CHAPTER II

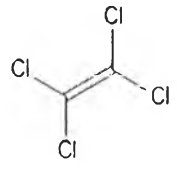
BACKGROUND AND LITERATURE REVIEWS

PCE does not occur naturally in the environment. It has been widely used in various industrial processes such as a dry cleaning in the textile industry, the scouring of the machines, and in fat extraction (Holliger, 1995; Sponza, 2003). There were suspicions of PCE risks as carcinogens (Hara et al., 2004). At many PCE spill sites, residual amount of PCE persist in pure liquid phase (commonly referred to as dense non-aqueous-phase liquids (DNAPLs)) within pore spaces or fractures. The slow dissolution of residual PCE results in a contaminated plume of soil and groundwater. Given that it is often not possible to locate and remove the residual PCE, remediation must focus on preventing further migration of dissolved contamination. This plume control must be maintained for a long period of time. Therefore, more economic and less expensive approaches are desirable for soil and groundwater remediation to provide for long-term control of contaminated soil and groundwater.

Current evidence suggests that PCE is recalcitrant to aerobic degradation, and can only be removed though reductive dechlorination process. Under sequential reductive dechlorinating conditions, PCE can be microbiologically transformed to less chlorinated compounds (trichloroethene; TCE, dichloroethene isomers; DCEs and vinyl chloride; VC). A chloride atom is replaced by a hydrogen atom at each step during reductive dechlorination. In general, the reaction rates for each sequence decrease as chloride is removed. Thus, reductive dechlorination is relatively facile for

PCE, but relatively slow for VC. In fact, the rate limiting step in the reductive dechlorination pathway appears to be the conversion of VC to ethene; ETH (Bradley and Chapelle, 1996). Meanwhile the daughter compounds of PCE (DCEs and VC) are generally degraded faster under anaerobic condition; they would be degraded in the aerobic zone at the own gradient edge of the plume. A number of organic compounds, including acetate, methanol, glucose, benzoate, phenol, methylamines, and alkyl benzenes, have been used as electron donors and carbon sources for reductive dechlorination under methanogenic conditions. Thus, in situ reductive dechlorination is a feasible technology to clean up PCE contaminated sites if organic substrates can be provided to the anaerobic zones continuously.

2.1 Properties of Tetrachloroethene

Tetrachloroethene (PCE)	
	
General characteristics	
Systematic name	1,1,2,2-Tetrachloroethene
Other names	Perchloroethylene, tetrachloroethylene, PCE
Molecular formular	C_2Cl_4
Molar mass	165.8 g/mol
appearance	Clear, colorless liquid
Properties	
Density and phase	1.622g/cm^3, liquid
Solubility in water	0.015g/100ml (20°C)
Melting point	-19°C (254 K)
Boiling point	121.1°C (394 K)
Viscosity	0.89 cP at 25°C

(Source: http://en.wikipedia.org/wiki/Tetrachloroethylene)

2.2 Remediation

Remediation is the improvement of a contaminated site to prevent, minimize or mitigate damage to human health or the environment. Remediation involves the development and application of a planned approach that removes, destroys, contains or otherwise reduces the availability of contaminants to receptors of concern. (Source: http://www.mostchoice.com/general-liability)

Chlorinated solvents are hazardous compounds that are common contaminant of soil, sediments and groundwater aquifer. The most prevalent chlorinated solvents are the chloroethenes, which frequently occur as dense non-aqueous phase liquids in groundwater. Some commonly found chloroethenes are tetrachloroethene, trichloroethene, dichloroethene and vinyl chloride. Because of their known adverse health effects, treatment is required when human exposure is possible. Potential treatments for in situ ground water remediation in current used include: pump-and-treat, solvent flushing/co solvent extraction, air sparging and bioremediation. Due to strong partitioning to the solid phase, the pump-and-treat method is very slow for hydrophobic compounds and can be hindered by DNAPL formation. Air sparging can be effective for VOCs but typically has high installation costs. The co-solvent extraction method has been shown to be effective but ultimately leaves a residue of the extraction solvent and contaminants that may require further treatment (Jayaraj et al., 2003).

