Usage of Water-Filled Trench in Improving Groundwater Quality

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ABSTRACT (1 page only)

The water-filled trench method is a simple and cost effective in situ groundwater remediation technique. The water-filled trench processes avoid the risk of unassisted natural attenuation by controlling plume migration and reducing the risk of off-site liability. Simultaneously, these treatment approaches avoid the high costs and other disadvantages associated with remedial systems like pump and treat. The mechanisms of water-filled trenches in cleaning up contaminated groundwater include biological degradation, volatilization, adsorption and chemical or biological oxidation and precipitation. Water-filled trenches make use of these mechanisms to clean up contaminated groundwater, thereby reducing levels of risk to human health and the environment. In Florida, groundwater is vulnerable to different kinds of contamination, in part because of the shallow depth to groundwater in most Florida regions. Conventional methods for treating deep groundwater contamination are largely unsatisfactory because of cost and other considerations. Active methods of groundwater surface treatment are most commonly used. The active methods most frequently used are so-called pump and treat methods. Because of the high costs of active systems using pump means, interest has increased in passive systems that can treat groundwater without a need for actual removal of the water. The water-filled trench can

efficiently treat contaminated groundwater as part of a low-cost passive treatment system, which is simple and inexpensive.

This research explores the possibility of the usage of water-filled trenches in removing the organic and inorganic contaminants from the groundwater. During water-filled trench processes, the contaminants may be removed by one or more mechanisms, with certain mechanisms dominating over the others. Therefore, knowledge of the physicochemical and biological processes that are responsible for groundwater decontamination in water-filled trenches is required in order to promote field applications. This research investigates whether, and if it is yes, to what extent, water-filled trenches can be utilized in groundwater decontamination in Florida. From this research, it is demonstrated that water-filled trench is an effective means for groundwater remediation. Evaporation, adsorption and biodegradation are the three mechanisms involved in the water-filled trench processes. Among these three mechanisms, evaporation and adsorption dominate over biodegradation owing to the short reaction time. Since both evaporation and adsorption belong to physicochemical processes, water-filled trench thus mainly relies on physic-chemical treatment to remove the contamination. Owing to the physicochemical processes, water-filled trench is a simple, cheap and efficient treatment method. It avoids the risk of unassisted natural attenuation by controlling plume migration and reducing the risk of off-site liability.

EXECUTIVE SUMMARY
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Objective:
This research explores the possibility of the usage of water-filled trenches in removing the organic and inorganic contaminants from groundwater. During water-filled trench processes, the contaminants are removed by the following mechanisms, namely, evaporation, biodegradation, adsorption and oxidation and precipitation. This research investigates whether, and if it is yes, to what extent, water-filled trenches can be utilized in groundwater decontamination in Northwest Florida. In addition, the dominating mechanisms are identified, which play the key role in groundwater decontamination during water-filled trench processes.

Methodology:
Custom-made experimental setups of a water-filled trench and a water-filled trench with an aerobic filter were tested for the treatment of simulated groundwater that contains organic contaminants and heavy metals. The mechanisms of organic compound decomposition, volatile organic compound vaporization, organic contaminant adsorption on the filter media, and heavy metal transformation and precipitation were investigated in this research.

Results:
Near all the BTEX and TCE can be removed after around 150 minutes' evaporation. Among these compounds, xylene was the easiest compound to be evaporated; TCE was most difficult compound to be evaporated. Toluene and

benzene were in between. Under aerobic conditions, only BTEX was found to be degraded by the cultured aerobic microorganisms. TCE cannot be degraded under aerobic conditions. Depending on the input concentration, around 67% to 78% of benzene, 60% to 67% of toluene, 38% to 45% of xylene, and 18% to 20% of TCE was removed from the simulated groundwater using the water-filled trench followed by aerobic filtration using mulch as the filter material. In a separate experiment, the removal of BTEX and TCE by adsorption was investigated. Around 42% of benzene, 28% of toluene, 12% of xylene and 11% of TCE was removed by adsorption. During water-filled trench experiments, around 80% of iron was removed when the aerated groundwater passed through the biofilter at pH 8.0. For pH 7.52 and 7.04, around 60% and 50% was removed.

Rationale:
In Florida, groundwater is vulnerable to different kinds of contamination, in part because of the shallow depth to groundwater in most Florida regions. Organic compounds and heavy metals are the most common contaminants present in the groundwater. Water-filled trenches can clean up groundwater by means of biological degradation of organic contaminants, volatilization of volatile organic compounds, organic compound adsorption, and chemical or biological oxidation and precipitation of heavy metals. In situ, passive approaches of water-filled trenches that aim to accelerate the rate of decontamination of groundwater represent a sensible alternative for groundwater remediation.

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1. Introduction

A variety of natural physical, chemical, and biological processes can reduce the amount, toxicity, mobility, and concentration of contaminants in the groundwater. The challenge that hydrologists and environmental engineers are facing is to apply these processes to in situ remediation of contaminated groundwater in a simple, cheap and efficient manner. The introduction of water-filled trenches attracts significant attention as a means of natural attenuation of groundwater contamination (45). The water-filled trench method is a simple and cost effective in situ groundwater remediation technique. The water-filled trench processes avoid the risk of unassisted natural attenuation by controlling plume migration and reducing the risk of off-site liability. Simultaneously, these treatment approaches avoid the high costs and other disadvantages associated with remedial systems like pump and treat. The mechanisms of water-filled trenches in cleaning up contaminated groundwater include biological degradation, volatilization, adsorption and chemical or biological oxidation and precipitation (45, 113). Water-filled trenches make use of these mechanisms to clean up contaminated groundwater, thereby reducing levels of risk to human health and the environment. In Florida, groundwater is vulnerable to different kinds of contamination, in part because of the shallow depth to groundwater in most Florida regions. For instance, at Patrick Air Force Base, soil and groundwater contamination caused by benzene, toluene, ethylbenzene, and xylene (BTEX) has been known to occur near the BX Service Station (Site ST-29). At other locations in Florida, groundwater is also easily to be contaminated by contaminants associated with petroleum hydrocarbon releases. Since 1983, over 28,000 facilities have reported discharges of petroleum products from storage tank systems in Florida. Such discharge includes BTEX, which become dissolved in groundwater. On the other hand, at Hurlburt Air Force Base, perchloroethylene (PCE) and trichloroethylene (TCE) were the major contaminants released over a long period of time. Also nearby some commercial dry-cleaning facilities, PCE and TCE concentrations in the groundwater well exceeded the drinking water criteria (108). Additionally, groundwater in Northwest Florida is easily to be contaminated by heavy metals such as iron. For nonvolatile organic compounds, the preferred pathway is biodegradation, especially if this results in complete mineralization of the organic compounds to CO_2. Volatile organic compounds can be removed by volatilization and heavy metals can be removed by chemical or biological oxidation and precipitation (108).

1.1 Nonvolatile Organic Compound Removal
Water-filled trenches can biodegrade the organic contaminants such as BTEX, pesticides, and polycyclic aromatic hydrocarbons (PAHs), etc. in the groundwater. In situ, passive approaches of water-filled trenches that aim to accelerate the rate of biodegradation of organic contaminants in the groundwater represent a sensible alternative for groundwater remediation. For biodegradation of the organic contaminants to occur, an electron acceptor, nutrients and other constituents are required. Organic compounds such as BTEX have been proven to be biodegradable in the groundwater. Specifically, under aerobic conditions, benzene is readily to be biodegraded (59, 61, 112). Bacteria are able to attack the ring using oxygen, forming catechol as a product (139). Under strictly anaerobic conditions, however, benzene is regarded as recalcitrant (25). Its symmetrical

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ring structure is believed to be resistant to cleavage as functional groups, which act to disrupt the electron symmetry of ring structures, are not present (52). Degradation of benzene in "real-world" groundwater environments has often been reported (20, 79). Synoptic reviews of hundreds of gasoline release sites show that over 90% of the benzene plumes studied decreased to less than 5 ppb at a distance of less than 260 feet from the source in California (100) and 90% of the benzene plumes decreased to less than 10 ppb in less than 380 feet from the source in Texas (119). These sites are almost always shallow groundwater sites and the biodegradation of benzene is likely due to aerobic biodegradation at the plume periphery.

1.2 Volatile Organic Compound Removal

Water-filled trenches can also vaporize volatile organic contaminants. The water-filled trenches can be pressurized either by air or liquid fluids to improve the rate of evaporation of the volatile organic contaminants. The most common volatile organic pollutants are the petroleum products, such as gasoline and jet fuels ((19, 21, 77). Especially, some of the constituents of gasoline, such as BTEX fall within this category (90). Most importantly, it has been demonstrated that water-filled trenches can also effectively volatile chlorinated hydrocarbons, notably PCE and TCE from the contaminated groundwater (26, 88). It should be noted that the volatility of these compounds is temperature-dependent since higher temperature causes these substances to have a higher vapor pressure (85, 90, 131). In the contaminated groundwater, it is common for the majority of the organic pollutants to exist as separate liquid phases. A portion may dissolve into groundwater or may evaporate into the gas phase. The water-filled trenches create an air phase, which volatilizes the volatile organic contaminants contained in the groundwater, and removes these contaminants as a vapor (128).

1.3 Metal Removal

Inorganic contaminants in the groundwater, such as heavy metals, may be transformed to different chemical forms and precipitate in water-filled trenches. In Northwest Florida, groundwater is usually observed to have elevated levels of iron concentrations (140). Water-filled trenches can be used to oxidize and precipitate iron from the groundwater (38, 124). For metals to precipitate, raising pH or creating redox conditions is required. For the metal precipitation processes, it is very important to gain control of the pH of the groundwater because pH affects the solubility of metals and the kinetics of the oxidation and hydrolysis processes (5, 102, 134). In addition, the relationship between pH and metal removal processes varies among metals and also between biotic and abiotic processes. For common metals, i.e., Fe, Al, and Mn, chemical oxidation or aerobic biological oxidation processes, with or without an alkaline agent are the most commonly practiced applications (24, 47, 56, 120, 132). Another major player is the usage of alkaline agents, most commonly lime (23, 34, 92). With the development of biotechnologies, microbial mediated treatment methods have been advocated. When groundwater passes the water-filled trenches, CO_2 will be stripped and pH will increase, which enhances both chemically and biologically mediated metal oxidation and precipitation.

1.4 Effect of Alkalinity on Contaminant Removal

Alkaline agents can raise the pH, thus neutralizing the effects of alkalinity loss during organic degradation and metal oxidation and precipitation (4, 16, 75). Bicarbonate (HCO_3^-), carbonate (CO_3^{2-}) and hydroxide ions (OH^-) are the three major alkaline substances to react with generated acidity. This is most important for heavy metal oxidation and precipitation. When the alkalinity of the groundwater is not enough to neutralize generated acidity, addition of external alkaline agents is required (13, 62, 104, 117, 118). By far the most commonly used alkaline agent for reducing acidity and precipitating heavy metals in the groundwater is lime or limestone (calcium carbonate) (7, 99, 105). Limestone is selected due to its availability and inexpensive cost. In addition, limestone is also rich in calcite. Once the pH of the groundwater is raised, metals can precipitate more easily to form hydroxides and oxyhydroxides. Other commonly used alkaline agents include hydrated lime (calcium hydroxide), soda ash (sodium carbonate), caustic soda (sodium hydroxide), and in some cases ammonia (17, 130).

1.5 Water-Filled Trench with an Aerobic Filter

To enhance biodegradation, aerobic filtration has been introduced to water-filled trenches, which appears to be promising as compared to the conventional water-filled trench techniques (114). Similar technologies such as biodegradable slurry are also widely utilized, which is generally made of powdered guar bean (35). Billions of indigenous microorganisms inherent within the filter media convert the organic compounds to carbon dioxide and water (137). These naturally occurring microorganisms consume the offending compounds in a safe, moist, and oxygen-rich environment. Thus, water-filled trenches with associated filters are more efficient in removing biodegradable organic contaminants (36, 74, 89). It should be noted that sufficient delivery of electron acceptors (i.e., oxygen) and nutrients (i.e., nitrogen and phosphorus) is required in order to maintain the decontamination efficiency. The organic contaminants will serve as the carbon source. For recalcitrant organic compounds that can only be degraded by co-metabolism, other energy sources (i.e., carbon) are required (33, 63). Generally, electron acceptors and nutrients are the two most critical components for the efficient operation of the system. Oxygen can be provided during the water-filled trench processes. For filters that use mulch as the filter material, mulch can supply the necessary nutrients. The mechanisms of such processes include a combination of contaminant adsorption and microbial degradation (37). Microorganisms contained in the filter continually metabolize the contaminants, as they are adsorbed, converting them ultimately to water, carbon dioxide and salts. Similar filter materials include compost and other high organic matter content materials such as wood-product based material (27). Some other filter materials such as lime stone have also been popularly utilized (145). These materials can help increase groundwater alkalinity for the microbial mediated organic degradation. When large quantities of organic contaminants need to be removed, manual addition of nutrients (e.g. nitrogen and phosphorous compounds) may be required in order to sustain the microbial life. Water-filled trenches with an aerobic filter can also volatize volatile organic compounds from the contaminated groundwater, which is achieved during the water-filled trench processes.

1.6 Water-Filled Trench Applications

Conventional methods for treating deep groundwater contamination are largely unsatisfactory because of cost and other considerations. Active methods of groundwater surface treatment are most commonly used (28, 143). The active methods most frequently used are so-called pump and treat methods (3, 9, 57). Because of the high costs of active systems using pump means, interest has increased in passive systems that can treat groundwater without a need for actual removal of the water. The water-filled trench can efficiently treat contaminated groundwater as part of a low-cost passive treatment system, which is simple and inexpensive. The factors that impact water-filled trench applications include volume of contaminated groundwater that needs to be treated, groundwater flow rate and direction, seasonal variation of groundwater flow, height and profile of water table at steady state, expected concentration of contaminants in the groundwater, and environmental effects (i.e., volatile organic emission to the atmosphere), etc. These hydrogeologic conditions will determine the elevation or positioning of the water-filled trenches as well as the operation of the water-filled trenches.

1.7 Objectives

This research explores the possibility of the usage of water-filled trenches in removing the organic and inorganic contaminants from the groundwater. During water-filled trench processes, the contaminants may be removed by one or more mechanisms, with certain mechanisms dominating over the others. Therefore, knowledge of the physicochemical and biological processes that are responsible for groundwater decontamination in water-filled trenches is required in order to promote field applications. This research investigates whether, and if it is yes, to what extent, water-filled trenches can be utilized in groundwater decontamination in Florida. We designed the experimental devices and conducted laboratory experiments during this research. Specific objectives of this project include:

Objective 1. We simulated water-filled trench processes in the laboratory to investigate the possible decontamination of organic compounds and inorganic heavy metals in groundwater. We expected to observe organic decomposition, volatile organic compound vaporization, and metal oxidation and precipitation in these experiments. The decontamination mechanisms for different contaminants were identified and the removal rate was quantified. The effect of initial contaminant concentration and pH on groundwater decontamination was investigated.

Objective 2. We further investigated organic decomposition, volatile organic compound vaporization, organic compound adsorption and metal oxidation and precipitation using a water-filled trench with an aerobic filter. The filter material used for this part of research was mulch. We quantified the contaminant removal in this process. In addition, we investigated iron reduction nearby landfills and the consequently released ferrous iron adsorption on *S. putrefaciens*.

2. Background

2.1 BTEX

Organic contamination is a major problem in groundwater, which creates a hazard to public health and the environment. The monoaromatic hydrocarbons, abbreviated BTEX, which stands for benzene, toluene, ethylbenzene, trimethylbenzenes and the three xylene isomers, are aromatic hydrocarbons commonly found in gasoline contaminated groundwater (15, 18, 39, 48, 53). BTEX have in recent years attracted much attention, since they constitute one of the most common and serious threats to groundwater reservoirs and indoor climate deriving from contaminated sites (51, 58, 78, 80). This is mainly due to the potential effects of benzene, which is considered a strong carcinogen, and which is highly mobile in the soil and groundwater environment, which is also the case for the other BTEX (72, 141, 142). BTEX are currently found in numerous sites, including areas used for fuel operations, refineries, gasoline stations, and gasification sites (127). One of the most common sources for BTEX-contamination of soil and groundwater are spills involving the release of petroleum products such as gasoline, diesel fuel and lubricating and heating oil from leaking oil tanks. Because of their polarity and very soluble characteristics, the organic chemicals of petroleum products will be able to enter the soil and groundwater systems and cause serious pollution problems. The physic-chemical properties of BTEX are listed in Table 1 (110).

