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White Paper

Electron donor selection in Enhanced Reductive Dechlorination remediation projects

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Introduction

Enhanced Reductive Dechlorination, ERD in short, is an *in situ* bioremediation method for treating chlorinated solvents like tetrachloroethene (PCE) and others under anaerobic conditions. Dechlorination is achieved by both direct (respiratory) and cometabolic biotic degradation pathways. Certain bacteria are known to respire specific chlorinated solvents and grow in the process, e.g. the genus *Dehalococcoides*. Certain chlorinated solvents are also degraded by accident while bacteria are degrading another compound, this phenomenon is known as cometabolic degradation. Table 1 shows a list of chlorinated volatile organic compounds (CVOCs) commonly detected in groundwater and which biodegradation mechanism is applicable.

Table 1. Biodegradation mechanisms for selected CVOC's

Contaminant	Direct	Cometabolic
Chlorinated Ethenes		
tetrachloroethene (PCE)	•	•
trichloroethene (TCE)	•	•
cis-1,2-dichloroethene (cDCE)	•	•
trans-1,2-dichloroethene (tDCE)	•	•
1,1-dichloroethene (DCE)	•	•
vinyl chloride (VC)	•	•
Chlorinated Ethanes		
1,1,1-trichloroethane (1,1,1-TCA)	•	•
1,2-dichloroethane (1,2-DCA)	•	•
1,1-dichloroethane (1,1-DCA)	•	•
chloroethane	x	x
Chlorinated Methanes		
carbon tetrachloride (Freon-10)	×	•
chloroform (Freon-20)	•	•
methylene chloride (Dichloromethane /DCM)	•	•
chloromethane (Refrigerant-40)	×	×

• Known to occur in natural and/or engineered systems; × Not known to occur; Modified from U.S. Environmental Protection Agency (U.S. EPA), 2000.

In order to create conditions under which biotic dechlorination can occur amendments are injected into the subsurface. These processes occur in the saturated zone of the subsurface (aquifer). Amendments used for biostimulation are electron donors, pH buffers and in some cases nutrients[1].

Reductive dechlorination is a process in which chlorine atoms are step by step removed from the carbon molecule. In the case of PCE, a common contaminant of concern (COC) in contaminated aquifers, the complete chlorine removal results in the formation of ethene and hydrochloric acid. In order for this reaction to occur hydrogen is required.

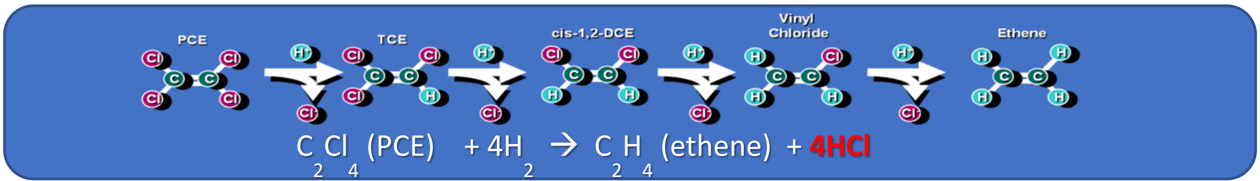

Figure 1. Degradation pathway of PCE

The direct addition of hydrogen in gaseous form is possible but requires significant safety measures above ground due to the explosive nature of hydrogen gas. Hydrogen gas is poorly soluble, even at a head pressure of 4,5 meter (15 ft) and 15,24 meter (50 ft) hydrogen concentrations reach only 3 and

5 ppm respectively. In order to maintain these concentration levels in the aquifer continuous dosing of hydrogen gas is required from a secure above ground installation.

A safer and more sustainable method for the delivery of hydrogen in the target treatment zone (TTZ) is the addition of a fermentable organic substrate. Once injected this organic substrate will remove any available electron acceptors like oxygen, nitrate and sulphate as the substrate is used by aerobic, denitrifying and sulphate reducing bacteria. Once these electron acceptors are depleted and the ORP of the aquifer has dropped to below -100 mV the anaerobic bacteria will start to ferment the substrate which will release acetate and hydrogen (H_2). These two compounds are used by *Dehalococcoides, Dehalobacter, Dehalogemonas sp.* to remove the chlorine from the CVOCs.