The remediation mechanisms of chlorinated compounds can be classified as destructive and non-destructive processes. Destructive processes include biodegradation and hydrolysis. Non-destructive attenuation mechanisms include sorption, dispersion, dilution and volatilization. Dilution and dispersion are generally the most important non-destructive mechanisms, while biodegradation is the most prevalent destructive mechanism.

2.3 In Situ Bioremediation

Bioremediation is defined by the American Academy of Microbiology (AAM) as "the use of living organisms to reduce or eliminate environmental hazard resulting from accumulations of toxic chemicals and other hazardous wastes" (Gibson and Sayler, 1992). At the present time, bioremediation is often the preferred method for remediation of petroleum hydrocarbons because it is cost effective, and it converts the petroleum hydrocarbons into harmless by-products such as carbon dioxide and water.

The advantages of in situ bioremediation compared to or in combination with other remediation technologies include, but are not limited to, the following.
(Source: http://www.er.doe.gov)

1. In situ bioremediation can be used to completely degrade and detoxify some organic contaminants, thereby permanently removing liability for the contaminants

2. For some types of contaminants, physical and chemical methods of remediation may not completely remove the contaminants, leaving residual concentrations that are above regulatory guidelines. Bioremediation can be used as a cost-effective secondary treatment scheme to decrease the concentration of contaminants to acceptable levels. In other cases, bioremediation can be the primary treatment method, and followed by physical or chemical methods for final site closure.

3. In highly heterogeneous geologic environments, physical or chemical methods that rely on advective transport of remediation agents to the contaminants may be ineffective. However, in such environments, in situ remediation schemes that rely on diffusive transport of remediation agents (e.g. nutrients) to indigenous microorganisms for degrading or transforming the contaminants may be more effective.

4. For complex mixtures of contaminants requiring a combination or sequence of physical and chemical methods, bioremediation techniques that use microbial consortia to concurrently address all contaminants may be faster and more cost-effective

2.4 Aerobic and Anaerobic Biological Processes

Both aerobic and anaerobic biological processes have been investigated for in-situ remediation of chlorinated solvents. PCE is the only chlorinated ethene that resists aerobic biodegradation. Trichloroethene, dichloroethene, and vinyl chloride can be co-oxidized by many organisms. Anaerobic systems have demonstrated several potential advantages for PCE and TCE destruction. First, some anaerobic microbial systems have the ability to destroy both PCE and TCE to non-hazardous end-products such as ethylene as shown in Figure 2.1 while aerobic metabolisms cannot destroy PCE as shown in Figure 2.2. Second, the nutrients required for anaerobic system are extremely water-soluble, whereas the destruction capacity of aerobic systems is limited by the water solubility of oxygen. Finally, laboratory tests have demonstrated that anaerobic systems have the potential to destroy orders of magnitude more contaminant than aerobic systems for the same amount of added electron donor.

Anaerobic

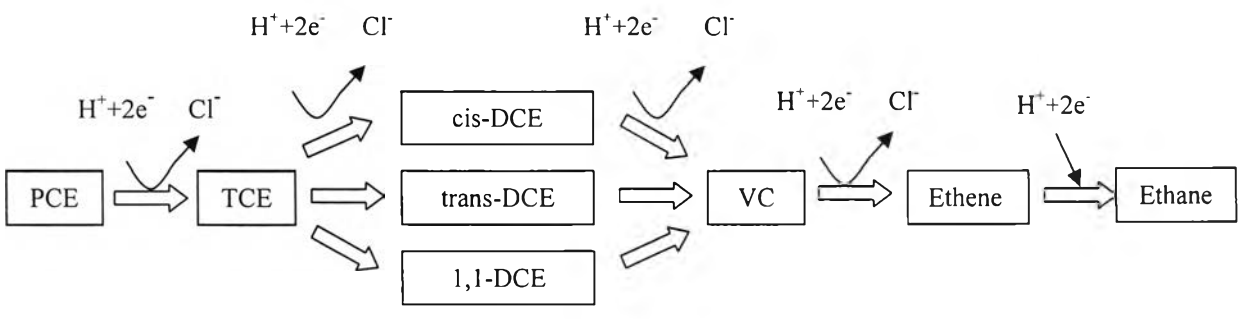


Figure 2.1 Anaerobic biological transformation of tetrachloroethene (PCE). (DiStefano et al., 1991)