Table 1. Physical and Chemical Properties of BTEX

	Benzene	Toluene	m-Xylene	o-Xylene	p-Xylene	Ethyl benzene
Chemical structure						
Chemical formula	C_6H_6	C_7H_8	C_8H_{10}	C_8H_{10}	C_8H_{10}	C_8H_{10}
Molecular weight [g/mole]	78	92	106	106	106	106
Water solubility [mg/l]	1700	515	-	175	198	152
Vapor pressure [mm Hg] (20^oC)	95.2	28.4	-	6.6	-	9.5
Specific density (20° C)	0.8787	0.8669	0.8642	0.8802	0.8610	0.8670
Octanol-water partition coeff. [log Kow] (20^oC)	2.13	2.69	3.20	2.77	3.15	3.15
Henry's law constant [kPa*m^3/mole] (25^oC)	0.55	0.67	0.70	0.50	0.71	0.80
Polarity	Non-polar	Non-polar	Non-polar	Non-polar	Non-polar	Non-polar
Maximum Contaminant Level (MCL) [mg/l]	0.005	1	10 [*]	10 [*]	10 [*]	0.7

Because of the relatively high water solubility and low K_{ow} values, BTEX tend to be dissolved in the water phase or evaporated into the air spaces of the soil. In addition, because of their relative hydrophilic nature, they are not attenuated very much in the groundwater (8, 10). In some sites, some BTEX are found several kilometres downstream the source (50). In groundwater, due to the low water solubility of oxygen, the flux of oxygen will in many cases not be enough to support aerobic degradation. Under anaerobic conditions, the biodegradation pattern for these compounds is rather complex, While most of them, including benzene, are shown to be degraded under strict anaerobic condition and sulfate reducing conditions, the biodegradation under denitrifying conditions are less favourable (1, 68, 96, 115, 135). Benzene cannot be degraded with nitrogen as terminal electron acceptor (129), and o-xylene often depend on the existence of primary substrates, either toluene or phenol to be degraded (46). In addition to this cometabolic behaviour, also prolonged lag periods for degradation of xylenes, ethylbenzene and 1,2,4-trimethylbenzene are often observed (64, 65). Biodegradation of BTEX under different redox conditions is summarized in Table 2 (20).

Table 2. Biodegradation of BTEX under Different Redox Conditions

Compound	Aerobic conditions	Denitrifying conditions	Sulfate-reducing conditions	Iron-reducing conditions	Methano-genic conditions
Benzene	++	-	+	-	+
Toluene	++	++	+	+	+
m-Xylene	++	++	+	+	+
p-Xylene	++	+	+		+
o-Xylene	++	+/-	-	-	+/-
Ethylbenzene	++	+/-		-	+/-
1,2,4-trimethyl-benzene	++				+/-

2.2 Remediation Techniques

To remove the contamination, natural attenuation or *in situ* remediation technologies such as bioremediation in the form of biostimulation with addition of nutrients and or electron acceptors, soil vapor extraction/bioventing, or reactive barriers can be utilized (32, 44, 82, 86, 94, 143). Volatilization affects the actual BTEX concentration. When analyzing gasoline contaminated groundwater, evaporation should be taken into consideration since BTEX will volatilize due to their high solubility, relatively low molecular weight and high vapor pressure. Concurrently with volatilization the gasoline will begin to dissolve into the groundwater. Compared to the other main group of components in gasoline, such as the aliphatics, BTEX are very soluble. The degradation of BTEX is another aspect that affects BTEX concentration in groundwater. The degradation techniques are sometimes

recommended as a natural means of remediation (intrinsic bioremediation). By adding nutrients and oxygen, it is possible to enhance the degradation process.

2.2.1 Biodegradation

Biodegradation is one of the techniques that can remediate BTEX in groundwater. Using this technique, microorganisms degrade BTEX into CO_2 and water. Oxygen and nutrients might be injected to promote the degradation rate. If nothing is being added, the biodegradation is called intrinsic. The degradation may occur under the use of different electron acceptors than oxygen. For instance, toluene may degrade via an anaerobic pathway using nitrate as an electron acceptor (71).

2.2.2 Air sparging

Air sparging is a physical and/or microbiological degradation process. By driving large quantities of air into the saturated zone this technique enables an aerobic degradation of organics. The injection of air to the saturated zone serves two purposes. First it drives the volatile organics into the unsaturated zone, from where they may be removed by vapor phase extraction. Secondly it increases the microbiological activity in the saturated zone (3, 70). For the removal of volatile organics from the unsaturated zone, vapor phase extraction with the application of vacuum is applied to volatilize the organic compounds. The vacuum ensures that the organic compounds are transported to the extraction wells.

2.2.3 Bioventing

Bioventing is a microbiological degradation process. Usually, both injection and extraction wells are installed. Nutrients may be added to stimulate a biological degradation of the BTEX. The process is able to remove both the volatile organics such as BTEX and the more heavy-end contaminants such as diesel and crude oil.

2.2.4 Activated Carbon Treatment

This treatment is used after groundwater has been pumped out of the aquifer. The contaminated water is passed through the activated carbon unit where BTEX are adsorbed and collected. This is accomplished through the adsorption of BTEX onto a carbon matrix. The effectiveness of this process is related to the amount of surface area on the activated carbon and the properties of the chemical substance. This process is often used to remove relatively low concentrations.

2.2.5 Air stripping

Air stripping is a pump-and-treat method that uses a countercurrent flow of upward air-solvent gas and downward water-solvent liquid to physically strip the contaminant out of the water. Often activated carbon systems are used as a secondary step to this procedure.

2.3 PCE and TCE

Groundwater contamination by the chlorinated hydrocarbons tetrachloroethene (PCE) and trichloroethene (TCE) is a major problem throughout the United States. Due to their widespread use and because of poor handling and disposal practices, these compounds are frequently found as contaminants in groundwater (43, 91, 93, 103). A 1980 study of drinking water systems throughout the United States reported that 31 municipal wells in

California were closed due to TCE contamination and that wells in 15 Pennsylvania communities were closed for the same reason (6). Furthermore, vinyl chloride (VC), an intermediate in the PCE and TCE degradation pathways, is a known carcinogen. Since PCE and TCE are suspected carcinogens, and because of the toxicity of VC, these compounds are regulated by the United States Environmental Protection Agency and the Safe Drinking Water Act of 1986 (121). A common problem associated with the contamination of groundwater systems by PCE and TCE stems from the formation of dense-nonaqueous-phase-liquids (DNAPLs). PCE and TCE form DNAPLs that sink through permeable groundwater aquifers until a non-permeable zone is reached at which point they pool (2, 146).

Tetrachloroethylene (PCE) and trichloroethylene (TCE) are effective solvents for a variety of organic materials (66). For many applications, these chlorinated solvents offer the best technical solution. Vapor degreasing, for example, is recognized as the most effective way of cleaning metal components. Other examples include the manufacture of medical equipment, pharmaceuticals, car braking systems, high-specification plastics, aircraft wings and hydraulics, and in crack testing and dry cleaning (101). There may be alternatives, but they do not necessarily offer a better environmental performance. Water-based cleaning, for instance, may result in contamination of the aqueous environment. Most importantly, for these reasons, they are widely used in dry cleaning and in the automotive and other metalworking industries for degreasing metal parts. The demand for TCE as a degreaser began to decline in the 1950s in favor of the less toxic 1,1,1-trichloroethane. However, 1,1,1-trichloroethane production has been phased out in most of the world under the terms of the Montreal Protocol, and as a result trichloroethylene has experienced some resurgence in use as a degreaser. The physical and chemical properties of PCE and TCE are listed in Table 3.

Table 3. Physical and Chemical Properties of TCE and PCE

	Trichloroethylene	Perchloroethylene
Density	1.463 g/ml (25°C)	1.6311 g/ml (25°C)
Vapor Density	4.5 (vs Air)	5.7 (vs Air)
Vapor Pressure	61 mm Hg (20°C)	18.47 mm Hg (25°C)
Refractive Index	N20/D 1.476 (lit.)	
Storage Temperature	0 – 6°C	
Water Solubility	0.11 g/100 ml	< 1 mg/l

PCE and TCE have been widely used for many years, and methods of use have been developed which mean their impact on the environment and on health can be kept to the very minimum. The persistence of chlorinated solvents such as TCE and PCE makes them not easy to be biodegraded (54). A developing strategy for the remediation of PCE and TCE contaminated aquifers is anaerobic biodegradation. PCE and TCE form dense-nonaqueous-phase liquids (DNAPLs) which can provide a persistent groundwater contamination source. Under saturating PCE and TCE conditions, these chlorinated

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ethenes were rapidly converted to ethene with little or no accumulation of VC. These results suggest that anaerobic remediation can potentially be used in the remediation of PCE and TCE DNAPLs. From a TCE contaminated groundwater site, microorganisms were enriched with the ability to anaerobically convert PCE and TCE completely to ethane (81, 144). Kinetic studies performed with this culture showed that degradation of PCE, TCE, and vinyl chloride (VC) was first order with respect to substrate concentration up to their solubility. It was also shown that, although VC inhibited TCE degradation, a finite degradation rate could be achieved in the presence of high VC concentrations.

According to the Agency for Toxic Substances and Disease Registry (ATSDR), over 1,309 Superfund sites in the U.S. have soil or groundwater that is contaminated with PCE; 1,460 Superfund sites are contaminated with TCE. This does not include thousands of other sites under the jurisdiction of state agencies and undergoing cleanup as part of RCRA Corrective action, which are not listed on the National Priority List. In many cases, people living near these sites have been exposed to these chemicals in domestic-use water, from ingestion, skin contact or inhaling chemicals that volatilize from water. However, what has become a greater concern is vapor intrusion, a pathway where these chemicals volatilize from soil or groundwater, migrate through soil near building foundations and are drawn into indoor air through egresses in foundations.

2.4 Iron in Groundwater

Iron in rural groundwater supplies is a common problem: its concentration level ranges from 0 to 50 mg/l, while WHO recommended level is < 0.3 mg/l. The iron occurs naturally in the aquifer but levels in groundwater can be increased by dissolution of ferrous borehole and handpump components. Iron-bearing groundwater is often noticeably orange in color, causing discoloration of laundry, and has an unpleasant taste, which is apparent in drinking and food preparation.

Iron dissolved in groundwater is in the reduced iron II form. This form is soluble and normally does not cause any problems by itself. Iron II is oxidised to iron III on contact with oxygen in the air or by the action of iron related bacteria. Iron III forms insoluble hydroxides in water. These are rusty red and cause staining and blockage of screens, pumps, pipes, reticulation systems etc. If the iron hydroxide deposits are produced by iron bacteria then they are also sticky and the problems of stain and blockage are many times worse. The presence of iron bacteria may be indicated by rusty slime inside headwork's, reduced water flow form the bore and unpleasant odor from water pumped from the bore, slimy deposits blocking main and lateral lines, severe staining on pavements, walls foliage [9].

2.5 Treatment Technologies for Heavy Metals

Traditional treatments rely on conventional, well-recognized technology to raise pH or create redox conditions to oxide and precipitate iron in the groundwater. There are many biotic and abiotic processes that lead to metal precipitation that are commonly practiced in treating groundwater. For all of these treatment processes, it is very important to gain control of the pH of the groundwater because pH affects the solubility of iron and the kinetics of the oxidation and hydrolysis processes (138). The most predominate treatment process is oxidation, dosing with alkali, and sedimentation. Another major player in the

passive treatment of groundwater with high iron content are alkaline agents, most commonly lime, although the application of lime to reduce acidity is not particularly innovative. On the other hand, some of the ways to expose the acidic water to the alkaline agent are innovative. Others methods in groundwater with high iron content include: sulfidization, biosedimentation, sorption and ion exchange, and membrane processes such as filtration and reverse osmosis, etc. (49, 60, 76).

With the development of biotechnologies, microbial mediated treatment methods have been developed (12, 87, 126). Bio-reactors and permeable reactive barriers are the common technologies. Constructed wetland can also treat groundwater with high iron content, depending on the oxidation conditions.

2.5.1 Limestone Drains

Alkaline agents can raise the pH, thus promoting iron oxidation and precipitation from groundwater (42). It is expected that the bases can dissolve in the water to produce hydroxide ions (OH^-) to react with the hydrogen ions (H^+) and neutralize the water in combination with carbonate ions (CO_3^{2-}). By far the most common application for reducing acidity and precipitating iron is through addition of lime or the usage of limestone (calcium carbonate). Limestone is selected due to its availability and inexpensive cost. In addition, limestone is also rich in calcite. Once the pH of groundwater is high enough, iron can precipitate more easily to form hydroxides and oxyhydroxides. Other commonly used alkaline agents are hydrated lime (calcium hydroxide), soda ash (sodium carbonate), caustic soda (sodium hydroxide), and in some cases ammonia.

Anoxic limestone drains treat groundwater through an underground pathway that is packed with crushed limestone. Treated water from anoxic limestone drains typically is discharged into a settling pond or wetland to allow metals to precipitate and settle (30). However, anoxic limestone drains often experience armoring, i.e., strong adhesion and complete pacification by encrustation, causing the limestone to become inactivated and potentially clogging of the drain (31, 122). For effective operation, dissolved oxygen and Fe^{+3} concentrations are suggested to be less than 1 mg/L, though some authors suggested that Fe^{+3} and concentrations can be as high as 5 mg/L (29). It is suggested that the armoring of limestone can be substantially offset by incorporating sandstone into the drain (125). It is observed that the majority of metallic oxides precipitated onto sandstone rocks when iron contaminated groundwater discharged onto both exposed limestone and sandstone (125). This suggested a preferred precipitation media. In order to validate that the observed precipitation was not merely coincidence or mechanical, laboratory and field test at other mine drainage locations were conducted and similar results were recorded. It seems that sandstone has an order of magnitude higher of iron precipitation than limestone (125). If this preference is fairly consistent, the addition of crushed sandstone to limestone drains could reduce armoring of limestone. It might also be noted that these studies were not conducted at oxygen deficit locations, and so behavior in anoxic conditions should be investigated.

2.5.2 Successive Alkalinity Producing Systems

Successive alkalinity producing systems have the following basic elements: organic mulch layer, limestone layer, and a drainage system. Usually, a flushing system is included as well. This technology was created in the early 1990's by Kepler and McCleary (67). Groundwater flows into the tops of the cell, creating a top layer of water which prevents the infiltration of oxygen into the bottom layers. The organic layer removes dissolved oxygen from the water to provide anaerobic conditions, supporting the establishment of sulfate-reducing bacteria. The anaerobic environment is a reducing environment that changes Fe^{+3} to Fe^{+2}, thereby reducing the likelihood of iron hydroxide precipitation. Since these units encourage reducing conditions and establishment of sulfate-reducing bacteria, a major contribution to the treatment of the water, these units are sometimes referred to as reducing and alkalinity producing systems (67). Finally, the water enters the limestone region and the absence of oxygen prevents the armoring of limestone. Upon leaving the successive alkalinity producing systems the water is usually directed to an aerobic settling pond or wetland to allow metals to form precipitates and further water polishing (55). Many successive alkalinity producing systems include flushing systems to remove possible precipitates that can clog the cell. The flushing systems are generally operated by generating head differences that move water rapidly through the system. Successive alkalinity producing systems tend to be more efficient than anaerobic wetlands and require less space to provide the same level of treatment (55). Successive alkalinity producing systems require some maintenance, not only for periodic flushing, but also to prevent or correct the development of preferential flow paths.

2.5.3 Bioreactors

Bio-reactors and permeable reactive barriers utilize alkaline agents or metal oxidizing bacteria to oxidize and precipitate iron in groundwater (73). These technologies are designed to remove ferrous iron in groundwater and always involve the establishment of metal oxidizing bacteria. Passive bioreactors are lined trenches or pits that can contain a variety of materials, most commonly a mixture of cobbles, compost, other organic matter, and/or an alkaline agent. Sometimes above ground tanks containing any variety of materials including those described above are used to establish appropriate microorganisms to precipitate metals and adjust pH. They are all referred to as "bioreactors." Bioreactors are somewhere between a permeable reactive barrier and a wetland, treating contaminated water through natural reactions.

2.5.4 Permeable Reactive Barriers

Permeable reactive barriers are barriers to remove specific chemicals of concern that are placed in the path of groundwater flow (69, 98). Permeable reactive barriers have a subsurface reactive section for the groundwater to flow through and to be treated. In some cases, there are impermeable walls to direct the water flow to the reactive section. The reactive media is usually compost material that hosts metal oxidizing bacteria. Permeable reactive barriers have been used to remove iron with possible permeable materials such as rocks in the form of amorphous ferric oxyhyroxide (111).

3. Materials and Methods

3.1 Simulated Groundwater

Petroleum hydrocarbons are common groundwater pollutants as a result of leaking underground storage tanks and spills in Florida. Besides, considering the PCE, TCE and iron contamination of the groundwater in Florida, the simulated groundwater thus have a composition of benzene, 25 mg/L; toluene, 25 mg/L; *p*-xylene, 25 mg/L; TCE, 200 μg/L; glucose, 50 mg/L (to represent all other organic contaminants); and ferrous iron, 10 mg/L.