The objective of this paper is to provide a short insight into amendment selection and how amendments behave once injected as this is a key element in the success of ERD projects.

ERD Amendment Selection

A critical component of any ERD design is the selection and delivery of amendments. The selection of which amendments are used (single or multiple) will depend on the hydrogeology and geochemistry of the aquifer (lithology, effective porosity, K, gradient and other electron acceptors), results of bench-scale testing and the method of amendment delivery. Design considerations for common electron donors are provided in table 2.

Table 2. Design considerations for the application of Electron Donors for ERD

Substrate Type	Electron Donor Substrates	Typical Delivery Techniques	Form of Application	Frequency of Injection
Soluble Substrates	Lactate, Butyrate	DPT injection, injection wells, or recirculation systems	Acids or salts diluted in water	Continuous to monthly
	Methanol, Ethanol	DPT injection, injection wells, or recirculation systems	Diluted in water	Continuous to monthly
	Sodium Benzoate	DPT injection, injection wells, or recirculation systems	Dissolved in water	Continuous to monthly
	Molasses, High-fructose corn syrup	Injection wells	Dissolved in water	Continuous to monthly
	Whey (soluble)	DPT injection or injection wells	Dissolved in water or slurry	Monthly to annually
Slow-Release Substrates	Polylactate ester	DPT injection	Neat injection	Annually to biennially; some proprietary formulations 3-4 years according to vendor claims. Depends on contaminant mass and distribution (NAPL, sorbed).
	Vegetable oil	DPT injection or injection wells	Neat oil injection with water push or high oil concentration (>20%) emulsions in water	One-time application (typical). Depends on contaminant mass and distribution (NAPL, sorbed).
	Vegetable oil emulsions	DPT injection or injection wells	Low oil concentration (<10%) microemulsions suspended in water	Every 2 to 3 years (typical)

Adapted from U.S. EPA, 2013; ITRC, 2008a; and AFCEC et al., 2004.

Substrate Selection

Organic substrates can roughly be divided into two classes, soluble and insoluble substrates. It is important to understand how both types behave once injected into the aquifer.

Soluble substrates

Soluble substrates are in general readily biodegradable. Readily biodegradable means they can be 60% to 100% biodegraded through microbial activity within a period of 28 days. Soluble substrates can almost directly pass through the bacterial cell membrane without any prior hydrolysis. They are quickly taken up in the internal cell metabolism but because of their short carbon chain length result in a low acetate and hydrogen yield per unit of substrate.

Due to the high degree of biodegradability the microbial development will be rapid and electron acceptors will be quickly consumed resulting in a low ORP and anaerobic conditions. This means that when using soluble substrates conditions for optimal conditions for ERD will quickly emerge. However, the consequence of this rapid degradation is that substrate levels are quickly depleted, and multiple reinjection events are required in order to maintain the optimal conditions for ERD.

Insoluble substrates

Insoluble substrates take longer to biodegrade. Microorganisms have the ability to solubilize insoluble organics through a process called hydrolysis, an enzymatic process which uses an enzyme, e.g. lipase, to split the organic molecule into 2 parts.

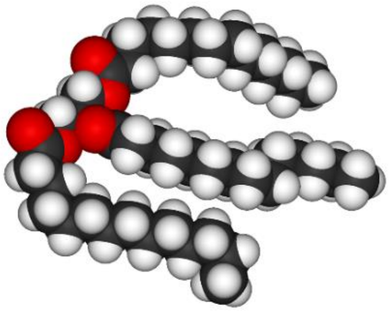

Figure 2. A typical triglyceride molecule consisting out of 1 glycerol (red) and 3 fatty acids connected by an ester linkage.

