sulfide, a reasonable level of control on sulfate dosing can help manage most of these potential complications.

### Calcium-Sulfate Interactions

In addition to the iron-sulfate relationships, calcium-rich aquifer systems can contribute to near instantaneous precipitation of gypsum solids following injection, where the sulfate concentrations being applied lead to an exceedance of the saturation index for that mineral. While gypsum is highly soluble and will still be a viable source of sulfate to sulfate-reducing bacteria, the precipitation of significant amounts of gypsum can result in temporary reductions in aquifer permeability that limit the ability to conduct effective repeat injections.

## Sulfate-Based ABOx Expectations and Observations

ABOx remediation strategies (sulfate-based or with other electron acceptors) have not seen widespread use in the past, mainly as a result of the superior thermodynamics (energy yield) and faster kinetics associated with aerobic pathways. Reaction rates reported for laboratory- and field-based analytical data for benzene, toluene, ethylbenzene, and total xylenes (BTEX) oxidation show that second to oxygen and in some cases nitrate, sulfate is the next kinetically fastest electron accepting process on average (Aronson and Howard, 1997).

In biological systems, the availability of a reactant can easily overwhelm differences in kinetics of this magnitude, and as previously discussed, sulfate can be made available in very high concentrations (far exceeding anything possible with oxygen). The question then becomes, what is the ideal sulfate concentration to support the ABOx process? Although most natural groundwater systems contain 10s to 100s of mg/L of sulfate, sulfate is usually consumed below 20 mg/L in systems impacted with PHC and below the sulfate concentration supporting optimal sulfate-reduction rates.

Classical microbial kinetic analyses show that at lower concentrations of a rate-limiting substrate [S], the



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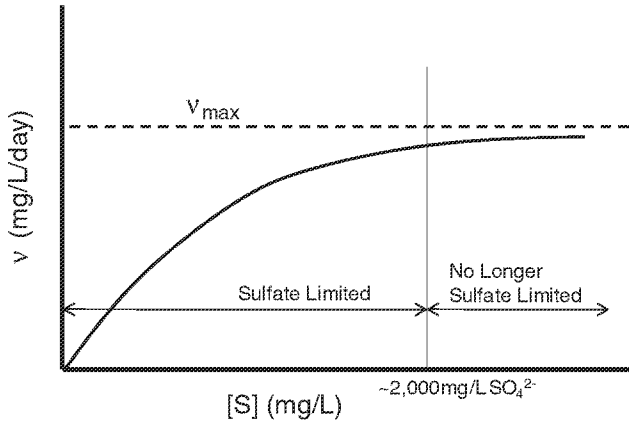


**Figure 1. The relationship between rate-limiting substrate concentration [*S*] and overall reaction rate (*v*) in microbial-mediated reactions. Rate-limiting sulfate concentration based on kinetic data presented in Roychoudhury and McCormick (2006).**

microbial-mediated rate of reaction (*v*) proceeds as first-order (Chapelle, 2001). As the concentration of the rate-limiting substrate is increased, *v* will approach zero-order where the rate of reaction is no longer dependent on substrate concentration (Figure 1). Engineered systems that increase sulfate availability can enhance the rate of PHC oxidation by more than one order of magnitude above intrinsic or non-engineered conditions. Generally, the recommended minimum threshold sulfate concentration to sustain treatment is approximately 100 mg/L, equivalent to the concentration at which microorganisms conduct intercellular sulfate storage and operate most efficiently (Habicht et al., 2005). As indicated on Figure 1, sulfate concentrations in excess of approximately 2000 mg/L are not expected to yield increased PHC oxidation rates.

Sufficiently short lag time associated with microbial stimulation and acclimation represents another prerequisite for ABOx of PHC to be a viable and effective remedy. Even where baseline site biogeochemical characterization data suggest little to no sulfate reduction activity occurring, sulfate reduction has been stimulated within 45 days (d) of sulfate addition (data not shown), which is consistent with sulfate-reducing microbial lag times reported in the literature (Table 2). This is helped by the fact that sulfate-reducing organisms are ubiquitous and robust in terrestrial systems. The phylogenetic and physiological diversity is broad, including wide ranges of potential electron donors, pH conditions, salinity, and temperature (Detmers et al., 2001). Based on the wide array of carbon compounds present at PHC-impacted sites and the large microbial communities present in these groundwater systems, the bio-oxidation process is likely a cooperative effort between multiple microbial species (Boopathy, 2004).