3.2 Soil Collection and Characterization

The soil used for this research was collected from four landfills located in Northwest Florida, including Franklin County Landfill, Quincy-Byrd Landfill (Gadsden County), Baker Landfill (Okaloosa County), and Santa Rosa Central Landfill (Santa Rosa County). Soil samples were collected 1 to 3 feet below the surface, 100 to 300 feet away from the landfills. The collected soil samples were immediately placed in a Styrofoam cooler and sealed. All the soil samples were delivered to the laboratory immediately and stored under refrigeration at $4^{\circ}C$ until usage in the experiments. To assess the soil iron content, soil samples were first partially thawed and placed in an anaerobic chamber with a maintained H_2-N_2 atmosphere. The samples were then ground and the weighed samples were placed in a glass reaction vessel and purged with CO_2-scrubbed air, after which the samples were acidified with hot, 5% perchloric acid to dissolve carbonate precipitates such as siderite, calcite, aragonite, and carbonate forms of green-rust. Evolved CO_2 gas was carried to the coulometer cell containing a CO_2-sensitive ethanolamine solution and quantitatively titrated. The samples were then reacted with 0.25 M hydroxylamine (NH_2OH) hydrochloride in 0.25 N HCl and incubated at 60 °C for 2 hours for iron extraction (97, 123). Following the extraction, soil iron content was determined using spectrophotometric analysis techniques by reacting Fe(III) with the thiocyanate ion to form a highly colored complex:

$$Fe^{3+}(aq) + 6SCN^-(aq) = [Fe(SCN)_6]^{3-}(aq)$$
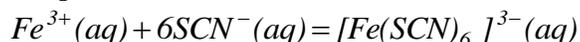
Because the thiocyanate complex is colored red, it absorbs at 447nm on the absorption spectrum. For this research, extracted iron was reacted with 1.5 M KSCN and the Fe(III) concentration was measured using a spectrophotometer at the wavelength of 447 nm (Shimadzu UV-1650 PC).

3.3 Aerobic Microbial Culture Cultivation

In an attempt to isolate a BTEX degrading culture, microorganisms were isolated from leachate samples collected from Santa Rosa Holley Landfill in Santa Rosa County, FL. Continuous cultivation and enrichment of the BTEX degrading culture were carried out in a 250 ml serum bottle containing 100 ml sterilized minimal salt medium amended with approximately 25 mg/l benzene, 25 mg/l toluene and 25 mg/l xylene. The mineral salts medium used in this study had the following composition: KH_2PO_4, 160 mg/l; K_2HPO_4, 420 mg/l; Na_2HPO_4, 50 mg/l; NH_4Cl, 40 mg/l; $MgSO_4 \cdot 7H2O$, 50 mg/l; $CaCl_2$, 50 mg/l; $FeC1_3 \cdot 6H2O$, 0.5 mg/l; $MnSO_4 \cdot 4H2O$, 0.05 mg/l; H_3BO_3, 0.1 mg/l; $ZnSO_4 \cdot 7H_2O$, 0.05 mg/l; $(NH_4)_6Mo_7O_{24}$, 0.03 mg/l. The medium pH was adjusted to 7.0 with 0.1 M HCl or 0.1 M NaOH. The growth bottles received constant air supply for approximately two

weeks until the formation of black precipitate at the bottom and on the wall of the serum bottles can be observed. Then 10 ml enriched culture was transferred into 100 ml fresh culture medium amended with approximately 25 mg/l benzene, 25 mg/l toluene and 25 mg/l xylene for the second phase culture enrichment. After the fourth phase enrichment was completed, bacterial cells were harvested and used for the BTEX degradation experiments. From these growth chambers, material was withdrew for identification both through morphology and PCR analysis. For the morphology identification, both mixed cultures and pure cultures were fixed on slides by heating and viewed under a bright field microscope. For the mixed culture, a small amount of soil/water material was withdrawn from the chamber and fixed onto the slide by heating.

3.4 Anaerobic Species Cultivation
The same culturing preparation used in the aerobic culturing was also used in the anaerobic culturing, with a few alterations. For anaerobic growth, the soil and media mixture were placed in a 750 ml flask and sealed. Approximately 5 ml of potassium hydroxide was placed in the arm tube to serve as the CO_2 entrapping device (Figure 1). For this research, 1 M KOH was used to entrap CO_2. This method helps to alleviate pressure in the system and closely mimic real world conditions. The system was allowed approximately one month of incubation before further analysis. The growth media had a composition (mg/l) of KH_2PO_4, 160; K_2HPO_4, 420; Na_2HPO_4, 50; NH_4Cl, 40; $MgSO_4 \cdot 7H_2O$, 50; $CaCl_2$, 50; $FeCl_3 \cdot 6H_2O$, 0.5; $MnSO_4 \cdot 4H_2O$, 0.05; H_3BO_3, 0.1; $ZnSO_4 \cdot 7H_2O$, 0.05; $(NH4)_6Mo_7O_{24}$, 0.03; glucose, 200; and ammonia chloride, 60. The pH of the media was adjusted to 7.4 with 1 M HCl or 1 M NaOH, after which the media were sterilized by autoclaving ($121^{o}C$ and 1 atm) for 20 min. Glucose was filter-sterilized and aseptically added to the autoclaved media. For the morphological identification, a small amount of water, soil mixture was removed from the system. To avoid introduction of oxygen to the system, a needle was inserted through the rubber stopper. The same steps were then followed as were done with the aerobic species.

3.5 *S. putrefaciens* Cultivation and Identification
S. putrefaciens was cultured using the sampled soil as the innocula under anaerobic conditions in an anaerobic chamber. 250 ml Teflon-sealed serum bottles equipped with CO_2 entrapping devices were used for the culturing. The serum bottles contained 100 mL mineral salts media that had a composition of KH_2PO_4, 160 mg/L; K_2HPO_4, 420 mg/L; Na_2HPO_4, 50mg/L; NH_4Cl, 40 mg/L; $MgSO_4 \cdot 7H_2O$, 50 mg/L; $CaCl_2$, 50mg/L; $FeCl_3 \cdot 6H2O$, 0.5 mg/L; $MnSO_4 \cdot 4H_2O$, 0.05 mg/L; H_3BO_3, 0.1 mg/L; $ZnSO_4 \cdot 7H2O$, 0.05 mg/L; and $(NH4)_6Mo_7O_{24}$, 0.03 mg/L. Glucose at a concentration of 0.2 g/L and $Fe_2(SO_4)_3 \cdot 7H_2O$ at a concentration of 500 mg/L were added to serve as the carbon source and electron acceptor to stimulate *S. putrefaciens* growth. Resazurin (1 mg/L) was added as a redox indicator to indicate contamination by molecular oxygen and cysteine (3.0 g/L) was added to reduce the trace amount of oxygen remaining in the media after autoclaving. The media pH was adjusted to 7.0 with 0.1 M NaOH. The headspace of the serum bottles was pressurized with ultra-pure nitrogen and the serum bottles were capped with butyl rubber septa and crimped with an aluminum seal. The inoculated serum bottles were put into a rotary-shaker (150 rpm at 35 ℃) in the dark for at least 1 week until the formation of black precipitate at the bottom and on the wall of the serum bottles was

Figure 1. Anaerobic Bacterial Culturing

observed. Then 10 mL enriched culture was transferred into 100 mL fresh culture media amended with 0.2 g/L glucose and 500 mg/L $Fe_2(SO_4)_3 \cdot 7H_2O$ for the second phase culture enrichment. After the fourth phase enrichment was completed, bacterial cells were harvested by centrifugation (6000 g, 15 min) and washed twice with a fresh electrolyte solution under an extra-pure nitrogen atmosphere. The electrolyte solution was prepared using analytical reagent-grade NaCl and nano-pure de-ionized water (NPDI, Barnstead, Dubuque, IA) at a concentration of 10^{-5} M and adjusted to pH 7. The concentrated cells were then re-suspended in a serum bottle containing fresh, electrolyte solution (10^{-5} M NaCl) to give a final concentration of approximately 5×10^9 cells/ml.

S. putrefaciens was identified based on polymerase chain reaction (PCR) analysis. Upon verification of the PCR reaction by viewing the gel bands, the PCR samples were purified using a QIAGEN QIAquick-spin PCR purification kit. After the purification, the samples were amplified and the resulted sequences were compared with the database of the National Center for Biotechnology Information (NCBI) based on the strands that have been previously identified as *S. putrefaciens*. The strains whose DNA codes matched the codes of *S. putrefaciens* were selected and enriched for further experiments.

3.6 BTEX and TCE Evaporation Experiments
A custom-made experimental setup was utilized for this part of research (Figure 2). The reactor had a working volume of 1.0 L. The simulated water was stirred in the reactor

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continuously mimicking water-filled trench operations for volatile organic compounds to be evaporated.
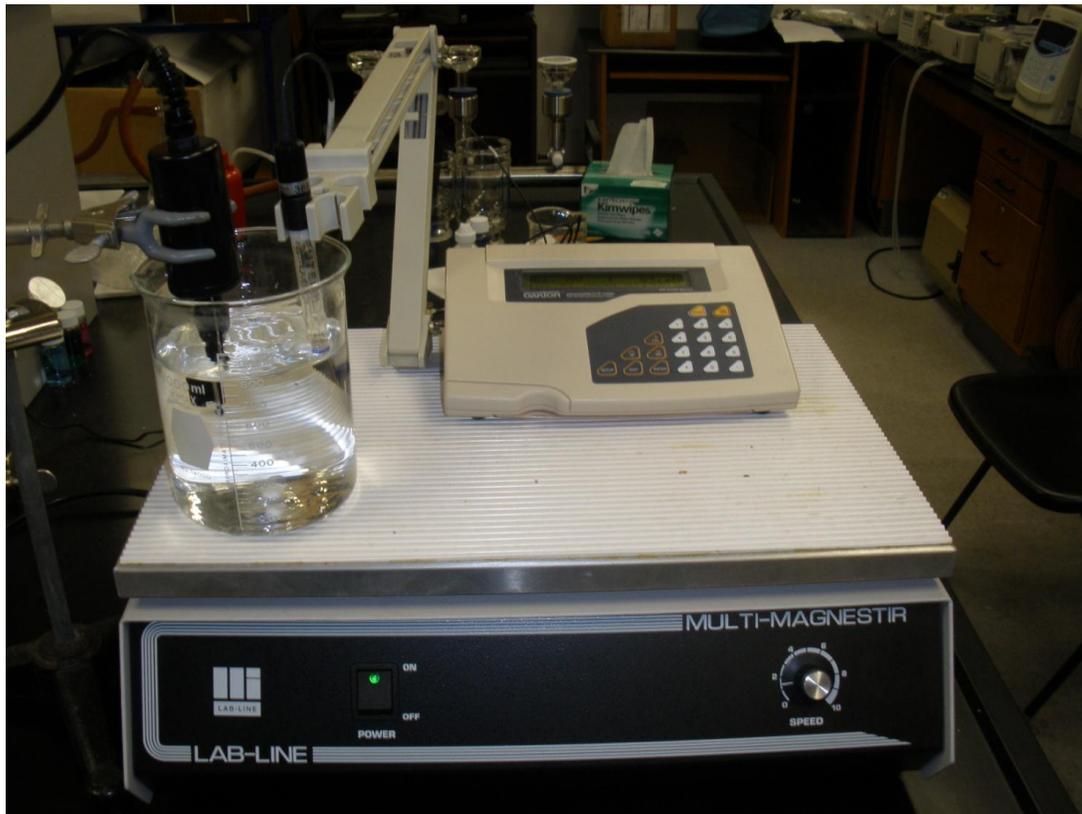
Figure 2. Evaporation Experiment Setup

3.7 BTEX and TCE Biodegradation Experiments

Biodegradation experiments for BTEX and TCE were performed in triplicate with the average values being reported using cultured aerobic microorganisms. The biodegradation experiments were conducted in a closed 500 ml amberglass reactor with a Teflon-lining septum sampling port (Figure 3). Oxygen was supplied from a pressure cylinder at a flow rate of 0.1 mg min^{-1}. Excess oxygen passed through the oxygen pressure regulator to provide an atmospheric seal and to maintain a constant pressure inside the reactor. Carbon dioxide produced was removed from the atmosphere by potassium hydroxide held in a glass tube inside the reactor. Each reactor contained 250 ml mineral salt medium with BTEX or TCE serving as the sole carbon source and was incubated at 30°C. The inoculum was 5 mg/l as COD. The contents of the flask were continuously stirred at 150 RPM throughout the course of the experiments. Samples were withdrawn periodically from the reactor and analyzed for BTEX and TCE concentrations and total protein for biomass determination.

Figure 3. BTEX and TCE Biodegradation Experiment Setup

3.8 Water-Filled Trench with Biofilter

After aeration, the simulated groundwater was transferred to a biofilter (Figure 4, Right Image). The success of water-filled trench with biofilter is dependent upon the proper range of the following parameters: dissolved oxygen concentration, redox potential, pH, and alkalinity. Dissolved oxygen is the most thermodynamically favored electron acceptor used in the biodegradation of fuel hydrocarbons. As a rule, the stoichiometric ratio of dissolved oxygen consumed by microbes to destroyed BTEX compound is 1.0 mg/L of dissolved oxygen consumed to approximately 0.32 mg/L of BTEX compounds destroyed. During aerobic biodegradation, dissolved oxygen levels are reduced as aerobic respiration occurs. Also, anaerobic bacteria (obligate anaerobes) generally cannot function at dissolved oxygen levels greater than about 0.5 mg/L. Therefore, higher values of dissolved oxygen indicate that aerobic biodegradation is likely to dominate. In addition, the high dissolve oxygen is also an indication that effective mass transfer between the air and groundwater is possible, which is the key for the volatilization of volatile organic compounds. The reduction/oxidation potential of groundwater is a measure of electron activity and is an indicator of the relative tendency of a solution to accept or transfer electrons. A solution with a higher redox potential will have a tendency to gain electrons from new species (i.e. oxidize them) and a solution with a lower redox potential will have a tendency to lose electrons to new species (i.e. reduce them). For biologically mediated processes, the redox potential influences rates of biodegradation. Some biological processes can only occur within a prescribed range of redox conditions. Redox potential of groundwater is also the indicator for the possibility of chemical processes such as metal transformation and precipitation. The redox potential of groundwater generally ranges from - 400 mV to 800 mV. The effective redox potential

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for biological and chemical processes can be increased by adjusting the dissolved oxygen concentration and pH value of the groundwater. In addition to its effect on redox potential, the pH of groundwater has an effect on the microbial activity. Microbes capable of degrading petroleum hydrocarbon compounds generally prefer pH values varying from 6 to 8 standard units. The total alkalinity of a groundwater system is indicative of the water's capacity to neutralize acid. Alkalinity is defined as the net concentration of strong base in excess of strong acid with a pure CO_2-water system as the point of reference. Alkalinity results from the presence of hydroxides, carbonates, and bicarbonates of elements such as calcium, magnesium, sodium, potassium, or ammonia. During metal precipitation, alkalinity will be consumed. Certain alkalinity level is required for both biological and chemical decontamination processes.

The filter material used for this research was shredded mulches obtained from Office Depot (Figure 4, Left Image). The mulches used for this research was pine bark mulches. These organic mulches decompose with time, releasing small amounts of nutrients and organic matter to the environment. Bark mulches are one of the most popular mulches around and have a capacity for water conservation since they provide a solid barrier against moisture evaporation. Most bark mulches come in large chips, which decompose slowly. Shredded bark mulches not only trap moisture, but also provide nutrients more easily since they decompose quickly. The filter was an acrylic column (5 inch × 25 inch) and BTEX, TCE and iron removal was investigated when passing through the medium of mulch in this filter (Figure 5). The column was oriented vertically and sealed at the bottom with a custom frit to permit the flow of water and retain the medium. Prior to starting each experiment, approximately 100 pore volumes of de-ionized water was eluted through the column by a peristaltic pump to stabilize the filter. Cultured bacteria were inoculated into the column by introducing 1 L logarithmic state bacterial suspension in minimal salt media (as described before) by the peristaltic pump from the top. The column was then supplied with minimal salt media alone for 12 hrs to stabilize the microbial community. The simulated groundwater was then introduced to the column at a flow rate of 40 ml/min. The elution was collected and quantified for BTEX, TCE and iron, respectively. Throughout the course of the experiments, pH and redox potential was monitored. In addition, microbial activities were traced by ATP assay (22).

Since there is a possibility for BTEX and TCE to be adsorbed to the media, a separate experiment was conducted to test the adsorption of BTEX and TCE on the shredded mulch. The simulated groundwater was introduced to the column filled with the mulch with no bacterial inoculation. The adsorption of BTEX and TCE on mulches observed in the filter was simulated using a math model to quantify the adsorption capacities of these compounds on mulches. As compared to biodegradation, adsorption is a physicochemical process, which should be more efficient. If biological process is taken consideration, the adsorbed organic compounds may be then degraded by the microorganisms inoculated in the media.