Most common insoluble substrates are on vegetable oil basis. The hydrolysis of these oils or triglycerides (see figure 1) and more specifically the ester linkages between the glycerol and fatty acid chains, releases glycerol and 3 long chained fatty acids (LCFA's). Glycerol solubilizes well and is therefore directly accessible to bacteria. As the glycerol only has a chain length of only 3 carbon it is quickly consumed. The LCFA's (e.g. linoleic acid or $C_{18}H_{32}O_2$), also soluble, are degraded via the β-oxidation pathway by bacteria in which each cycle step results the production of one Acetic Acid ($C_2H_4O_2$) and one Hydrogen (H_2). The complete degradation of Linoleic acid results in 9 Acetic Acid and 14 Hydrogen.

$$C_{18}H_{32}O_2 + 2H_2O \rightarrow C_{16}H_{30}O_2 + C_2H_4O_2 + H_2$$
$$C_{16}H_{30}O_2 + 2H_2O \rightarrow C_{14}H_{28}O_2 + C_2H_4O_2 + H_2$$
$$C_{14}H_{28}O_2 + 2H_2O \rightarrow C_{12}H_{26}O_2 + C_2H_4O_2 + H_2$$

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$$C_{18}H_{32}O_2 + 16H_2O \rightarrow 9C_2H_4O_2 + 14H_2$$

Figure 2. β-oxidation Reaction pathway of linoleic acid

Due to the slow hydrolysis step, which only occurs on the water/oil droplet interface, and the fact that LCFA's take much longer to be digested in the internal cell metabolism, the degradation of insoluble substrates therefore takes much longer than the degradation of soluble substrates.

This slow rate of biodegradation means the insoluble substrate will remain present in the aquifer much longer (2 to 3 years) compared to soluble substrates (1 to 2 months). The acetate and hydrogen yields of insoluble substrates is also much higher per mol of substrate compared to soluble substrates. Table 3 provides an overview of different insoluble electron donors and electrons released during anaerobic fermentation compared to common soluble substrates.

Table 3. Average Composition of Different Substrates and Electrons Released during Anaerobic Fermentation

	Atoms per Mole Substrate			Average Molecular Weight	H_2 Released per mole Substrate	Moles H_2 released per gram substrate
	C	H	O			
Acetate	2.0	4.0	2.0	60.1	4.0	0.0666
Lactate	3.0	6.0	3.0	90.1	6.0	0.0666
Glucose	6.0	12.0	6.0	180.2	12.0	0.0666
Soybean	56.3	99.5	6.0	873.1	156.5	0.1792
Corn	56.3	99.9	6.0	873.5	156.6	0.1793
Cottonseed	55.5	99.3	6.0	862.8	154.7	0.1792
Palm	54.2	100.8	6.0	848.5	152.8	0.1800
Peanut	56.8	102.7	6.0	881.4	158.9	0.1803
Olive	56.2	102.7	6.0	875.0	157.8	0.1804
Canola	57.1	102.3	6.0	884.6	159.3	0.1801
Butterfat	50.2	94.0	6.0	793.4	141.4	0.1782
Lard	55.2	102.4	6.0	862.4	155.6	0.1804
Beef Tallow	55.1	102.9	6.0	862.2	155.8	0.1807

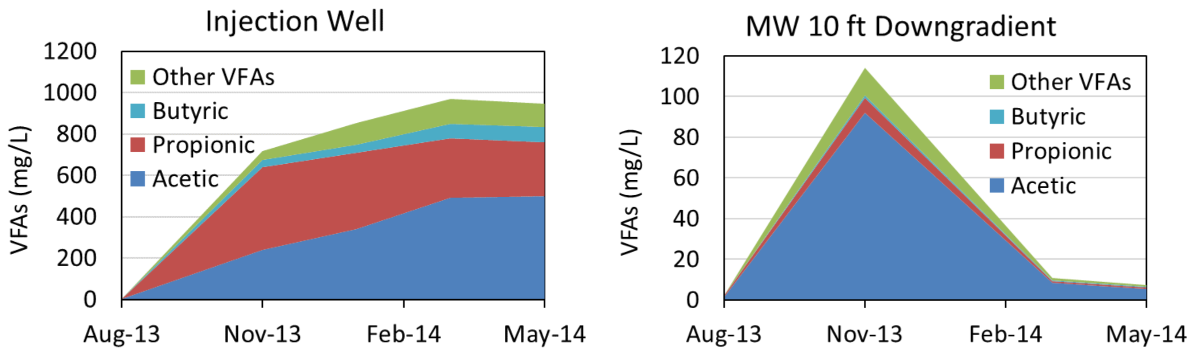
Source: ESTCP-Protocol for Enhanced In Situ Bioremediation Using Emulsified Edible Oil, May 2006, page 16

This analysis shows that there is essentially no difference in the amount of reducing power per gram of oil. However, all of the oils have much more reducing power than soluble substrates. For example, 45 Kg of oil has about the same reducing power as 122 Kg of acetate or sugar[2].

