BIOREMEDIATION SYSTEMS FOR CONTAMINATED SOIL AND GROUND WATER

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A brief outline of the bioremediation systems is presented. The success of operation in a bioremediation system depends on some factors that include contaminant characteristics, natural supplies of macronutrients and micronutrients, availability of electron acceptors, presence of indigenous bacteria capable of degrading the contaminants, and subsurface characteristics. Also a site remedial alternative discussed here - the intrinsic bioremediation witch is not equivalent to a "no action".

Keywords: bioremediation, ground water, soil.

1. Introduction

Bioremediation treatment systems can be grouped into two categories: in situ and ex situ.

In situ systems are those where the contaminated medium is not physically moved or transported from its original location. Further on, in situ systems can be classified as either intrinsic remediation (natural or passive bioremediation) or engineered systems.

Intrinsic bioremediation is essentially allowing nature to take its own course. It should be emphasized, however, that it does not entail a "not action" approach. There is a strict protocol to hold on when considering intrinsic bioremediation.

Engineered in situ bioremediation involves the design and installation of system designed for the purpose of supplying microbe-simulating materials into the subsurface. Engineered systems can, sequentially, be broadly categorized as either biostimulation or bioaugmentation systems. Biostimulation refers to the addition of oxygen in aerobic systems or the addition of both oxygen and nutrients to the subsurface. Bioaugmentations is the process of adding nonnative bacteria to the subsurface to work together with the indigenous bacteria in breaking down the contaminants. Bioaugmentation typically also includes adding electron acceptors and nutrients.

Ex situ systems involve bringing the contaminated medium to the surface for treatment. Ex situ bioremediation usually involves the design and construction

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of an above-ground bioreactor or biofilter for treatment of contaminated groundwater.

Bioremediation is a viable remedial technology, especially for the treatment of petroleum hydrocarbons. However, when it comes to the treatment of less easily degraded compounds, such as chlorinated solvents, the technology is still considered to be at a research stage.

2. Microbiology of bioremediation

The overall concept of bioremediation of petroleum-contaminated site is fairly simple. Carbon-consuming bacteria occurs naturally in ground, the indigenous bacteria population utilizes petroleum hydrocarbons as a source of food and energy, breaking them down to carbon dioxide and water and producing more biomass. Along with carbon, the bacterium requires an electron acceptor such as oxygen and nutrients such as nitrogen and phosphorus. When the petroleum hydrocarbons are gone, the majority of bacteria disappear.

The bacteria cell is made up of approximately (70 ÷ 90) % water and (10 ÷ 30) % dry mass by weight. Of the dry weight, approximately 92 % is composed of carbon, oxygen, nitrogen and hydrogen. Carbon is the major element making up between (45 ÷ 55) percent of the dry cell material. Based on the composition of the cell mass, the most widely accepted empirical formula for the bacterial cell is $C_5H_7O_2N$.

The requirements for the microbial growth are physical condition as temperature and pH, and chemical condition as energy, carbon and nutrients sources. In the most bioremediation systems, the source of both carbon and energy is the contaminate itself. Only the macronutrients (nitrogen and phosphorus) may or may not need to be added to an in situ system. Usually the micronutrients (sulfur, potassium and iron) are found in sufficient quantities in the soil as trace elements.

In addition to the above sources that sustain growth and reproduction, three other conditions must exist.

- An electron donor must be present ton act as the source of reducing power;
- An electron acceptor to oxidize the reducing agent to provide the means of releasing the energy stored in the molecules;
- Water must be present as it is an essential component of the metabolic process.

During the metabolism, several types of processes take place, including energy conservation, biosynthesis, assimilation and ingestion, and cell maintenance. The complete metabolism process is, however, far more complex to be covered here.

The cell uses the carbon from its external source to manufacture cell material. After that there must be a reducing agent present and an oxidation potential. Cell that feed on organic compound utilize the hydrogen from the compound itself as their source of reducing power whereas organisms that depend on carbon dioxide for their carbon source must reduce the carbon dioxide biomass.

