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Nitrate/Sulfate Addition and Syntrophic Biodegradation:

Kickstarting Petroleum Biodegradation

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The remediation management of petroleum-impacted sites in both active and monitored natural attenuation situations commonly relies on biodegradation. A large and diverse population of microbes is known to use petroleum hydrocarbons as a food source, which forms the basis of bioremediation for these contaminants[1]. Petroleum biodegradation is fastest when microbes have an ample supply of oxygen, which serves as the most favorable electron acceptor from a redox standpoint. For this reason, oxygen is quickly depleted in most petroleum-impacted sites (Figure 1). One of the next-best electron acceptors is nitrate, NO_3^-, which is also rapidly utilized in groundwater when petroleum or other carbon sources are present. After oxygen and nitrate, several other electron acceptors, including iron and sulfate, SO_4^{2-}, may be used by microbes. As the more favorable electron acceptors are consumed, an aquifer will become methanogenic, meaning that CO_2 serves as an electron acceptor to generate CH_4. Petroleum biodegradation proceeds slowly under these conditions.

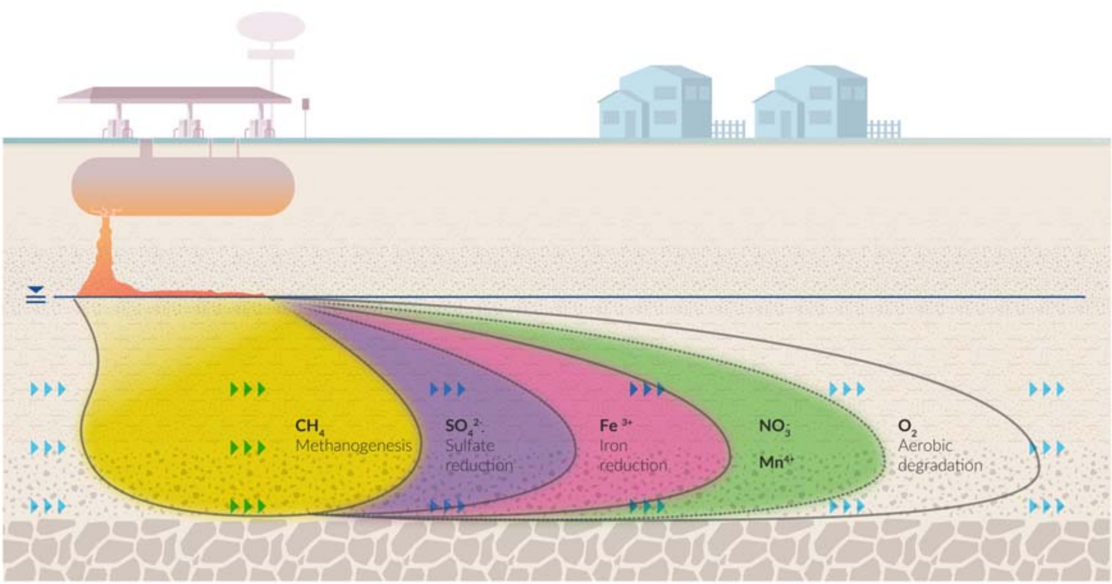

Figure 1. *Electron acceptors are utilized by petroleum degrading microbes during the biodegradation of petroleum hydrocarbons. The order that electron acceptors are consumed follows with their redox potential (R to L as shown in the figure). Oxygen has the most positive redox potential and is therefore depleted first.*

The mineralization of petroleum contaminants using electron acceptors like oxygen requires a stoichiometric amount of the electron acceptor, which often is an impractical amount or necessitates follow-up injections. This limitation is true for PetroFix™ or almost any other remedial amendment on the market. However, the addition of non-stoichiometric amounts of NO_3^- or SO_4^{-2} as electron acceptors can aid biodegradation even after their consumption by promoting syntrophy.