Substrate Transport & Retention in the subsurface

A critical component in ERD design is the behavior of the substrate once injected in the aquifer. In order for ERD to work effectively there has to be direct contact between amendment and CVOC's and sufficient contact time. As indicated above different substrates have different properties and it is important to understand how they behave in relation to the aquifer in order to achieve good transport and retention.

So why is good oil distribution and retention critical? Remembering that the fermentation of the oil produces acetate and hydrogen. From acetate it is known that it can travel some distance down gradient but hydrogen does not migrate any significant distance. In the case of PCE degradation, acetate stimulates PCE to TCE to cDCE degradation but there is stops. Hydrogen is needed for cDCE to Vinyl Chloride (VC) to ethene degradation. Hydrogen is not produced from acetate.



Graph 1. Fermentable carbon distribution of EOS Pro in injection well and 10 ft downgradient monitoring well.

Graphic 1 shows the distribution of LCFA's and acetate at the injection well and in a 10 ft downgradient monitoring well. The injection well received EOS Pro. EOS Pro is an emulsified organic substrate based on soybean oil in combination with soluble substrate and nutrients with a mean oil droplet size of 1 µm. In the injection well complete dechlorination from TCE to ethene occurred. 10 ft downgradient some soluble TOC was detected which was primarily acetate together with some other volatile fatty acids (VFA's). There was good degradation of TCE down to DCE but poor DCE to ethene degradation. As hydrogen is primarily produced out of the LCFA's it is therefore essential that the amendment is in close proximity of the COC in order for its fermentation products, acetate and more importantly hydrogen, to be available for the dehalogenating bacteria in order to consume the COC completely.

Delivery of insoluble substrates such as emulsified oil is therefore critical for adequate remediation. Where soluble substrates travel very easily through the pores, insoluble substrates can have transport limitations. Several factors will determine whether or not it will distribute evenly around the point of injection and how far it will travel. Factors governing this distribution in relation to aquifer porosity and lithology are:

- Neat or emulsified amendment
- Oil droplet size of amendment
- Type of surfactant used to create emulsion
- Zetapotential or emulsion stability

The injection of a neat amendment like pure vegetable oil can quickly clog the pores of the aquifer, limiting the distance it can travel from the injection well. The advantage of neat amendment injection is its longevity. Degradation will be slow because there is relatively little specific contact surface where biodegradation can occur, but on the other hand, the low distribution will be critical because acetate and hydrogen cannot reach the contamination.

Therefore, insoluble substrates like vegetable oils can be distributed in aquifers much more efficiently when injected as oil-in-water emulsions. Emulsification of oil is completely miscible with water so it can easily disperse with groundwater after injection[2].

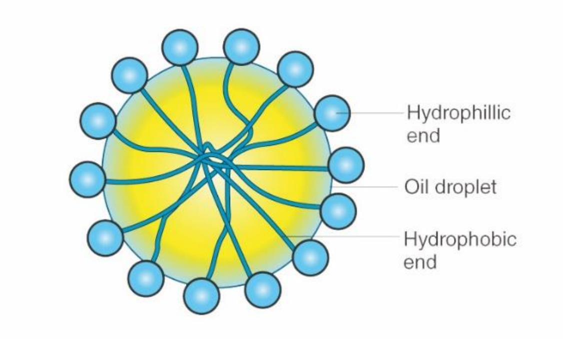


Figure 3. Representation of an oil-water micelle where the hydrophobic end of the surfactant attaches to the oil and the hydrophilic part attaches to water.