Aerobic

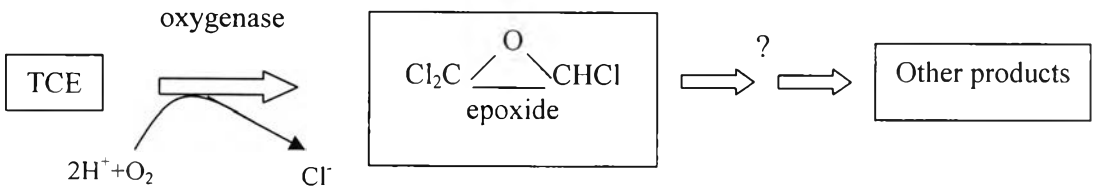


Figure 2.2 Aerobic biological transformation of trichloroethene (TCE). (Bouwer and McCarty, 1985; Fogel et al., 1986; Lee, 2002)

2.5 Anaerobic Degradation Process.

According to Tchobanoglous and Burton (1991), anaerobic biological conversion of the organic matter can be divided into three steps (see in Figure 2.3). The first step in the process involves enzyme-mediated transformation (hydrolysis) of higher-molecular-mass compounds into compounds suitable for use as a source of energy and cell carbon. One group of organisms is responsible for hydrolyzing organic polymers and lipids to basic structural building blocks such as monosaccharide, amino acids, and related compounds.