Figure 2 presents field data from a sulfate application site that was impacted with benzene. Following the initiation of sulfate application and groundwater recirculation, general increases in benzene concentrations are observed. This transient increase in benzene concentrations may be the result of hydraulic mixing, but is also consistent with solubility enhancements of non-polar, sparingly soluble compounds observed in other anaerobic bioremediation systems (Suthersan et al., 2011). Following peak benzene concentration observed during the operational period noted on Figure 2, benzene concentrations decrease by one-half to two orders of magnitude as a result of hydraulic mixing, sulfate distribution, and subsequent ABOx treatment of benzene.

At fuel release sites, aromatic hydrocarbons (i.e., BTEX) and higher

**Table 2**
**Sulfate Reduction Field-Scale Microbial Lag Times Reported in the Literature**

| Target Organic Compound | Electron Accepting Process | Observed Sulfate-Reduction Microbial Lag Time | Reference |
|---|---|---|---|
| Gasoline associated with an underground storage tank (UST) release | Concurrent nitrate and sulfate addition | Approximately 30 to 45 d, based on observed decreasing sulfate concentration trends during a field test with continuous flushing of nitrate- and sulfate-amended solution. | Cunningham et al. (2001) |
| Gasoline associated with a UST release | Sulfate reduction | >1 d, based on lack of microbial response observed following sulfate amendment to in situ microcosms | Roychoudhury and McCormick (2006) |
| Gasoline and BTEX amended at a field test site associated with a UST release | Nitrate reduction, followed sequentially by sulfate reduction | Approximately 30 d, based on observed decreases in toluene, ethylbenzene, and xylenes concentrations in a controlled push-pull in situ field test | Reinhard et al. (1997) |
| Landfill leachate impacted with elevated concentrations of dissolved organic carbon (DOC) | Sulfate reduction | Approximately 25 d, based on observed sulfate reduction colonization rates following microcosm sulfate amendment | Kneeshaw et al. (2011) |



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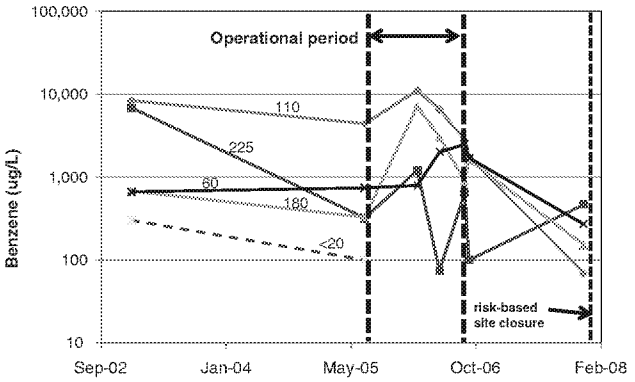


**Figure 2. Benzene concentration trends from a series of five monitoring wells influenced with sulfate during a large-scale sulfate application. The monitoring well benzene data represented by the red dashed line were non-detect following initial system operation and these data points are not plotted. Sulfate was applied during a 1-year period of groundwater recirculation. The maximum sulfate concentrations observed during the 1-year operational period are noted next to the benzene trend line for each well. Following operation and post-remediation monitoring, risk-based closure for the site was granted.**

molecular weight dissolved hydrocarbons (e.g., TPH-gasoline range organics [GRO], TPH-diesel range organics [DRO], etc.) are collectively the target compounds for bio-oxidation processes. In most instances, the total concentration of the broad range of dissolved hydrocarbons will be significantly higher than that of BTEX, thus reducing the sulfate available for BTEX transformation. These higher molecular weight constituents must therefore be considered during design and implementation of ABOx applications.