Droplet size of the emulsion in relation to the porosity of the aquifer will affect the distribution of the emulsion in the aquifer. Once injected, the oil emulsion droplets are transported through the aquifer pore spaces by flowing groundwater. Studies[3] have shown that that droplets larger than the sediment pores are rapidly removed by straining with a large, permanent permeability loss.

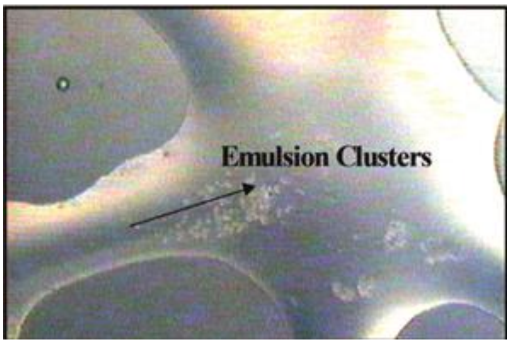


Figure 4. Small oil emulsion droplets passing through pores in clusters

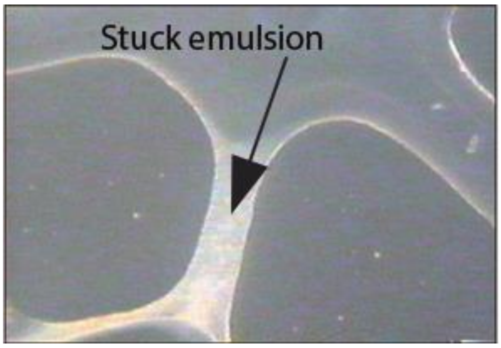


Figure 5. An emulsion stuck in the space between soil particles

The median pore size of a sand aquifer is typically over 100 µm[4]. Factory prepared vegetable oil-water emulsions have a droplet diameter of less than 2 µm so physical straining (figure 4) is not a significant retention mechanism in sands but field prepared oil-water emulsions have often larger droplet sizes, so physical straining of the large droplets can be significant with field prepared emulsions as can be seen in figure 5.

Type of surfactant used to create the emulsion in relation to the lithology will also influence the travel distance of the emulsion. Surfactants are available in ionic or non-ionic form. Ionic surfactants can be anionic (negatively charged), cationic (positive charge) and amphoteric (contains a positive and negative charge). Non-ionic surfactants contain no charge. Depending on the net ionic charge of the aquifer material ionic surfactants will form a bond with negative or positively charged soil particles. Especially in clay and silt soil particles which are ionically charged and ionic surfactants will attach to these charged particles.

When the small droplets of a factory prepared emulsion collide with sediment surfaces they stick because of an ionic bonding. This is a process referred to as interception. The Deep-bed filtration theory[5][6][7] describes the retention of small droplets by aquifer material. Droplet capture by the sediment surface is a function of:

1. The frequency that droplet collide with sediment surfaces;
2. The collision efficiency, which is a fraction of droplets colliding with the sediment surfaces that are actual retained[8].

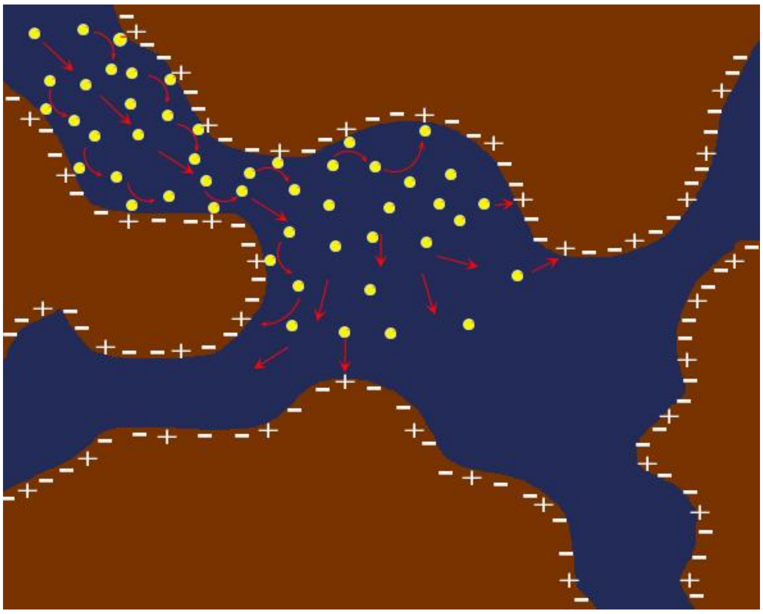


Figure 6. Drawing illustrating oil droplet transport and retention in porous media[8][9]