Figure 4. Mulch (Left Image) and Biofilter with Mulch as the Filter Media (Right Image)


Figure 5. Biofilter Setting Up

3.9 Iron Reduction Experiments

Laboratory iron reduction experiments were conducted using 100 g collected soil samples reacting with landfill leachate collected from corresponding landfills in the presence of *S. putrefaciens* (5 mL stock solution at a concentration of 5×10^9 cells/mL) (Figure 6). All of the experiments were performed in 250 mL Teflon-sealed serum bottles equipped with CO_2 entrapping devices (Figure 7). The headspace of the serum bottles was pressurized with ultra-pure nitrogen. As a control, sampled soil was reacted with corresponding leachate in the absence of *S. putrefaciens*. Throughout the course of the experiments, chemical oxygen demand (COD) and ferrous iron concentrations were monitored. COD measurements followed the standard method using a Hach DR5000 Spectrophotometer. For ferrous iron quantification, 1,10-Phenanthroline Method was utilized. In the presence of 1,10-phenanthroline ($C_{12}H_8N_2 \cdot H_2O$), ferrous iron formed a stable, orange-colored complex with the reagent:

$$Fe^{2+}(aq) + 3(ph)H^+ = Fe(ph)_3\ ]^{2+} + 3H^+$$
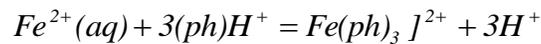
For this experiment, 0.0125 M 1,10-phenanthroline was used and ferrous iron concentrations were quantified using the spectrophotometer (Shimadzu UV-1650 PC) at a wavelength of 520 nm.
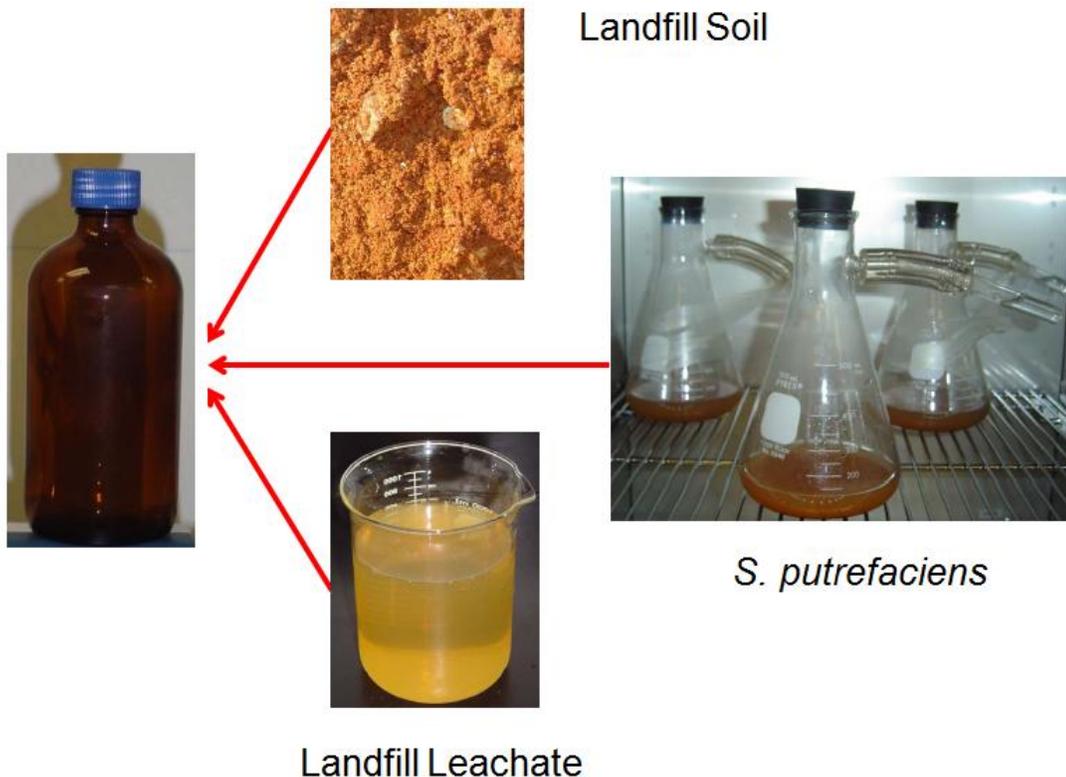
Figure 6. Iron Reducing Process by *S. putrefaciens*

Figure 7. Iron Reducing Experiments

3.10 Iron Adsorption Experiments

Ferrous iron adsorption on *S. putrefaciens* was carried out in autoclaved (121 °C and 1 atm for 20 min) high-density polyethylene centrifuge tubes in an anaerobic chamber in a nitrogen environment. *S. putrefaciens* was first harvested by centrifugation at 6000 *g* for 15 min after growth in the minimal salts media. The cell pellets were then washed and re-suspended in the electrolyte solution to make a bacterial solution. The mass of the bacterial cells was quantified using ATP assay (22). To investigate the kinetic adsorption of ferrous iron on *S. putrefaciens*, a series of centrifuge tubes containing ferrous iron chloride at concentrations of 0.25, 2.0, 3.5, 10.0, 15.0, and 30.0 mg/L as Fe^{2+} and 0.1 g *S. putrefaciens* (including blank controls) (sealed with Teflon-lined screw caps and pressurized with ultra-pure nitrogen) were agitated on a Wrist Action Shaker (Burrel Scientic, Model 75) for up to 24 hrs. The suspensions were then centrifuged at 6000 *g* for 15 min, after which ferrous iron concentrations in the supernatant were measured. The amount of ferrous iron adsorbed on *S. putrefaciens* was obtained using the following equation:

$$q = \frac{(C_i - C_e) \cdot V}{M}$$

where q is the amount of ferrous iron adsorbed onto *S. putrefaciens* (mg/g), C_i and C_e are the initial and equilibrium ferrous iron concentrations in the solution (mg/L), V is the volume of the aqueous phase (L), and M is the mass of *S. putrefaciens* (g).

4. Results

4.1 BTEX and PCE Evaporation

Near all the BTEX and TCE can be removed after around 150 minutes' evaporation. Among these compounds, xylene was the easiest compound to be evaporated; TCE was most difficult compound to be evaporated. Toluene and benzene were in between (Figure 8).
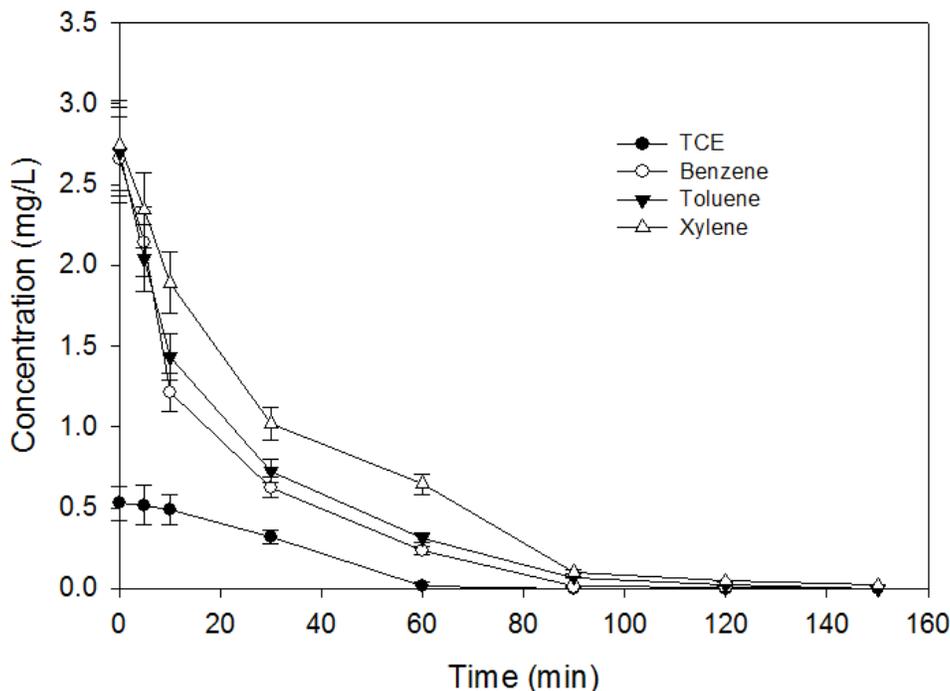

Figure 8. BTEX and TEC Removal by Evaporation

Removal data for BTEX and TCE were further manipulated to reflect percentage removal of these compounds as a function of time (Figure 9). As shown in this figure, above 70% of these compounds can be removed after one hour's evaporation. In fact, even one hour's evaporation is not practical owing to large quantity involved in groundwater treatment. Practically, evaporation should be limited to within 20 minutes. For the following biofiltration experiments, simulated groundwater containing BTEX and TCE was evaporated for 10 minutes before it was introduced to the biofilter. After 10 minutes' evaporation, nearly 55% benzene, 48% toluene, 32% xylene and 8% TCE was removed from the groundwater. Although around half of the BTEX and one tenth of TCE can be removed for this period of time, oxygen was transferred to the groundwater. Thus, the dissolved oxygen in the groundwater increased accordingly. According to our research, dissolved oxygen increased from ~ 2 mg/l to ~ 7 mg/l after 10 minutes' evaporation. The increase of dissolved oxygen in the groundwater is very important for the following steps of BTEX degradation and iron oxidation and precipitation.
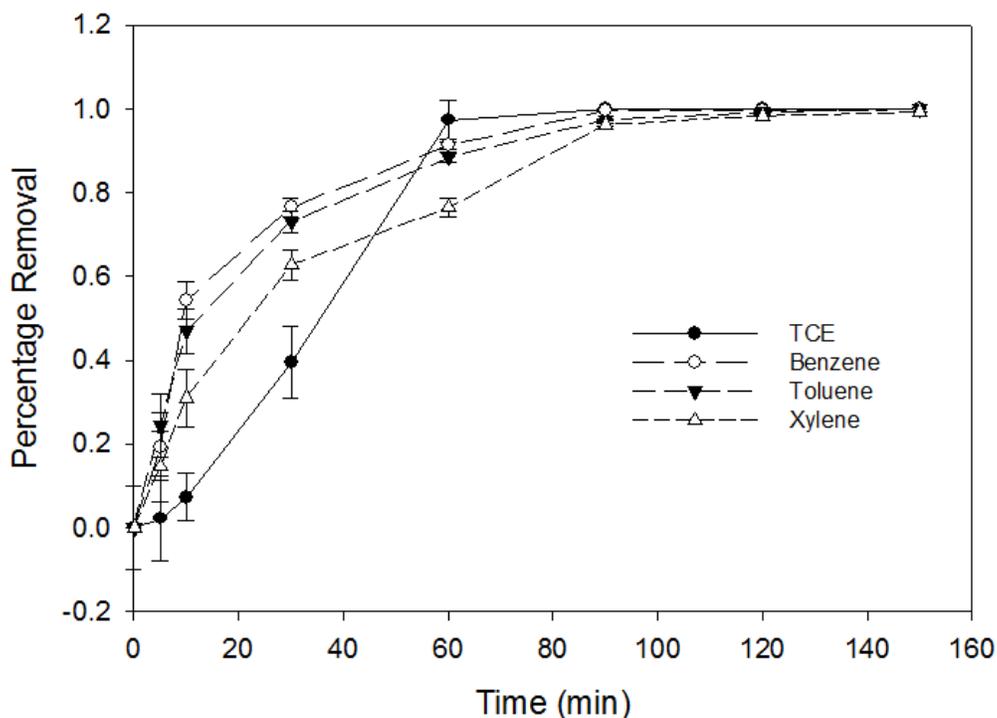
Figure 9. BTEX and TEC Percentage Removal by Evaporation

4.2 BTEX and TCE Biodegradation

Under aerobic conditions, only BTEX was found to be degraded by the cultured aerobic microorganisms (Figure 10). TCE cannot be degraded under aerobic conditions. This is supported by prior research (144). The initial concentrations of the BTEX compounds were chosen based on their solubility. Among the BTEX compounds, toluene was most degraded, i.e., around 61% of toluene was decomposed after 8 days. Following toluene was xylene, i.e., around 50% of xylene was degraded after 8 days. Benzene was least compound to be degraded, i.e., around 45% was decomposed after 8 days. Overall, all the BTEX components were removed by 45 to 60% by aerobic degradation. During the degradation, pH, oxidation/reduction potential (ORP) and dissolved oxygen were monitored. Figure 11 shows the changes of these parameters during benzene degradation. There was a general trend that oxidation/reduction potential (ORP) decreased with the proceeding of BTEX decomposition. There was no obvious change for dissolved oxygen since air was supplied throughout the course of the experiments. There was a slight decrease of pH with the degradation of BTEX compounds. This was because that alkalinity was consumed during BTEX degradation.

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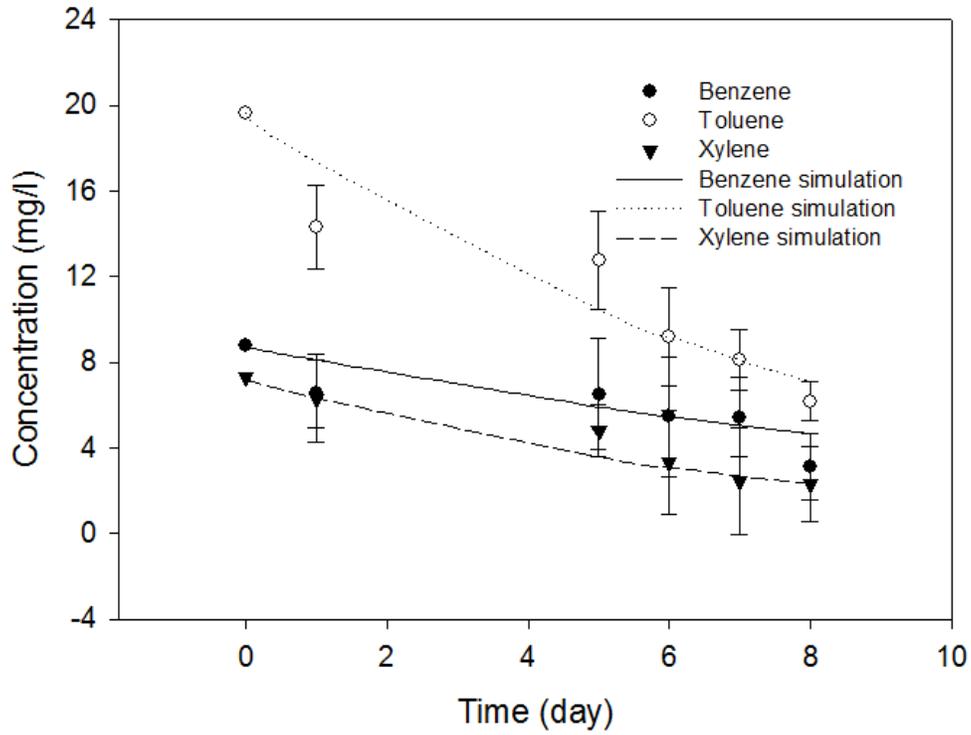
Figure 10. BTEX Degradation Curves
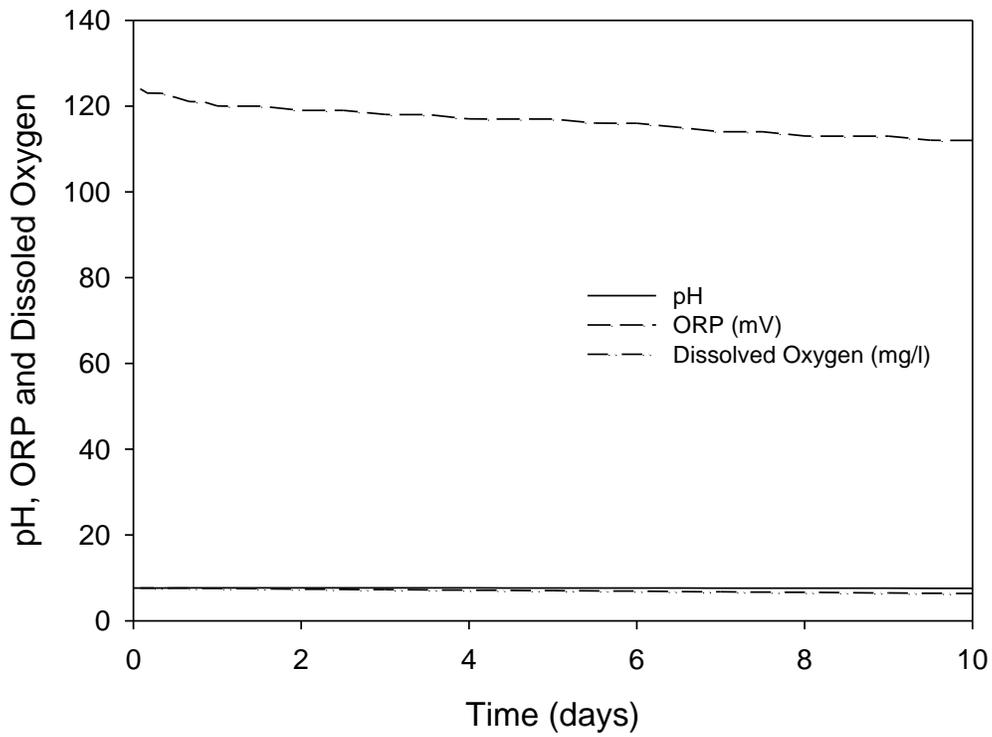
Figure 11. pH, ORP and Dissolved Oxygen during Benzene Degradation

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4.3 Water-Filled Trench with Biofilter