In aerobic system, oxygen is utilized as the acceptor and in anaerobic system, inorganic chemicals such as nitrate, manganese oxides, iron hydroxides, and sulfates are use as electron acceptors substitute. In addition to new cell mater, the by-products of aerobic respiration may include nitrogen gas, hydrogen sulfide, educed forms of metals, and methane, depending on the electron acceptor.

In the wastewater industry, bacteria have traditionally been classified based on their oxygen requirements: aerobic and anaerobic bacteria. Aerobic bacteria covert their food source to energy by transferring electrons from the compounds to oxygen, will anaerobic bacteria metabolize their food in the absence of oxygen and inside utilize inorganic chemicals such as nitrates, sulfates, carbon dioxide, or metals such as iron as substitute electron acceptors, and facultative bacteria, which can function in both aerobic and anaerobic environments.

3. Factors affecting bioremediation

In situ bioremediation is highly dependent on the site conditions and soil properties. Factors they play a significant role in the design and successful operation of a bioremediation system include contaminant characteristics, natural supplies of macronutrients and micronutrients, availability of electron acceptors, presence of indigenous bacteria capable of degrading the contaminants, and subsurface characteristics.

Simpler chemical structures are also easier to degrade. Branched structures degrade at a slower rate than the corresponding straight-chain hydrocarbons.

The microorganisms generally attributed for the bioremediation of contaminants are generally bacteria, but indigenous fungi are also responsible to hydrocarbon degradation. For complete degradation of the lighter hydrocarbons, multiple strains of bacteria are required. The indigenous bacteria population in the soil generally contains the necessary mixtures of bacteria species to perform that function.

The macronutrients required by the bacteria population are carbon, nitrogen, and phosphorus. The optimum carbon:nitrogen:phosphorus (C:N:P) ratio is 100:10:1. The carbon supply comes from the hydrocarbon contaminants themselves. Nitrogen and phosphorus may be naturally present in the ground in sufficient amounts.

Micronutrients that must be present in the soil include sulphur, potassium, sodium, calcium, magnesium, iron, manganese, zinc, and copper. Additional trace elements required for anaerobic metabolism include cobalt and nickel. Both macro- and micronutrients must be present, in the proper amounts, form, and ratios, for optimum sustained microbial growth and reproduction.

In aerobic metabolism, molecular oxygen acts as the terminal electron acceptor. Approximately thee pounds of available oxygen are required to cover one pounds of hydrocarbon to carbon dioxide and water. Using toluene (C_7H_8) as an example to illustrate stoichiometrically the breakdown of petroleum hydrocarbons aerobically, the chemical equation will be as follows:

$$C_7H_8 + 9O_2 \rightarrow 7CO_2 + 4H_2O \quad (1)$$

In anaerobic bioremediation, alternate or substitute electron acceptors are used in place of oxygen. These include, in order of preference, nitrate, manganese and iron oxides, respectively, sulphate and carbon dioxide. In general, the use of a particular electron acceptor is a function of its availability, the presence of the surrounding environment. The energy yield for microorganisms from hydrocarbon metabolism varies greatly, in the following order:

$$O_2 > NO_3^- > Mn^{4+} > Fe^{2+} > SO_4^{2-} > CO_2 \quad (2)$$

Nitrate Reduction

There are certain situations when is more adequately to use nitrate as an electron acceptor. Nitrate is more soluble than oxygen and it is less expensive. The advantage is that nitrate may be more economical to use from a practical standpoint, but on a negative side, nitrate is considered a pollutant, with a maximum contaminant level of 10mg/l.

$$C_7H_8 + 6NO_3^- \rightarrow 7CO_2 + 4H_2O + 3N_2 \quad (3)$$

Manganese Reduction

Insoluble manganese oxide is reduced to dissolved manganese by hydrocarbon degrading bacteria. The maximum level of manganese in ground water is 2mg/l.