Figure 6 shows how droplets move through the pores and collide with the soil particles. An ionic bond is formed between the droplet and the soil particle when the charges attract each other. At that moment that place is occupied and another droplet will no longer be able to attach itself there. At the same time droplets with the same ionic charge as the soil particle will be repelled and migrate further through the aquifer, gradually filling up the available attachment sites. In this way, the droplets are pushed further and further through the pore until they collide with a soil particle with an opposite ionic charge that is still unused[8]. Due to the small droplet size (<2 µm), sufficient space remains in the pore for transport at the same time. The transport and collision frequency between the droplets and soil particles depends on groundwater flow velocity (advection), diffusion and gravitational settling or floatation. For vegetable oil emulsions at typical groundwater velocities, the lowest collision frequency occurs at a particle size of 0.5 to 2 µm[9]. A low collision frequency means further transport of the droplets.

Another factor which can affect transport and retention of insoluble substrates on vegetal oil basis is the stability of the created emulsion. The zeta potential which is the potential difference between the bulk of the fluid and the stationary fluid layer attached to the particle surface, is a common measure of suspension or emulsion stability. Highly negative or positively zeta potential charged particles will not flocculate. At zeta potentials close to zero attractive forces may exceed the electrostatic repulsion and the emulsion may break and flocculate[8].

Typical rules of thumb for negatively charged emulsions (zeta potential < 0) are displayed in table 4.

Rapid flocculation	0 mV	<	zeta potential	<	-5 mV
Incipient instability	-10 mV	<	zeta potential	<	-30 mV
Moderate stability	-30 mV	<	zeta potential	<	-40 mV
Good stability	-40 mV	<	zeta potential	<	-61 mV
Excellent stability			zeta potential	<	-61 mV

Table 4. Emulsion stability in relation to zeta potential

Experiments[10] have demonstrated that dissolved cation concentration (Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}) can have a major impact on zeta potential and oil retention. In aquifers with a high total dissolved solids or with carbonate minerals high levels of dissolved cations will naturally occur and *in situ* bioremediation processes can increase cation concentrations by release of dissolved Mn^{2+} or Fe^{2+} or when pH is adjusted using alkaline materials. These factors can push the zeta potential of the emulsion once injected in the direction of zero and hence break the emulsion.

Laboratory column, sandbox and field studies have shown that emulsified vegetable oil (EVO) can be transported substantial distances through fine silty or clayey sand and fractured rock. However, once oil droplets attach to soil surfaces, they are strongly retained and do not migrate further. Much effort has focused on developing EVO formulations with low retention to reduce the amount of oil required to treat a given volume of aquifer. However, in some cases, higher oil retention is required to treat very high permeability gravels or fractured rock. In these cases, EVO with large oil droplets can be used. The large droplets increase oil retention by straining and by oil droplet buoyancy which causes the large droplets to collide with the roof of the sediment pores[8].

Amendment requirement calculation

Amendment requirement can be calculated in two ways;

1. A stoichiometric calculation of the amount of electron donor based on the amount of electron acceptors present in the aquifer (COC and others such as oxygen, nitrate, etc ..);
2. A calculation based on the retention of the amendment in the specific aquifer (oil retention).

Whereas consultants quite often calculate the amount of total organic carbon (TOC) needed to supply enough electrons for reductive dechlorination, practice often shows that the need for amendment is determined on the basis of its distribution and retention as it is essential that the amendment is close to the contamination in order to be effective.