Depending on the input concentration, around 67% to 78% of benzene, 60% to 67% of toluene, 38% to 45% of xylene, and 18% to 20% of TCE was removed from the simulated groundwater (Figure 12). There was a general trend that the removal increased with the increase of input concentration for BTEX. On the other hand, the removal decreased with the increase of TCE input concentration. Since around 10 minutes' evaporation was performed before the groundwater was introduced to the biofilter, there were three possible mechanism that accounted for BTEX and TCE removal, i.e., evaporation, biodegradation and adsorption. Based on the evaporation experiments, around 55% of benzene, 48% of toluene, 32% of xylene and 8% of TCE was removed from the groundwater. For this part of research, the duration of the filtration process was around 2 hours. Based on the biodegradation process, less than 5% of BTEX can be degraded. Therefore, the dominating mechanism during biofiltration was through adsorption.
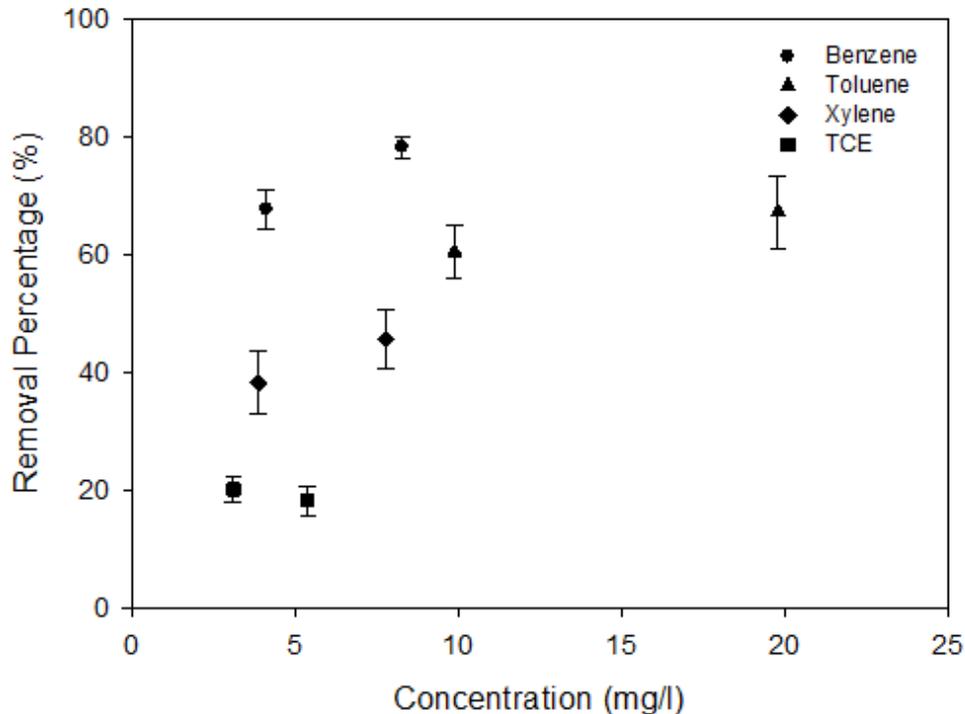
Figure 12. BTEX and TCE Percentage Removal during Water-Filled Trench with Biofilter

To further investigate BTEX and TCE removal through adsorption, the simulated groundwater was transported at the same flow rate through a uninnoculated biofilter without aeration. In the absence of the BTEX degrading microorganisms, the removal of BTEX and TCE was solely owing to adsorption. Around 42% of benzene, 28% of toluene, 12% of xylene and 11% of TCE was removed by adsorption (Figure 13). Based on mass balance, less than 10% of BTEX removal was accounted for biodegradation, which was in the range based on BTEX biodegradation results.
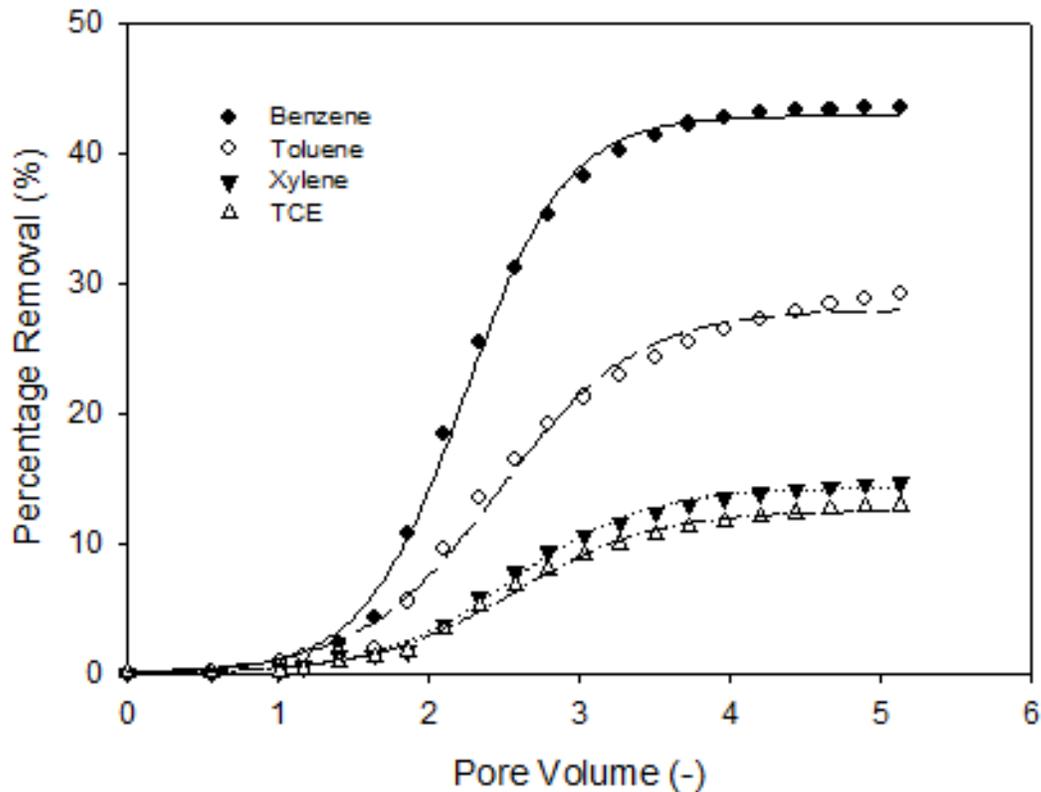
Figure 13. BTEX and TCE Removal by Adsorption

Iron removal is also very important for contaminated groundwater in Florida. Owing to the low oxygen content, iron exists in the form of ferrous iron accordingly. When exposed to the air, ferrous iron will be oxidized to ferric iron accordingly. The oxidation of ferrous iron to ferric iron is pH dependent. As shown in Figure 14, high pH favored ferrous iron transformation. At pH 8, around 85% to 97% of iron existed in the form ferric iron. The oxidation of ferrous iron to ferric iron also depended on the reaction time, which was more pronounced for low pH. At pH 7.04, within the first two minutes, only around 5% of ferrous iron was oxidized. After 10 minutes, around 50% was oxidized. Since ferrous iron was colorless and ferric iron had the orange or red color, the oxidation of ferrous iron can be easily observed based on the color change (Figure 15). Usually, ferric hydroxide ($Fe(OH)_3$) was the direct result of ferrous iron oxidation and precipitation. Microorganisms may also involve in iron oxidation. The microbial mediated rate of iron oxidation may be dominating if enough nutrients are available. The chemolithotrophic prokaryotes of our interest are *Acidithiobacillus ferrooxidans* (or *Thiobacillus ferrooxidans*) and *Leptospirillum ferrooxidans*, which are known to able to oxidize ferrous iron to ferric iron. Owing to the low nutrient contents in our groundwater, microbial mediated iron oxidation was not the dominating mechanism for this research.

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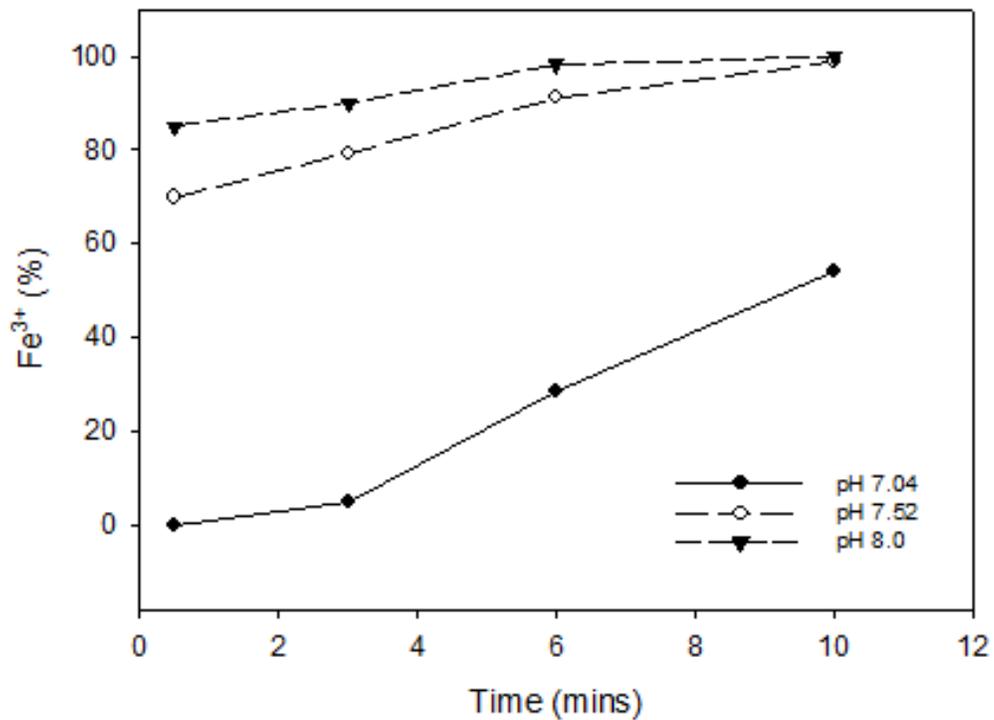
Figure 14. Ferrous Iron Oxidation as a Function of pH and Reaction Time

Figure 15. Ferrous Iron Transformation

During water-filled trench experiments, around 80% of iron was removed when the aerated groundwater passed through the biofilter at pH 8.0 (Figure 16). For pH 7.52 and 7.04, around 60% and 50% was removed. Again, the removal seemed not to be a function of input concentration. But it was a function of pH. The higher the pH, more iron was removed. The dependence of iron removal with pH was related to the iron transformation. At high pH, iron tended to be existing in the form of ferric iron, consequently, ferric iron was formed, which can be easily precipitated and filtered out during the bilfiltration process.
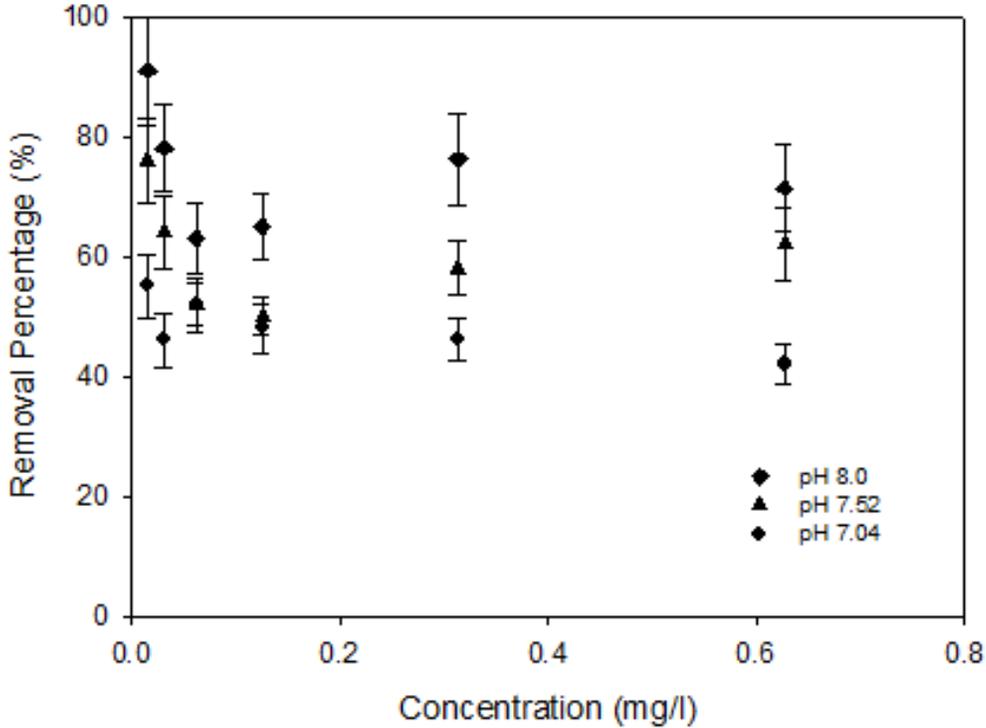
Figure 16. Iron Percentage Removal against pH and Input Concentration

The removal of BTEX, TCE and iron during water-filled trenches processes can be quantified in terms of removal coefficient, K_c:

$$\frac{K_c L}{v} = -Ln(1 - fr)$$

where K_c is the removal coefficient (min^{-1}); L is the length of the column (cm); v is the velocity (cm/min); and fr is the percentage removal (-).

By plotting BTEX, TCE and iron removal coefficient against input concentration, similar trend was observed as reported in terms of percentage removal (Figure 17 and Figure 18). However, the quantification of the removal coefficient eliminated the effect of velocity of the removal of BTEX, TCE and iron during biofiltration process. The removal coefficient was in the range of 0.004 min^{-1} to 0.012 min^{-1} for BTEX, 0.0018 min^{-1} to 0.002 min^{-1} for TCE and 0.05 min^{-1} to 0.02 min^{-1} for iron.

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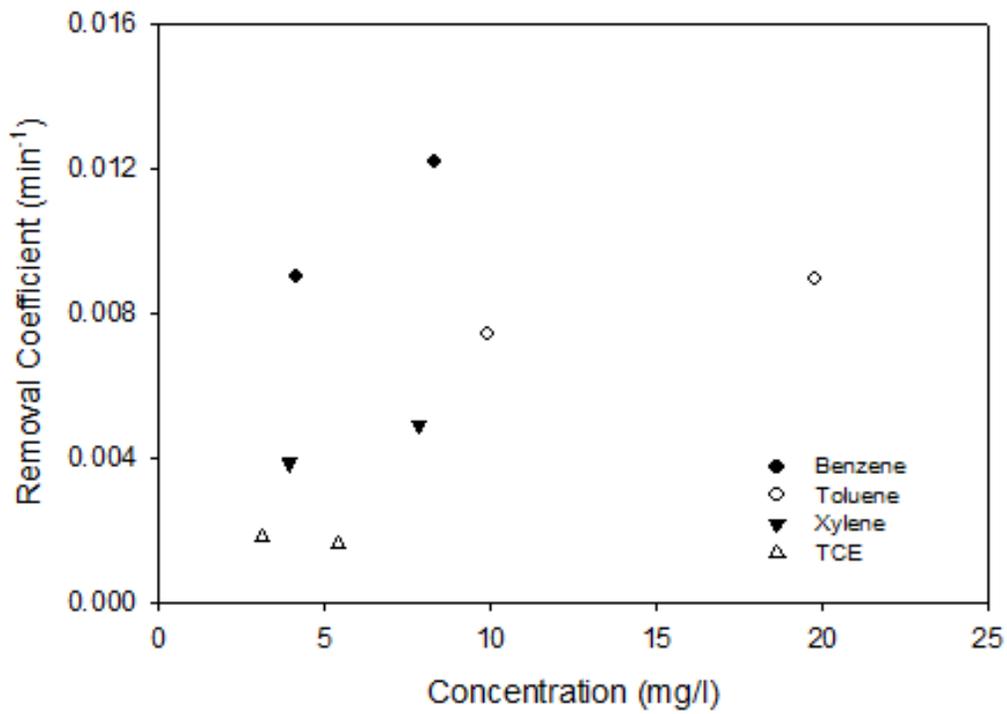
Figure 17. BTEX and TCE Removal Coefficient against Input Concentration
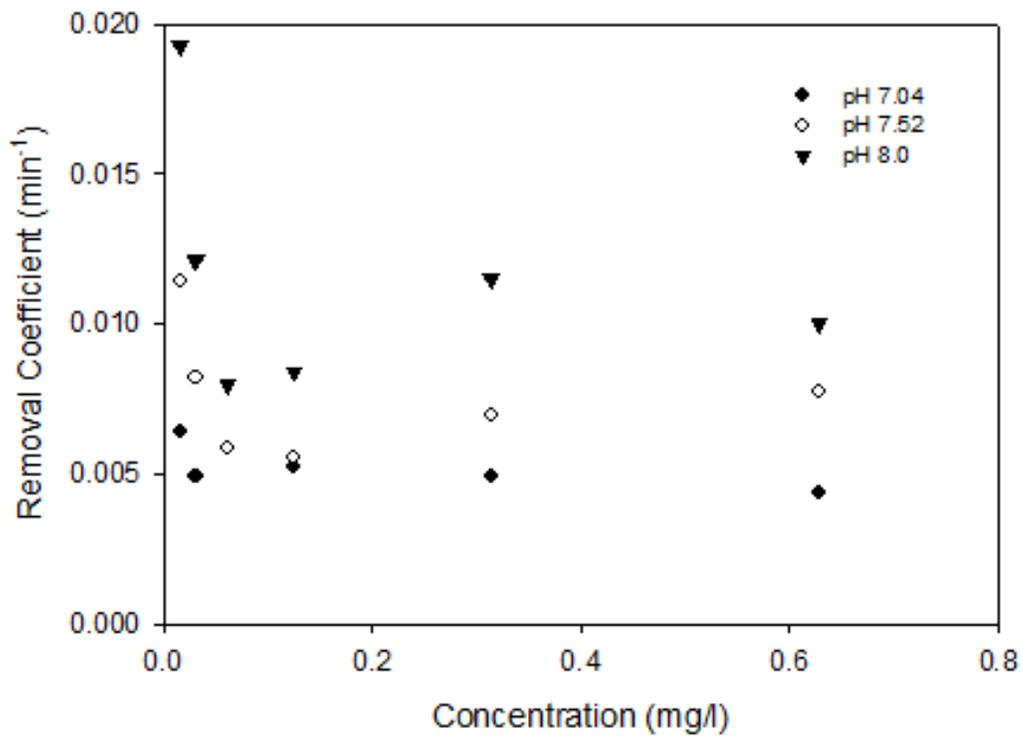
Figure 18. Iron Removal Coefficient against pH and Input Concentration

4.4 Soil Characterization and *S. putrefaciens* Identification

The soil samples were characterized based on sieve analysis and were identified as loamy or fine sand. Based on sieve analysis, all the soil samples exhibited a poor grading, i.e., the soil particles were in general similar in size range. The finest particles were screened out by sieve 200 (~ 75μm). Santa Rosa County samples had the highest percentage fines of 6.01%. Gadsden County samples were determined to have the lowest percentage fines of 0.53%. Franklin County and Okaloosa County samples had medium values of 1.31% and 4.12% respective. The soil iron content for all the samples ranged from 39.4 mg/g (Franklin County) to 119.9 mg/g (Okaloosa County). The average iron content was 77.1 mg/g. It should be noted that only reducible iron contributes to the quantified iron content. There was a general trend that the soil iron content increased with the increase of percentage of finer particles. This is due to the increase in surface area available for iron accumulation.