$$C_7H_8 + 9MnO_2 \rightarrow 7CO_2 + 4H_2O + 9Mn^{2+} \quad (4)$$

Iron Reduction

Iron is found in the subsurface as either dissolved ferrous, reduced iron, or insoluble ferric, oxidized iron. Significant amounts of precipitated ferric iron may be present in the aquiver, especially under aerobic conditions.

$$C_7H_8 + 36Fe(OH)_3 \rightarrow 7CO_2 + 22H_2O + 36Fe^{2+} + 72OH \quad (5)$$

In the biodegradation of hydrocarbon, carbon consuming bacteria, in the process of oxidizing the organic compounds, will reduce ferric iron to soluble ferrous iron. Under normal conditions, the maximum level of dissolved iron in ground water is 10mg/l, but the reduction of iron in hydrocarbon-contaminated aquifer could lead to concentration high as 10 to 100 mg/l.

Sulfate Reduction

After dissolved oxygen, nitrate, manganese, and iron have been epleted, sulfate may be used as an electron acceptor for biodegradation.

$$4C_7H_8 + 18SO_4^{2-} + 12H_2O \rightarrow 9H_2S^- + 28HCO_3^- + H^+ + 9HS \quad (6)$$

The sulfanogenesis represent an anaerobic microbial reduction of sulfate to hydrogen sulfide gas.

Metanogenesis

This process represents the conversion of low molecular fatty acids, alcohols, carbon dioxide, and hydrogen to methane gas, and it follows sulfate reduction. Both processes are accomplished by a group of bacteria known as methanogenic bacteria.

$$2C_7H_8 + 10H_2O \rightarrow 5CO_2 + 9CH_4 \quad (7)$$

In general, noncohesive and permeable are the most favorable for bioremediation, as gravel and sands.

4. Feasibility studies

Like most long term treatment methods in the remediation of contaminant sites, a feasibility determination of the proposed technology should always be performer prior to detailed design and construction.

In in situ bioremediation, microbial cleanup can also be attributed to other factors, such as volatilization, if oxygen is introduced into the subsurface as an electron acceptor, and off-site migration, especially in highly permeable material.

Measurement of in situ bioremediation success is further compounded by the inherent difficulty in analyzing a process that is taking place beneath the ground surface. In general, the times period for bioremediation studies varies from three weeks to six months.

A perform procedure for evaluating the feasibility and effectiveness of in situ bioremediation was compiled in 1993 by National Research Council. This procedure consists in:

- Demonstrate a reduction in contaminant concentration.
- Prove that the native microorganisms at the site can degrade the contaminants.
- Evidence that biodegradation is indeed taking place at the site.

5. System design

The design of a bioremediation system involves several steps, including:

- Determining what needs to be added to the subsurface to stimulate microbial activity and in what quantities.
- Predicting changes in the chemistry and microbiology in the subsurface as a result of biostimulation and incorporating provisions in the design to counteract them.
- Designing delivery system.
- Implementing a monitoring program, which serves both as a measure of the success of the program and as an ongoing information collection system for adjusting the operation of the system.

In an aggressive approach of bioremediation, the system will be incorporate well and trenches to hydraulically control groundwater flow, and units for treating the extracted groundwater and returning nutrient amended and ph-adjuster water to the aquifer. Also, the sparge wells for providing oxygen or hydrogen peroxide will be necessary.

In a no aggressive approach of bioremediation, a groundwater recovery system may not be necessary. If containment of the migrating plume is all that is required, a "bio-barrier" wall or a trench will be sufficient. In certain cases, the intrinsic bioremediation is accepted as the treatment alternative. But in this case long-term monitoring will be the only remedial measure implemented.

From the results of the pilot test, determinations can be made regarding the amount of electron acceptors and nutrients that must be added to the site to stimulate microbial activity. In some cases, it may not be necessary to provide certain nutrients if continuous testing indicates that the particular nutrients are not lacking at the site. For most sites, however, electron acceptors need to be provides to the system. Dissolved oxygen levels, in hydrocarbon-impacted water-bearing zones can be as low as (0.1 ÷0.2) mg/l, with a corresponding high carbon dioxide range of (30 ÷150) mg/l. The objective here is to balance oxygen supply with oxygen demand. Generally, the calculation of oxygen requirements is based on the following statement: "three pounds of oxygen are needed to convert one pound of hydrocarbon to carbon dioxide and water", which derive from mass balance calculations based on the stoichiometry.