Furthermore, reports have demonstrated improved outcomes when both electron acceptors are used together as a result of the following benefits of co-application[2]:

1. Reduced competition for available electron acceptors. Separate groups of microbes utilize each species, meaning there is less direct competition.

2. Sulfate reducers and denitrifying bacteria metabolize BTEX components in slightly different ways. This feature again lowers competition for resources and promotes complete BTEX degradation.

3. Microbial community diversification may help stimulate syntrophic conditions that can assist in sustaining petroleum degradation after consumption of the NO_3^- and SO_4^{2-} (described below).

Syntrophic Metabolism

Syntrophy describes the process in which a community of microbes 'feed together', simultaneously using carbon sources and their byproducts in an ecological partnership[3].

A simplified illustration of syntrophy is shown in Figure 2. On petroleum-contaminated sites, BTEX and other hydrocarbons will be metabolized to acetate and hydrogen by syntrophs. This process can continue to occur so long as the produced hydrogen and acetate are removed by a community of methanogens. Under these conditions, contaminant degradation can proceed without the need for an additional electron acceptor. Researchers have shown that syntrophic arrangements are common and critical to the success of natural attenuation on many petroleum-impacted sites.

Syntrophic Metabolism - Continued

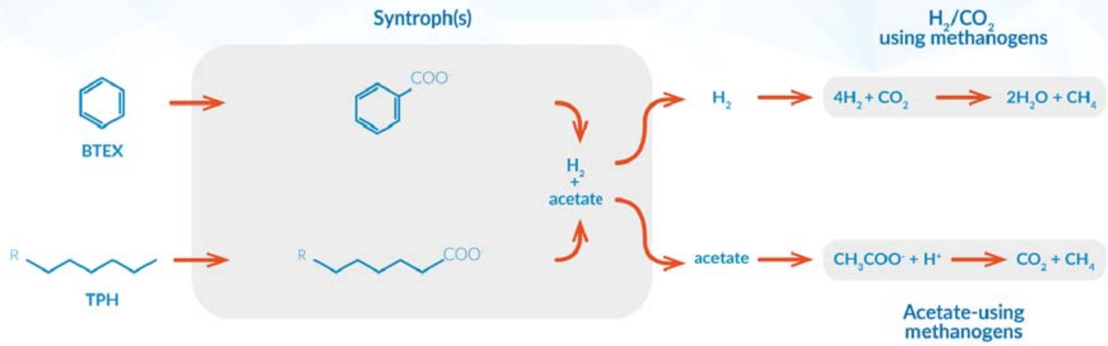

Figure 2 *Syntrophic metabolism on petroleum-impacted sites proceeds in two general steps. The first group of microbes metabolize BTEX and other hydrocarbons into simpler substrates like acetate and hydrogen, H_2. These metabolites are mineralized by subsequent microbes, whether methanogens (shown) or reducers of other electron acceptors, when available. By working together, these microbes maintain an ecological balance that facilitates efficient petroleum degradation. Figure adapted from Ref 3.*

Conclusion

The remediation strategy employed when applying PetroFix is to adsorb contaminants on activated carbon and kickstart biodegradation by adding a SO_4^{2-}/NO_3^- blend to establish a diverse microbial community. After the added NO_3^- and SO_4^{-2} are exhausted, petroleum degradation will continue via syntrophic processes, meaning the continuous addition of electron acceptors is not required. The combination of an injectable form of activated carbon that can adsorb contaminants and contain them in a finite zone with electron acceptors that will initially degrade the contaminants via anaerobic pathways and promote syntrophic conditions that sustain degradation will expedite the remediation of petroleum-impacted sites.

References:

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3. Gieg, L. M., Fowler, S. J. & Berdugo-Clavijo, C. Syntrophic biodegradation of hydrocarbon contaminants. *Curr. Opin. Biotechnol.* **27**, 21–29 (2014).

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(949) 366-8000
www.regenesis.com

1011 Calle Sombra
San Clemente, CA 92673