In the iron rich soil, *S. putrefaciens* played an important role in the cycling of carbon, trace metals, and nutrients. *S. putrefaciens* has the potential to oxidize complex sedimentary organic matter to carbon dioxide with Fe(III) serving as the sole electron acceptor. In northwest Florida, owing to the high iron content in the soil, Fe(III) would serve as the dominating electron acceptor. In this research, *S. putrefaciens* was identified by means of PCR analysis from the culture cultivated from the soil samples. PCR analyses indicated that the excised Fe(III)-reducing band contained a single polypeptide with an apparent molecular mass of 91 kDa. The 91-kDa heme-containing protein identified the strain as *S. putrefaciens*.

4.5 Iron Reduction Experiment

Landfill leachate collected from Okaloosa County had the highest COD value, followed by Santa Rosa County, Gadsden County and Franklin County. With the proceeding of the experiments, COD values decreased for all the landfill leachate investigated (Figure 19). After 20 days' reaction, 42% to 68% of the organic content of landfill leachate was decomposed. With the decomposition of leachate, ferrous iron release was observed, which linearly increased with the decomposition of the leachate (Figure 20). The average ferrous iron production was 2.2 mg per mg COD consumed. Okaloosa County had the most ferrous iron production, followed by Santa Rosa County, Gadsden County and Franklin County.

4.6 Ferrous Iron Adsorption

Metal-reducing bacteria which use solid substrates such as Fe(III) as the terminal electron acceptor for anaerobic respiration must be able to transport the electrons across the outer membrane between large particulate metal oxides (e.g., Fe_2O_3) and the electron transport chain in the cytoplasmic membrane (133). As confirmed by bioelectrochemical method, *S. putrefaciens* had approximately 80% of the membrane-bound cytochromes localized in its outer membrane when grown under anaerobic conditions (11, 14). This cytochrome distribution plays a key role in the ability to mediate Fe(III) reduction during anaerobic respiration (109). With the production of ferrous iron, ferrous iron may accumulate on the outside cell surfaces (95). The accumulation of ferrous iron on *S. putrefaciens* surfaces interferes the electron transport across the outer membrane between large particulate
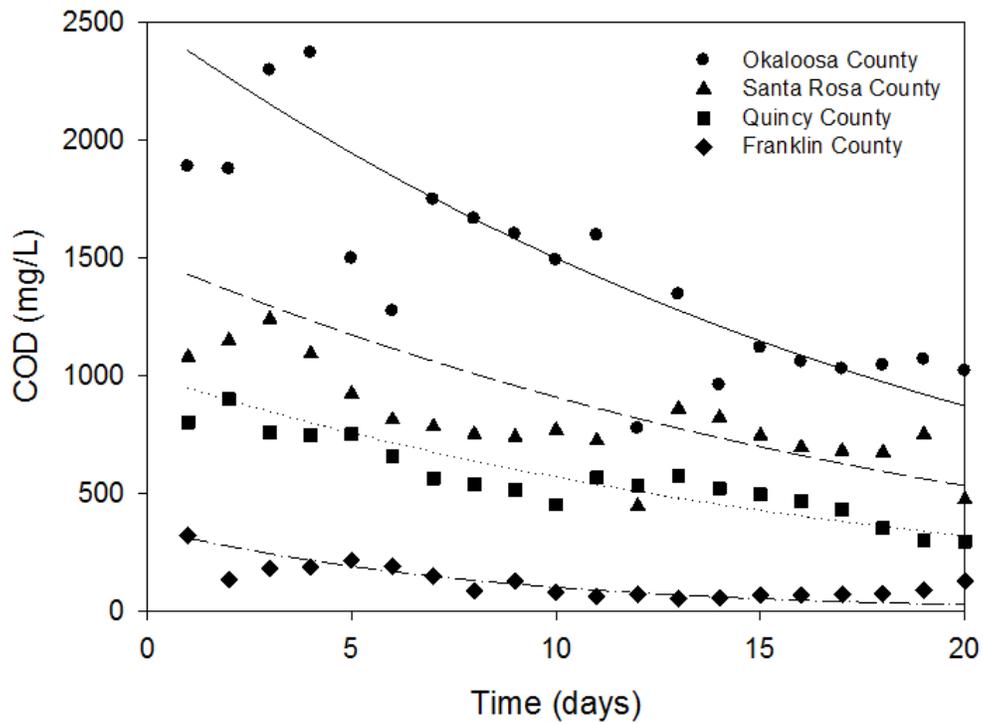
Figure 19. Landfill Leachate Decomposition
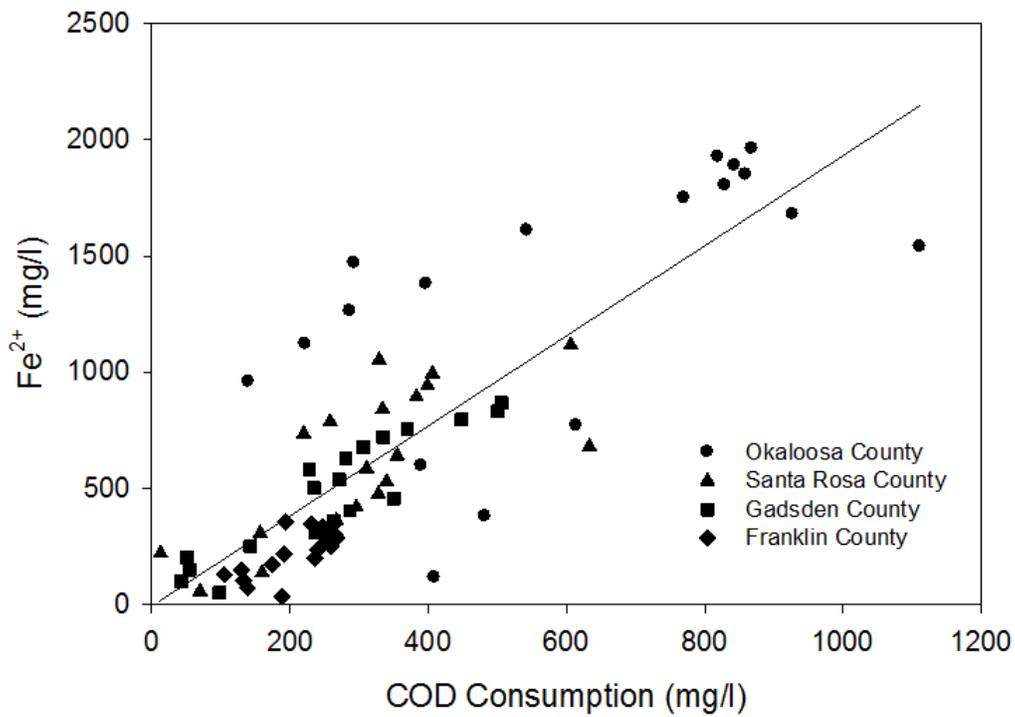
Figure 20. Ferrous Irion Release with Landfill Leachate Decomposition

metal oxides (e.g., Fe_2O_3) and the electron transport chain in the cytoplasmic membrane. This is evidenced that with more ferrous iron adsorbed on the bacterial surface, the specific growth rate decreased accordingly after 10 days when the remaining iron is around 50% of the total iron. Results of ferrous iron kinetic adsorption at concentrations of 0.25, 2.0, 3.5, 10.0, 15.0, and 30.0 mg/L were presented in Figure 21. Ferrous iron adsorption on *S. putrefaciens* increased with the increase of the reaction time and more ferrous irons were adsorbed for higher initial solution ferrous iron values. Based on the kinetic investigation, it was found that ferrous iron adsorption reached the maximum value and becomes stable after 200 minutes. The results demonstrated that equilibrium was reached after three hours' of adsorption reaction for most cases.

S. putrefaciens is one of very few isolated microorganisms that are able to use iron(III) as an electron acceptor. Thus it plays an important role in iron transformation in the environment. Based on the laboratory observation, it seemed that sorption of ferrous iron on *S. putrefaciens* increased linearly with reaction time until around 100 minutes. After 100 minutes, sorption of ferrous iron on *S. putrefaciens* became moderate.
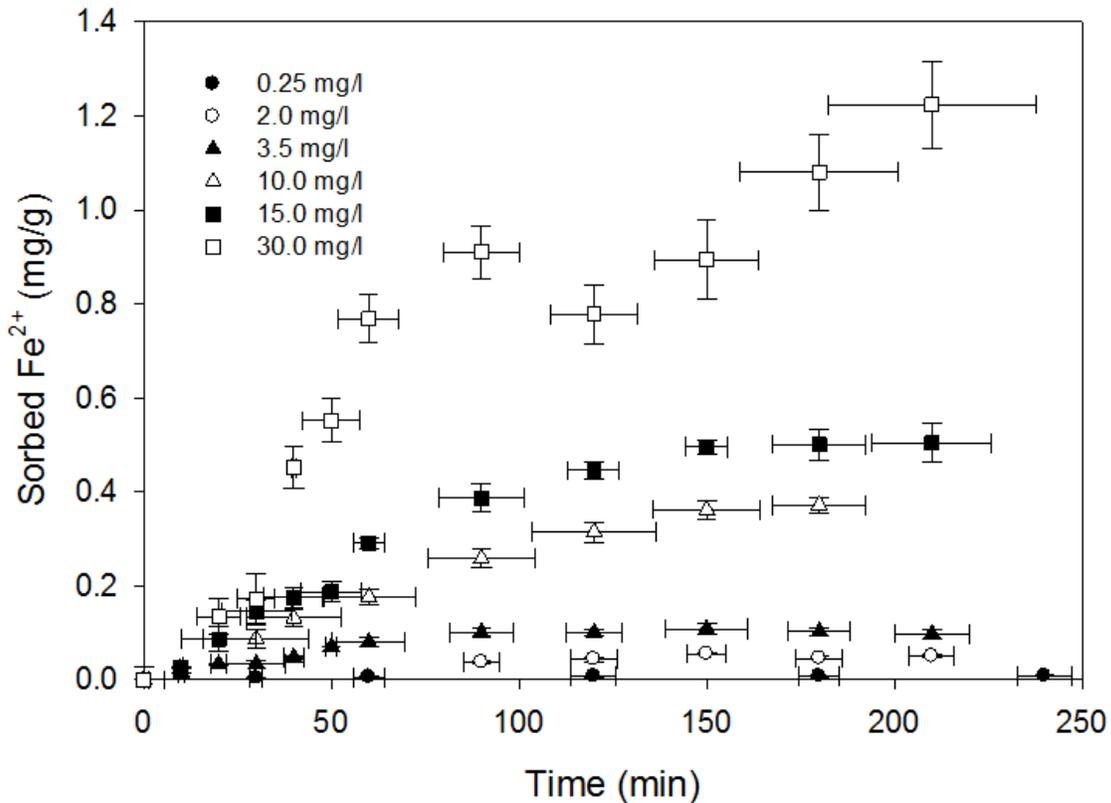
Figure 21. Ferrous Iron Kinetic Adsorption on *S. putrefaciens*

Effect of pH on ferrous iron equilibrium adsorption on *S. putrefaciens* was further investigated. Based on the speciation analysis, ferrous iron does not precipitate in the pH range of 3 to 9 (Figure 22). Therefore, the effect of precipitation on ferrous iron adsorption on *S. putrefaciens* was minimal. For microbial mediated iron reduction, *S. putrefaciens* conserves energy for growth with the structure Fe(III) in the soil as the sole electron acceptor (83, 84):

$$CH_2O + 2Fe_2O_3 + 3H_2O = CO_2 + 4Fe^{2+} + 8OH^-$$
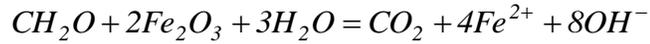
Based on above equation, OH⁻ was produced during iron reduction. Therefore, the reaction was favored with a low pH. Iron reduction under different pH conditions was investigated using the soil samples collected from Okaloosa County, Santa Rose County, Gadsden County and Franklin County (Figure 23-26). From all of these experiments, it was found the lowest pH produced the most ferrous iron. With the increase of pH, ferrous iron production decreased accordingly.