For example, considering the biodegradation of toluene (eq.1), the mole ratio of oxygen to toluene is 9:1, so 9 moles of oxygen are required to serve as electron acceptors by the microbes to metabolize one mole of toluene. The molar masses of toluene and molecular oxygen are:

$$C_7H_8 = 7\times 12 + 8\times 1 = 92; \quad O_2 = 2\times 16 = 32; \tag{8}$$

From the mole ratio and molar masses, the mass ratio of oxygen to toluene is then:

$$\frac{32\times 9}{92\times 1}=3.13 \qquad (9)$$

Similar stoichiometric calculations can be repeated for benzene (mass ratio 3.08), xylene and etylbenzene (mass ratio 3.17 in both cases). Assuming equal distribution of benzene, toluene, xylene and etylbenzene concentrations in total petroleum hydrocarbons plume, the average mass ratio of oxygen to total petroleum hydrocarbons is 3.14:1.

6. Intrinsic bioremediation

Intrinsic bioremediation or natural attenuation is gaining acceptance. With the push for sensible remediation, natural attenuation is finally considered a legitimate site remedial alternative. But it is important to remember that intrinsic remediation is not equivalent to a “no action” course of response. While it is true that no active remediation system will be installed, intrinsic remediation requires extensive data collection, numerical model simulation, and long-term monitoring to support its implementation.

The key to an intrinsic bioremediation program is gathering data to provide evidence that biotransformation is taking place and is capable of remediating the site. There are essentially three ways to provide evidence to support intrinsic bioremediation: demonstrate degreasing contaminant concentration over time; document loss of electron acceptors, both aerobic and anaerobic over time, and illustrating an increase in levels of by-products associated with the metabolic processes of petroleum degrading microbes.

In intrinsic bioremediation, it may not be as easy to differentiate between biotransformation of the contaminant from the mechanisms of dispersion, sorption, volatilization, and advection. Two techniques have been developed to determine if contaminant loss is due to intrinsic biodegradation: a conservative tracer or recalcitrant chemical and demonstrate mass loss over time.

Measuring dissolved oxygen concentrations and those of other electron acceptors can provide a key indicator of natural attenuation. Reduced oxygen, nitrate, and sulphate concentrations are considered to be strong evidence of intrinsic bioremediation.

The production of carbon dioxide, hydrogen sulphide, and methane and the accumulation of dissolved iron are additional indicators of biological attenuation at intrinsic bioremediation sites.

It is a demonstrate fact that natural attenuation is capable of remediatiating the site or at least limiting further migration of the plume. This is obtained by performing mass balance calculations on equations describing the stoichiometry of petroleum hydrocarbons degradation using electron acceptors under both conditions (aerobic and anaerobic).

The total petroleum hydrocarbons assimilative capacity of the aquifer is determined by adding the individual concentrations calculated for each electron acceptor. By multiplying this value with the available volume of fresh groundwater in the aquifer, a determination can be made on the quantitative assimilative capacity of the aquifer. By comparing this quantity with the calculated mass of the total petroleum hydrocarbons in the plume, a determination can be made if intrinsic bioremediation alone is sufficient to remediate the site.

For example, if the oxygen is use like acceptor, the average mass ratio of oxygen consummated to total petroleum hydrocarbons degraded is 3:1. This means that approximately 0.32 mg of petroleum hydrocarbons is biodegraded to carbon dioxide and water for every 1.0 mg of dissolved oxygen consumed. By multiplying the average background dissolved oxygen concentration, in mg/l, by 0.32, the concentration of total petroleum hydrocarbons that the aquifer can assimilate aerobically can be semi quantitatively determined.

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