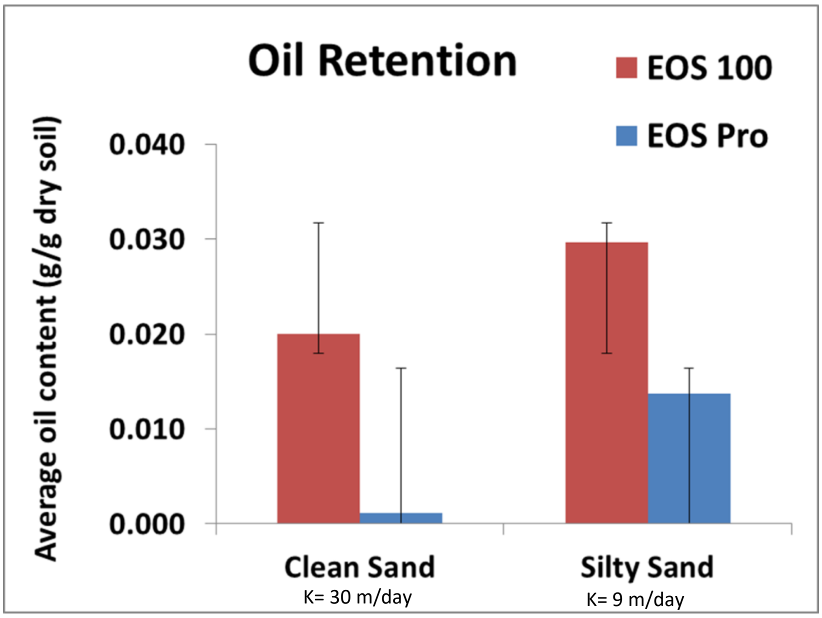
The formula for calculating the amount of EVO needed based on the oil retention:

$$\text{Mass of oil required for treatment} = x * y * z * ne * \rho_B * O_R$$

x = Treatment zone length parallel to GW flow (m)
y = Design width perpendicular to GW flow (m)
z = height (m)
ne = Effective porosity (unit less)
ρ_B = Sediment bulk density (Kg/m^3)
O_R = Oil retention (wt./wt.)

As discussed earlier, oil retention is determined by the droplet size and charge of the droplet and the aquifer grain. Oil retention data is available in the literature, but it is recommended to determine it empirically by column testing using aquifer material from the contaminated site in combination with the substrate envisioned for use. A small percentage of clay or silt in a sandy soil can already cause a significant deviation in the oil retention of a particular type of substrate.

As an example on how this can vary we compared two EVO substrates, EOS 100 and EOS Pro, in a clean sand and silty sand column.

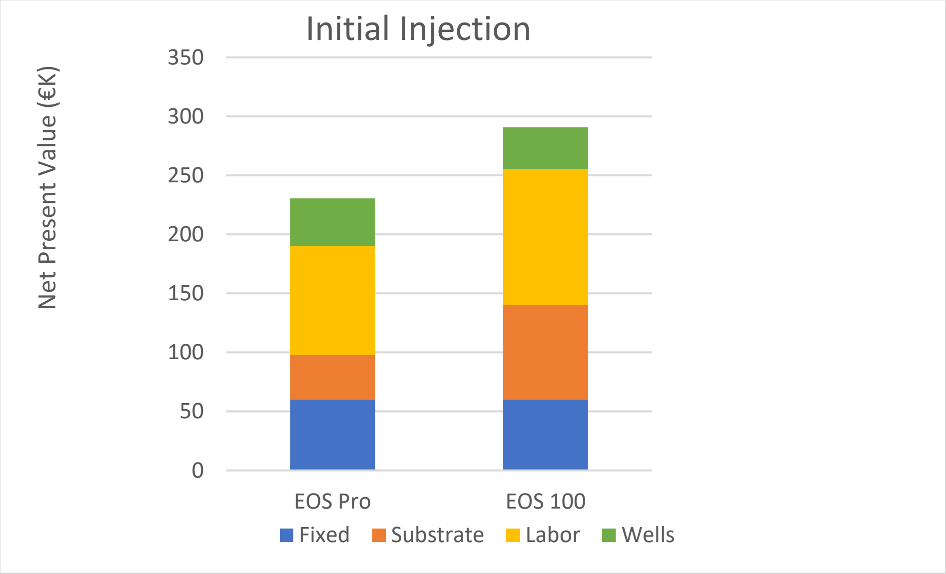


Graph 2. Oil retention comparison between EOS 100 and EOS Pro in a clean sand and silty sand aquifer