Ferrous iron had linear isotherms on *S. putrefaciens* under the pH range investigated for this research (Figure 26). From isotherm experiments, the average partition coefficient was found to be 0.073 L/g, 0.059 L/g, 0.050 L/g, 0.039 L/g, 0.035 L/g, 0.033 L/G and 0.026 L/g for pH of 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 respectively. These data were obtained based on the assumption that equilibrium of ferrous iron adsorption on *S. putrefaciens* was reached after 24 hours.
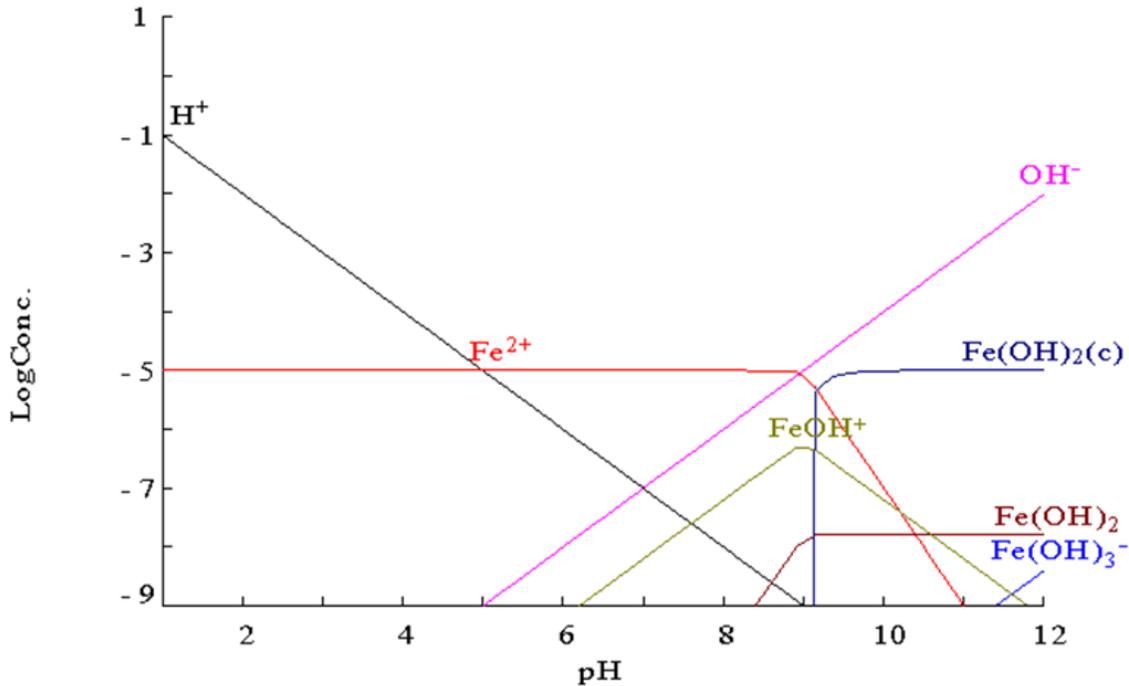
Figure 22. Ferrous Iron Speciation as a Function of pH
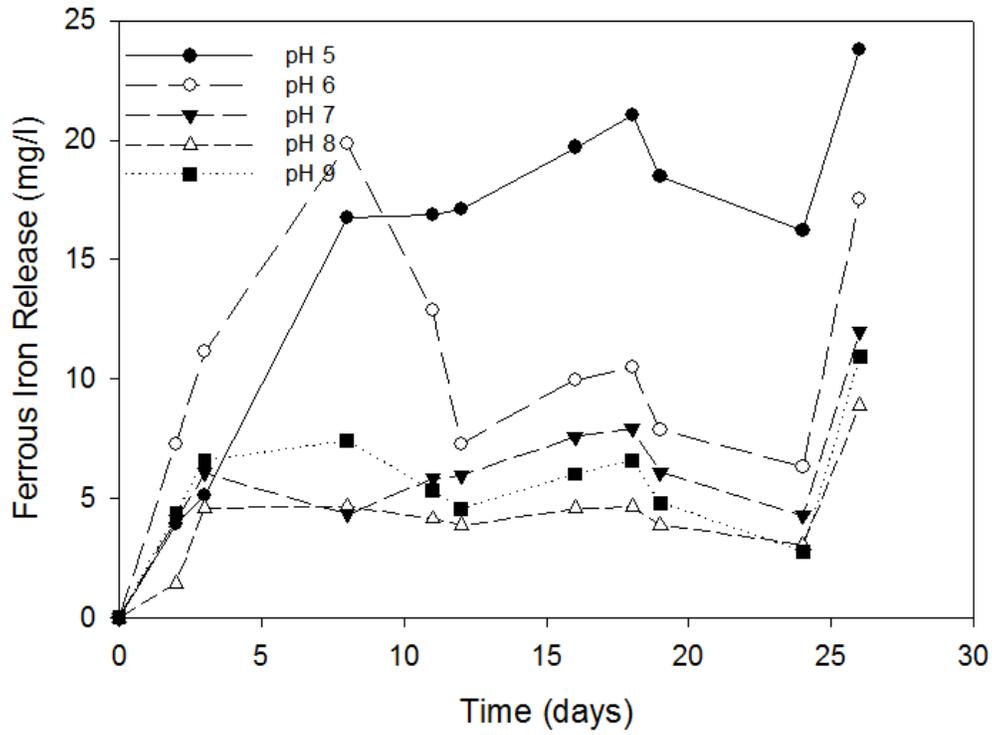
Figure 23. Ferrous Iron Release for Okaloosa County
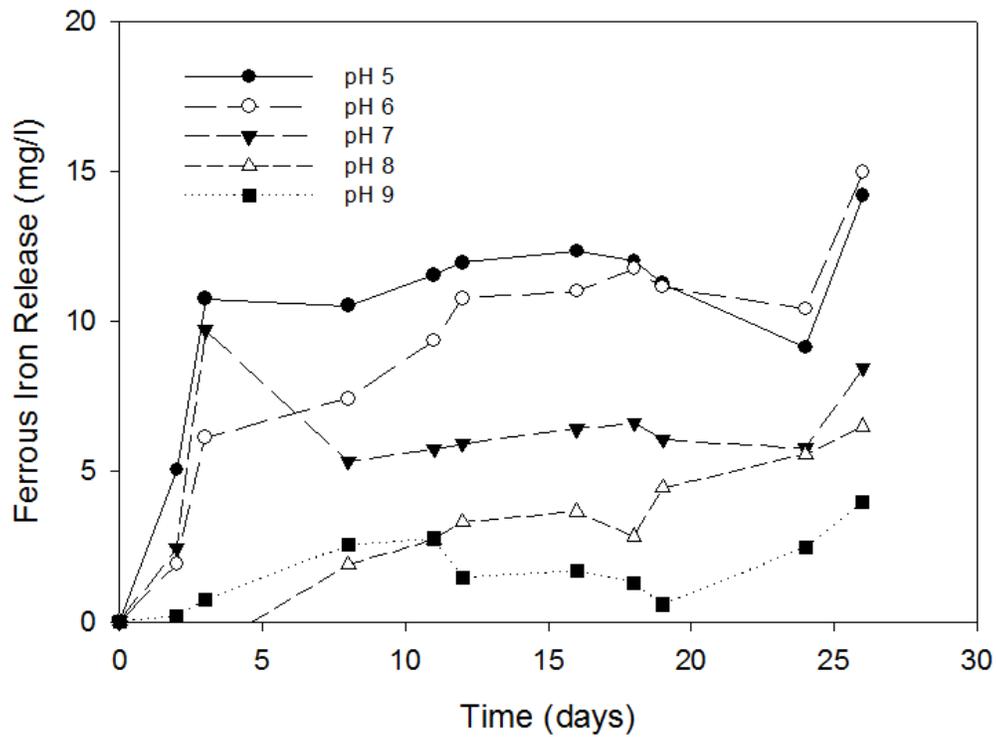
Figure 24. Ferrous Iron Release for Santa Rosa County
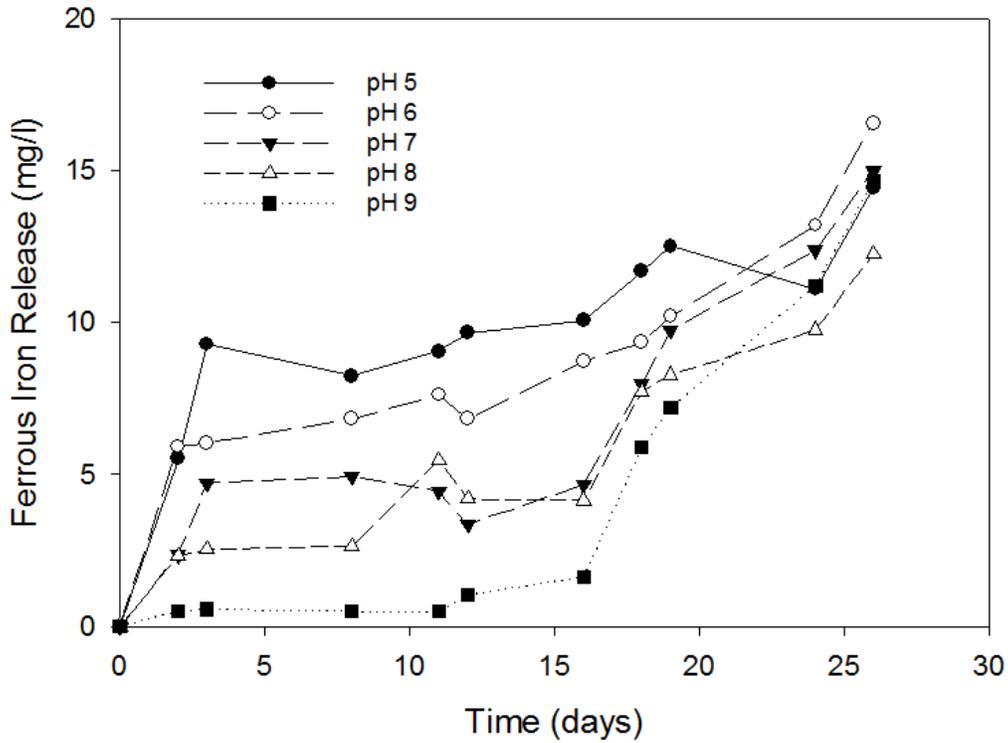
Figure 25. Ferrous Iron Release for Gadsden County
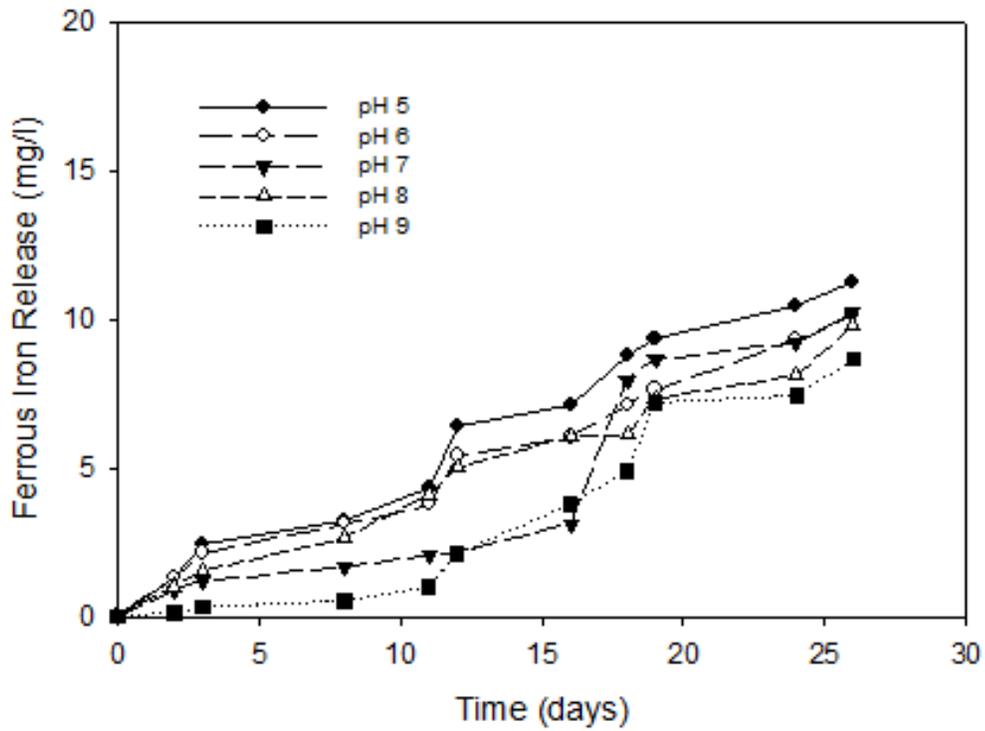
Figure 26. Ferrous Iron Release for Franklin County

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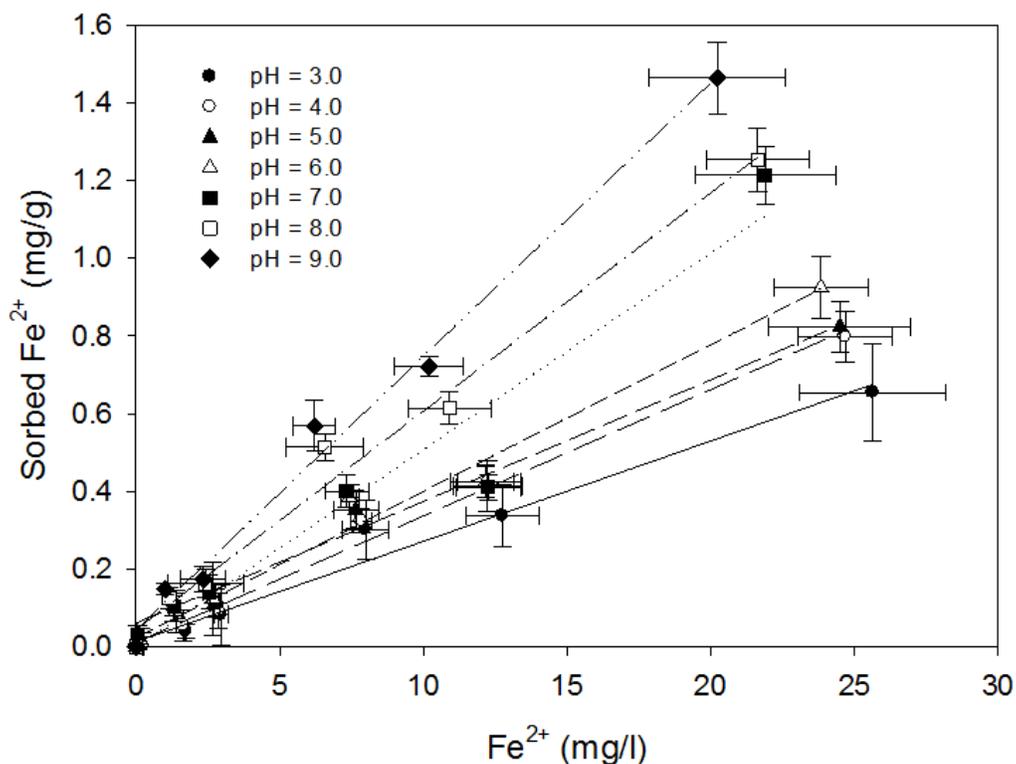
Figure 27. Ferrous Iron Adsorption on *S. putrefaciens* as a Function of pH

The driving force of ferrous iron adsorption on *S. putrefaciens* was the attractive electrostatic interactions. The ζ potential of *S. putrefaciens* was found to be -24.2 $\pm$ 0.4 mV as estimated by means of electrophoretic mobility measurements based on dynamic light scattering (Zetasizer 3000HAS, Malvern Instruments Ltd., Malvern, UK). During the ζ potential measurements, *S. putrefaciens* was suspended in the sterilized electrolyte solution (10^{-5} M NaCl) at a concentration of 5×10^{9} cells/mL. The negative ζ potential indicated that *S. putrefaciens* was negatively charged, which supported the attractive electrostatic interactions with positively charged ferrous iron. The chemical structure of *S. putrefaciens* was further analyzed using infrared spectroscopy to gain insight into the surface properties of *S. putrefaciens*. The surfaces of *S. putrefaciens* were found to be dominated by functional groups of carboxylic acids ($RCOO^{-}$) (1600 cm^{-1}) (32.6%) and carbonyl groups (CH_3CO-) (1320 cm^{-1}) (47.8%), which were responsible for the negative charges.

5. Discussion

5.1 BTEX and TCE Evaporation
Evaporation is a popular remediation tool for the removal of dissolved and free-phase volatile organic compounds from the groundwater. Pollutant removal from the aqueous phase to the gas phase is dependent on various mass transfer mechanisms. In the absence of nonaqueous phase liquids (NAPLs), removal of dissolved phase VOCs from groundwater are affected by advection, dispersion and volatilization. If NAPLs are present, dissolution of the nonaqueous liquids and direct volatilization into the air phase may affect mass removal. From this research, it was demonstrated that evaporation was an effective way for BTEX and TCE removal. As a physical means, evaporation is cost-effective. However, to reach a high removal rate, long reaction time is required, which is not practical. Therefore, evaporation is usually combined with other means to work together for BTEX and TCE removal.

5.2 BTEX Degradation
During batch BTEX biodegradation experiments, microbial growth and substrate depletion were monitored over time. Samples were periodically withdrawn from the reactors and analyzed for biomass and BTEX concentrations. The temperature of the reactors was maintained at $25^{o}C$.

If bacterial growth is coupled with substrate depletion and Monod-type kinetics are assumed to describe bacterial growth, substrate and biomass concentrations over time can be described by following equations (107):

$$\frac{dS}{dt} = -\frac{1}{Y}\frac{\mu_m SX}{K_s + S}$$

$$\frac{dX}{dt} = \frac{\mu_m SX}{K_s + S} - \frac{bX}{K_s + S}$$

where S is the organic substrate concentration, which is usually expressed in terms of COD (mg/L); μ_m is the maximum specific growth rate (hr^{-1}); X is the bacterial concentration (g/L); t is the elapsed time (hr); Y is the growth yield coefficient (g biomass per g substrate); K_s is the half-saturation coefficient (g/L); and b is the bacterial decay coefficient (hr^{-1}). By ignoring the decay rate coefficient, Y can be used to estimate the bacterial production based on organic substrate depletion, such that:

$$Y = -\frac{\Delta X}{\Delta S}$$

$$X = X_0 + Y(S_0 - S)$$

By substituting equation (8) into equation (5), substrate depletion can be expressed as:

$$\frac{dS}{dt} = -\frac{1}{Y}\frac{\mu_m S[X_0 + Y(S_0 - S)]}{K_s + S}$$

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The simulated half-saturation coefficient K_s (mg/l), growth yield coefficient Y (g biomass per g substrate), and maximum specific growth rate μ_m (day^{-1}) are listed in Table 4. Both benzene, toluene, and xylene had similar K_s values, indicating that the culture had similar affinity to these BTEX components. In addition, the Y and μ_m values were also similar. As compared to toluene and xylene, benzene had a slightly greater Y and smaller μ_m. Based on thermodynamic analysis, the calculated standard molar Gibbs energy for benzene, toluene and xylene is 133.6 kJ/mol, 126.7 kJ/mol and 125.5 kJ/mol, respectively. Owing to its greater molar Gibbs energy value, more carbon from benzene can go into cell synthesis. Therefore, benzene was observed to have slightly greater Y and smaller μ_m values.