When present, sulfate utilized to deplete higher-weight constituents will generate increasing quantities of lower-weight molecules that will serve as additional electron donor. In the application of ABOx for the treatment of TPH-DRO, review of TPH chromatograms of samples influenced by ABOx processes show the compositional shift of the TPH-DRO (Liu et al., 2008). TPH-DRO composition is initially observed as being dominated by compounds with longer gas chromatograph (GC) retention times and higher-molecular weight and, over time with exposure to ABOx processes, the TPH-DRO composition is comprised of compounds with shorter GC retention times and lower-molecular weight.

Many of these non-differentiated dissolved hydrocarbon compounds are not quantified individually in standard compound-specific analyses, but may be captured in bulk compound analyses (e.g., TPH-GRO and TPH-DRO). While the bulk concentration results may indicate that hydrocarbon concentrations are relatively unchanged (or slowly declining) in an engineered ABOx system, analysis of the GCs can be used as qualitative indicators that degradation and transformation of these compounds is ongoing. There are also several degradation intermediates that might offer direct evidence of biological transformations (i.e., phenol for benzene, cresol for toluene, etc.).

## Sulfate Delivery Strategies

As in all biologically mediated treatment approaches, selecting the most cost-effective means by which to provide a steady, sustained source of reagent within the target treatment area is critical to success. Delivery strategies must account for the site-specific rate of depletion to sustain sulfate concentrations within a targeted range, in the targeted area of PHC impacts. Dissolved-phase delivery mechanisms via batch injections into permanent wells, groundwater recirculation via injection and extraction wells, direct-push injection via temporary points, and solid-phase application via placement in open excavations or direct application of sulfate to the ground surface are all potentially viable sulfate delivery strategies.

Because of the geochemical considerations previously discussed, sulfate application strategies that employ repeat injections at highly elevated concentrations may not be as effective as sulfate delivery strategies that achieve relatively steady sulfate concentrations over time in the range of 100 to 2000 mg/L. This can increase the efficiency of the process while limiting the geochemical footprint of the reactions.

As the majority of petroleum liquids are less dense than water, they tend to initially accumulate at or near the water table and over time water level fluctuations create a "smear zone." The presence of a smear zone, where porosity is consumed to varying extents by residual separate-phase PHCs, can present a challenge to effective reagent delivery. The smear zone lateral and vertical extent should be defined and considered along with site hydrostratigraphy in designing the sulfate delivery strategy most likely to succeed during ABOx implementation.

## A Shifting Paradigm and Future Challenges

Sulfate-based ABOx has been demonstrated to be a significant process in the natural degradation of PHCs at many PHC release sites. The engineered enhancement of this process is emerging as a technology option that may provide an attractive alternative to the more common aerobic biodegradation-based approaches. As advancements are made in the implementation of this technology, a few things are becoming clear:

- The amount of residual source mass present in a given system is a key consideration in evaluating the potential for ABOx to be effective. Significant source mass can be self-limiting and result in sulfate mass loading requirements that are impractical and uneconomical. Consequently, application after source reduction has been implemented which is likely ideal to expedite depletion of residual smear zones and shorten overall remedial timeframes.
- Compared to lower molecular weight fractions of PHCs (e.g., BTEX), effective treatment of water-soluble composite analytes (i.e., TPH-GRO and TPH-DRO) will likely require prolonged



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exposure to elevated sulfate concentrations, which should be considered in the remedy selection and design processes.

- Dosing strategies that achieve moderate and more consistent concentrations are best to achieve optimum treatment efficiency and limit secondary geochemical effects.
- Careful evaluation of the geochemical effects of the treatment is an important consideration to a successful remedy.

With the understanding that water soluble PHCs are readily amendable to ABOx through sulfate reduction and that sulfate-reducing microbial lag phases are appropriate for remedy implementability (generally less than 30 to 50 d), the current ABOx technology development focus is on optimization of the microbial reactive zone, including reagent delivery. Moving forward, the challenge for designers in developing successful ABOx strategies is providing sufficient electron acceptor through the most appropriate dosing strategy to meet the demand of a given system, while limiting the potential consequences of microbial inhibition and secondary water quality effects.

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