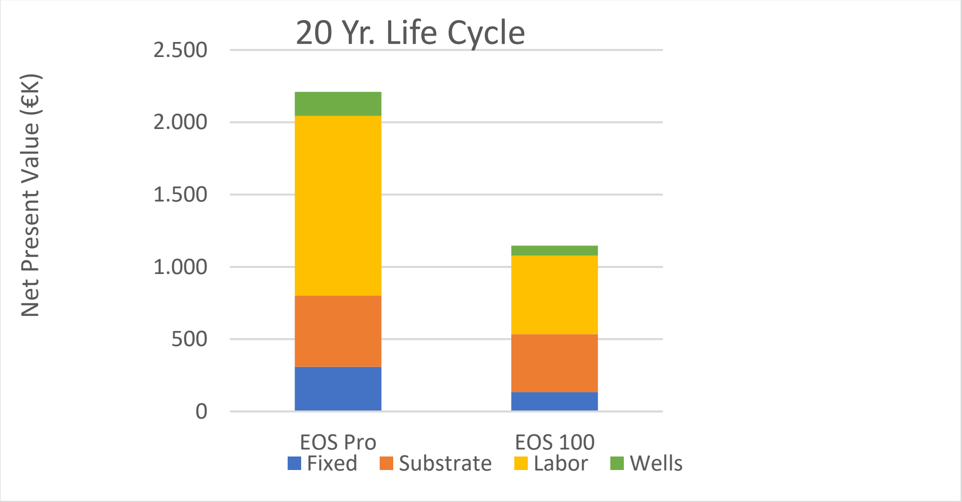
EOS 100 is field prepared emulsion with droplet sizes in the range of 10 µm and EOS Pro is a factory prepared EVO using non-ionic surfactants to form the emulsion with a <1 µm droplet size.

Graph 2 shows the difference in oil retention between the two lithologies and substrates. There is a dramatic difference in oil retention for especially EOS Pro. In the silty sand column the oil retention of EOS 100 increased due to the larger droplet size of the EOS 100 but for EOS Pro there is an increase in a couple of orders of magnitude. This demonstrates that a variation in aquifer lithology in relation to substrate selection can have major consequences in respect of oil retention and amount of EVO needed.

How this can affect the overall project costs was simulated in the ESTCP Emulsion design toolkit for these 2 substrates for a 61 m permeable reactive barrier (PRB) configuration in a medium sand with silt aquifer and a groundwater velocity of 0,49 m/day. Graph 3 demonstrates that initially an injection with EOS Pro is more economic as less of it is needed to achieve good distribution in the aquifer, due to its lower oil retention. However, when projected over a 20 year project life cycle it becomes apparent that injection with EOS 100, although initially more expensive, in the long term is more economical due to fewer injection rounds. This is because under these aquifer conditions a higher oil retention is better suited for long-term remediation and results in less labor and substrate costs. It is evident that it is important to understand how a substrate behaves in a particular aquifer as it can have significant effects in respect of total project costs.



Graph 3. Initial overall project costs with EOS Pro and EOS 100.



Graph 4. Total overall project costs with EOS Pro and EOS 100 over a 20 year project life cycle.

Summary

The selection of the type of amendment will depend on the behavior of the amendment once injected and the goal of the injection. If the goal is to quickly activate the microbiology so that optimal conditions for ERD are rapidly reached, which is useful at the start of a ERD program, a soluble substrate is most effective, provided that the groundwater does not flow too quickly and that it remains in the desired location long enough. If the goal is to provide a long-term source of electrons in order to keep the number of injection events to a minimum, an insoluble amendment is more appropriate. A substrate incorporating both properties will further contribute to reducing the number of injection events.

Bench scale laboratory testing such as column test can already reveal a lot of information on how a substrate will behave in a particular aquifer material in respect of transport and retention and this data will assist in the selection of the right amendment in that particular case and help to establish its dose.

Once in the field the selection of injection techniques and strategies will also contribute to the proper distribution of the amendment.

Literature

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