Table 4. BTEX Degradation Parameters

	K_S	Y	μ_{max}
Benzene	203.6	0.0758	0.00837
Toluene	174.4	0.0673	0.0107
Xylene	169.8	0.0663	0.0110

5.3 BTEX and TCE Adsorption

Benzene, toluene, xylene and TCE breakthrough curves had a broad and diffuse infiltration front (Figure 13). This behavior demonstrated that the retention of BTEX and TCE on pine mulches increased with time. When steady state was reached, around 42% of benzene, 28% of toluene, 12% of xylene and 10% of TCE were recovered from the the mulch column. Because the filter was not inoculated with BTEX and TCE degrading microorganisms and the hydraulic time was small, BTEX and TCE loss due to biodegradation was assumed minimal. Therefore, the unrecovered BTEX and TCE were assumed to be absorbed by the mulch inside the column. Retardation of benzene, toluene, xylene and TCE was manifested by delayed breakthroughs. Just by examining the breakthrough curves, there was no obvious difference in terms of lag among these four compounds. In order to different the difference of the lag, the breakthrough curves were simulated using the one dimensional transport model to account for the advective-dispersive movement and equilibrium reactions between the aqueous phase and the solid medium phase (106):

$$(1 + \frac{\rho_b K_s}{\theta})\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} - k_d C$$

where C is the benzene, toluene, xylene or TCE aqueous concentration (mg/L); K_s is the partition coefficient between the liquid phase and the solid phase (L/kg); ρ_b is the bulk density (kg/L); θ is the porosity (L/L); t is the elapsed time (hr); D is the longitudinal dispersion coefficient (cm^2/hr); v is the pore water velocity (cm/hr); x is the coordinate parallel to the flow (cm); and k_d is the deposition coefficient (hr^{-1}). It was assumed that benzene, toluene, xylene or TCE retention in the mulch filter was owing to their adsorption in the column. The simulation was proceeded by an implicit, finite-difference scheme and the simulation process was optimized by minimizing the sum of squared

differences between observed and fitted concentrations using the nonlinear least-square method. Benzene, toluene, xylene and TCE transport in the mulch filter were well described by above equation. The migration of benzene, toluene, xylene and TCE in the filter was described by the retardation factor, which is defined as the ratio of the solution velocity to the contaminant velocity. The retardation factor for any particular chemical is attributed to all of the interactions between the chemical species and the solid surfaces of the porous media. These interactions tend to retard the migration of the chemical relative to the water. To model or predict contaminant transport and plan remediation activities at a site, retardation factors need to be known. Retardation factor of benzene, toluene, xylene and TCE in the mulch material was 2.58, 2.64, 2.71 and 3.12.

5.4 Iron-Reduction Experiments

Organic substrate depletion by *S. putrefaciens* with the soil structure Fe(III) serving as the electron acceptor was simulated by means of non-linear regression of simplex optimization of least squares against equation. *S. putrefaciens* had similar maximum half saturation coefficient values for all the soil samples and the average half saturation coefficient was 243.8 mg/l (284.9 mg/L for Okaloosa, 216.5 mg/L for Santa Rosa, 234.9 mg/L for Quincy and 238.8 mg/L for Franklin). The similar half saturation coefficient values indicated that *S. putrefaciens* had similar affinity to the organic components. Similarly, specific growth rate and growth yield coefficient values were also in a similar range. The average maximum specific growth rate was 0.008 hr^{-1} (0.0105 hr^{-1} for Okaloosa, 0.0078 hr^{-1} for Santa Rosa, 0.0074 hr^{-1} for Quincy and 0.0068 hr^{-1} for Franklin) and the average yield coefficient was 0.173 mg/g (0.141 mg/mg for Okaloosa, 0.116 mg/mg for Santa Rosa, 0.178 mg/mg for Quincy and 0.252 mg/mg for Franklin). Based on the soil analysis, the iron content was 119.9 mg/g, 83.2 mg/g, 68.8 mg/g and 39.4 mg/g respectively for Okaloosa County soil, Santa Rosa County soil, Gadsden County soil and Franklin soil. Thus, the iron content was not the limiting factor during iron reduction. The variation in maximum specific growth rate among different landfill leachate and soil samples was attributed to variable concentrations of easily degradable organic compounds of the leachate. Okaloosa landfill leachate had the highest COD value and consequently most easily degradable organic compound content, thus *S. putrefaciens* had the greatest maximum specific growth rate among these samples.

5.5 Anaerobic Degradation of BTEX using Iron-Reducing Enrichment Cultures

There is a possibility for BTEX decomposition and iron reduction may occur the same time. In a separate experiment, the potential for BTEX degradation was investigated in the presence of cultured iron reducing bacteria. Benzene, toluene and xylene were found to be able to be degraded under iron-reducing conditions. This work provided the evidence of BTEX degradation by dissimilatory iron-reducing bacteria. The iron reducing bacteria adapted to benzene, toluene, and xylene without lag phase, suggesting that the same group of bacteria could be involved in the removal of more than one BTEX compound. Due to the fact that water-filled trench is operated under aerobic conditions, anaerobic degradation of BTEX may not be the dominating mechanism. Thus, this part of work was not the focus of this research, but it is an important process in the subsurface of Northwest Florida.

Anaerobic degradation of aromatic compounds plays a major role in gasoline contaminated groundwater remediation and increased knowledge on the anaerobic oxidation of aromatic compounds has been acquired recently (40, 41, 116, 136). Besides the reduction of soluble electron acceptors such as oxygen, nitrate, and sulfate, iron(III) reduction is thought to play a major role for the reduction of aromatic hydrocarbons at contaminated sites, especially in the iron rich Northwest Florida. Biodegradation of benzene, toluene, and xylene was achieved in this research used the microorganisms cultured with the collected soil nearby landfills as the innocula and BEX as the carbon source and ferric iron as the electron acceptor. Specially, the enrichment cultures were grown in a carbonate-buffered mineral medium with a pH between 7.2 and 7.4. The medium was anoxically transferred to 100-ml serum bottles, after which the bottles were purged with an 80/20 (vol/vol) mixture of N_2-CO_2 gas and sealed with butyl rubber stoppers. 20 g landfill soil samples were added as the bulk electron acceptor to each bottle. One of the different BTEX compounds, i.e., benzene, toluene or xylene, was injected through the stopper with a gastight syringe as the sole carbon and energy source at a concentration of 100 mM. The bottles were equilibrated for 1 week before incubation. All enrichments were inoculated with 5 ml of culture. The bottles were sealed with rubber stoppers and iron(II) production and BTEX degradation were measured over time. Sterile control cultures were set up for each BTEX substrate without addition of enrichment cultures. Of special interest were the experiments with benzene-degrading enrichment cultures because benzene is known to be the most stable BTEX compound with respect to anaerobic degradation. The degradation of benzene, toluene and xylene started immediately without any significant lag phase.

6. Conclusions

From this research, it was demonstrated that water-filled trench is an effective means for groundwater remediation. Evaporation, adsorption and biodegradation are the three mechanisms involved in the water-filled trench processes. Among these three mechanisms, evaporation and adsorption dominate over biodegradation owing to the short reaction time. Since both evaporation and adsorption belong to physicochemical processes, water-filled trench thus mainly rely on physic-chemical treatment to remove the contamination. Owing to the physicochemical processes, water-filled trench is a simple, cheap and efficient treatment method. It avoids the risk of unassisted natural attenuation by controlling plume migration and reducing the risk of off-site liability.

It was also demonstrated in this research that BTEX could be aerobically decomposed by microorganisms. However, under aerobic conditions, TCE cannot be decomposed. The biodegradation for BTEX took around several days before an effective result could be observed. One of the possibilities for the biodegradation to be involved in the water-filled trench processes was that BTEX was first adsorbed on the filter media, and then they were decomposed by the microorganisms inoculated in the media.

Groundwater contaminated with iron is very common in Northwest Florida owing to the high iron content in the soil. For the removal of iron from groundwater, evaporation followed by a mulch filter was an effective way. pH played an vey important role in this process. A high pH resulted in high removal efficiency.

Under anaerobic conditions, *S. putrefaciens* reduced iron oxide in the iron rich soil of Northwest Florida with landfill leachate serving as the carbon source. *S. putrefaciens* had similar maximum specific growth rate and half saturation coefficients for all the leachate and soil samples investigated in this research. The average maximum specific growth rate was $0.008 \ hr^{-1}$ and the average half saturation coefficient was 243.8 mg/l. Averagely, 2.2 mg ferrous iron was generated per mg COD consumed. In addition, adsorption of reduced ferrous iron on *S. putrefaciens* was further characterized. Ferrous iron adsorption on *S. putrefaciens* was a kinetic process, which increased with the increase of the reaction time. Equilibrium ferrous iron adsorption on *S. putrefaciens* can be reached after three hours. Ferrous iron had linear adsorption isotherms on *S. putrefaciens* for the pH range of 3 to 9. This research results provided guidelines for landfill leachate management in terms of iron release.

Benzene, toluene and xylene were also found to be able to be degraded under iron-reducing conditions. This work provided the evidence of BTEX degradation by dissimilatory iron-reducing bacteria. Due to the fact that water-filled trench is operated under aerobic conditions, anaerobic degradation of BTEX may not be the dominating mechanism. However, in the iron rich Northwest Florida, owing to the high iron content in the soil, it is important to understand this process. The degradation of benzene, toluene and xylene started immediately without any significant lag phase.

7. Recommendation

Water-filled trench is an effective means for groundwater remediation. From this research, it is demonstrated that evaporation and adsorption dominate over biodegradation owing to the short reaction time. Thus water-filled trench mainly relies on physic-chemical treatment to remove the contamination.

Although above 70% of groundwater contaminants investigated in this research can be removed after one hour's evaporation, one hour's evaporation is not practical owing to large quantity involved in groundwater treatment. We recommend groundwater containing BTEX and TCE be evaporated for 10 minutes before it is introduced to the biofilter. After 10 minutes' evaporation, nearly 55% benzene, 48% toluene, 32% xylene and 8% TCE can be removed from the groundwater. Around 42% of benzene, 28% of toluene, 12% of xylene and 11% of TCE is then to be removed by adsorption if a following-up biofilter is used. Combined together, 73.9% of benzene, 62.5% of toluene, 40.1% of xylene and 18.1% of TCE will be removed. At the same time, around 80% of iron can be removed when the aerated groundwater passes through the biofilter at pH 8.0.

8. Future Work

The oxidation kinetics of ferrous iron are well known to be pH dependent, with the slow oxidation kinetics of ferrous iron at low pH being part of the early basis for considering the importance of microbial iron oxidation when groundwater is exposed to the air. There are many known iron-oxidizing microorganisms in environments where the abiotic oxidation of iron is fast enough that the microbes must effectively compete with the abiotic process. Additionally, microbes must compete with each other for the available ferrous iron as substrate, with organisms able to utilize the iron faster in a particular environment making up the predominant part of a community including iron oxidizers. The elimination of the ferrous iron, by physical-chemical way, is obtained by raising the water redox potential by oxidation thanks to oxygen of the air and simple evaporation. Thus, the ferrous iron is oxidized to ferric iron, which precipitates as iron hydroxide, $Fe(OH)_3$. The precipitate is then separated from water by filtration on sand or decantation.

Ferric hydroxide ($Fe(OH)_3$) is the direct result of ferrous iron oxidation and precipitation. With time, ferric hydroxide is mineralized. The principal forms of mineralized ferric iron are:

- amorphous hydrous ferric oxide ($Fe_2O_3 \cdot XH_2O$),
- maghemite (gamma-Fe_2O_3),
- lepidocrocite (gamma-FeOOH),
- hematite (alpha-Fe2O3), and
- goethite (alpha-FeOOH).

The above iron oxides are listed in order of decreasing solubility, which also reflects increasing crystallinity. Amorphous hydrous ferric oxide at neutral pH and oxidizing oxidation/reduction potential conditions has a solubility of 0.6 μg/L, which is three orders of magnitude greater than that of goethite. However, a high concentration of iron in groundwater is not rare. Obviously, iron oxidation would not stop at that stage.

Iron readily undergoes reduction or oxidation, depending upon surrounding conditions. As a consequence there are many microbiological metabolic pathways that utilize redox couples between ferrous and ferric iron. Organic material can be biodegraded with ferric iron as the terminal electron acceptor, resulting in the production of reduced soluble ferrous iron. Anaerobic conditions are required as is the presence of ferric iron in a suitable form. As the crystallinity of the ferric iron mineral increases (as discussed above) the microbiological availability decreases. Or contrarily, iron fixing bacteria can oxidize ferrous iron to ferric iron, even under oxygen poor conditions, by many methods including: the extraction of carbon dioxide (for microbial energy systems) from ferrous bicarbonate leaving insoluble ferric hydroxide; and the utilization of iron bearing organic acid complexes as a carbon source, leaving precipitated ferric hydroxide. These are the reactions that are typically responsible for iron fouling of well screens, piping systems and air strippers that are used to remediate iron rich groundwater.

Our future research will be focused on the identification of the forms of mineralized ferric iron after ferrous iron oxidation, namely, amorphous hydrous ferric oxide ($Fe_2O_3 \cdot XH_2O$), maghemite (gamma-Fe_2O_3), lepidocrocite (gamma-FeOOH), hematite (alpha-Fe2O3), and goethite (alpha-FeOOH). We understand that some of these iron species may work as a catalyst for the continuation of ferrous iron oxidation. We will use Visual MINTEQ 2.51 to quantify the iron speciation in the water. The MINTEQ 2.51 computer code is an equilibrium chemical reaction model. In addition to support evaluations of speciation, MINTEQ 2.51 computer code can be used to calculate aqueous complexation to determine the ionic state and composition of the dominant species for the system. We will also use MINTEQ 2.51 to predict technically defensible maximum concentration limits for iron as a function of pH and index cations and realistic bounding values for the maximum concentration attainable in the system. In addition, we will use this code to analyze initial and final geochemical conditions to determine if the measurements have been affected by processes such as metal precipitation which might have compromised the derived values. Figure 27 shows the speciation of iron in an anaerobic non-carbonate system.
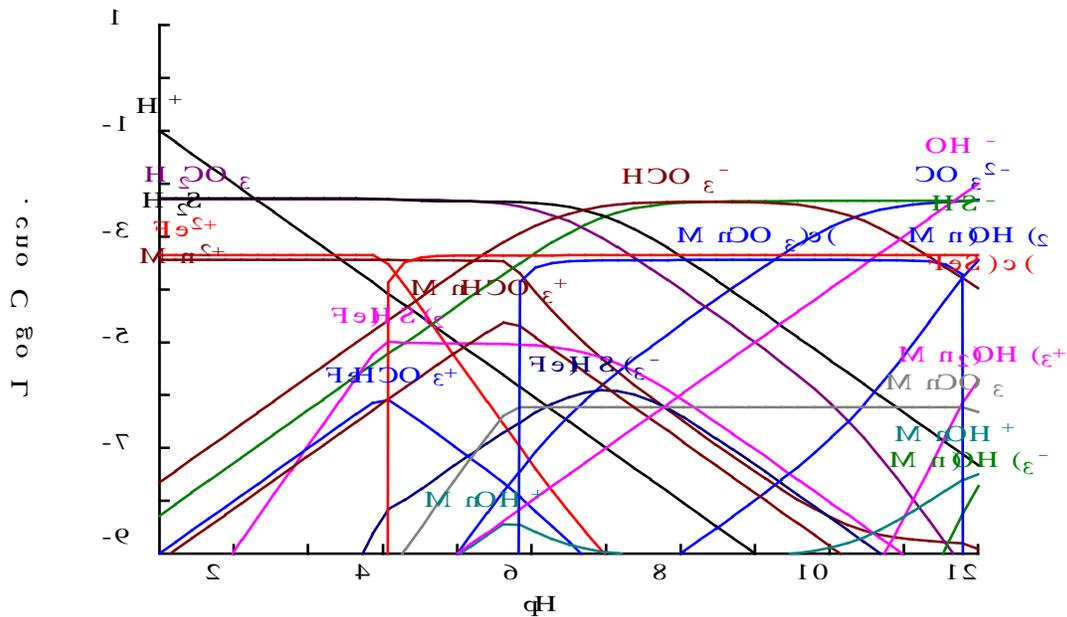
Figure 28. Iron Speciation in an Anaerobic Non-Carbonate System

There has also been a major impetus to investigate the ability of microorganisms to biodegrade hydrocarbons in the absence of oxygen. This is motivated by the fact that groundwater usually has low oxygen content. Studies of microbial degradation of monoaromatic hydrocarbons have resulted in the identification and isolation of a number of different anaerobic bacterial strains capable of degrading one or more monoaromatic hydrocarbons. Of these compounds, the anaerobic biodegradation of toluene is probably

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the most comprehensively understood. Toluene is biodegradable with nitrate, Mn(IV), Fe(III), humic substances, sulfate, and CO_2 as terminal electron acceptors. More recently, it has been demonstrated that toluene can also be assimilated anaerobically as a carbon source by anoxygenic phototrophs. We plan to continue our research with respect to BTEX anaerobic biodegradation.

8. Acknowledgements

Peer-reviewed journal paper publication:
1. Subramaniam, P. K., Martin, L., Grasel, P. and Chen, G., Iron Reduction and Adsorption on *Shewanella putrefaciens* nearby Landfills in Northwest Florida, Environmental Technology, under review.

Conference presentation:
1. Sandip, P., Subramaniam, P. K. and Chen, G., Iron Reduction and Adsorption on *Shewanella putrefaciens* nearby Landfills in Northwest Florida, Ninety-Fifth Annual ASM Southeastern Branch Conference, November 6-7, 2009, Savannah, Georgia.

9. References

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