Theoretical states

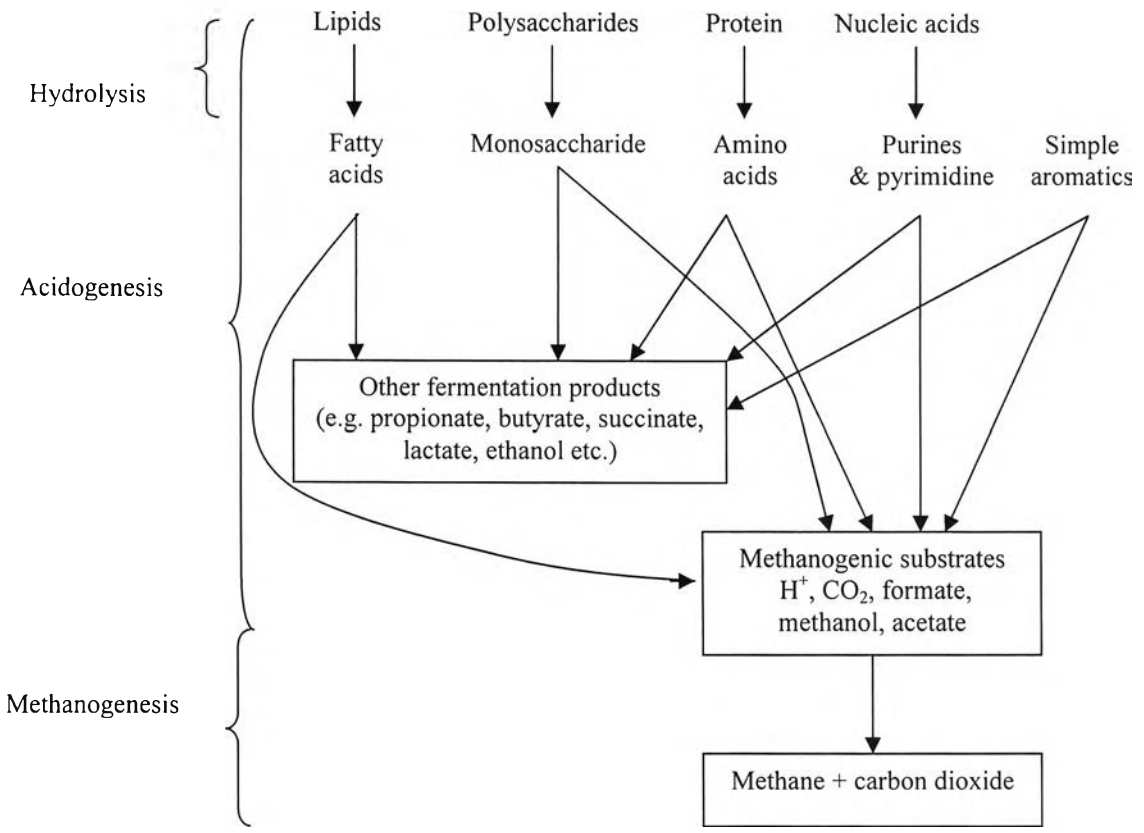


Figure 2.3 schematic diagrams of the patterns of the carbon flow in anaerobic (Tchobanoglous and Burton, 1991).

The second step (acidogenesis) involves the bacterial conversion of the intermediate compounds. The second group of anaerobic bacteria ferments the breakdown products to simple organic acids, the most common of which in anaerobic digester is acetic acid. The group of microorganisms, described as nonmethanogenic, consists of facultative and obligate anaerobic bacteria. Collectively, these

microorganisms are often identified in the literature as "acidogens," or "acid formers."

The third step (methanogenesis) involves the bacterial conversion of the intermediate compounds into simpler end products, principally methane and carbon dioxide. Microorganisms of a third group convert the hydrogen and acetic acid formed by the acid formers to methane gas and carbon dioxide. The bacteria responsible for this conversion are strictly anaerobes and are called methanogenic bacteria. They are identified as "methanogens," or "methane formers." It is important to note that methane bacteria can only use a limited number of substrates for the formation of methane. Currently, it is known that methanogens use the following substrates $CO_2 + H^+$, formate, acetate, methanol, methylamines, and carbon monoxide for their growth. The methanogens are able to utilize the hydrogen produced by the acidogens because of their efficient hydrogenase. Because the methanogens are able to maintain an extremely low partial pressure of H^+, the equilibrium of the fermentation reactions is shifted towards the formation of more oxidized end products (e.g., formate and acetate).

2.6 Dechlorination

Dehalogenation is a mechanism that removes halogen from halogenated compounds but dechlorination is specific to chloride removal from chlorinated compounds (Figure 2.4). There are two types of microbial mediated reactions involved in the removal of halogen substituents from organic molecules. Oxidative

and reductive reactions involve the transfer of electrons from or to the halogenated compound, respectively. Due to the electronegative character of halogen substituent groups, highly halogenated compounds are more oxidized than lesser-chlorinated isomers and thus are less susceptible to oxidative reactions; rather, they will undergo reductive reactions for thermodynamic reasons (Adriaens and Vogel, 1995).

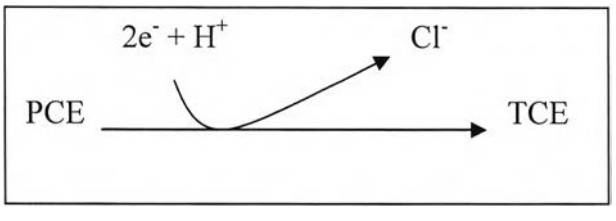


Figure 2.4 Mechanism of reductive dechlorination of chlorinated compounds

Under anaerobic conditions, reductive dehalogenation is the dominant mechanism for chloride removal. Reductive dechlorination of chlorinated organic compounds takes place in reduced environments such as deep soils and sediments and is mediated by native microbial consortia acclimated to existing contaminants. The result of reductive dechlorination is less chlorinated compounds congener, which is less toxic, less likely to bioaccumulate, more soluble and volatile than the polychlorinated compounds. Thus, more mobile and more susceptible compounds for microbial attack are produced (Adriaens and Vogel, 1995).

2.7 Dechlorination by Halorespiring Microorganisms

Halorespiration, also referred to as dehalorespiration, occurs when the organic compound acts as an electron acceptor (primary growth substrate) during reductive dechlorination. During halorespiration, the chlorinated organic compounds are used directly by microorganisms (termed halorespirators), such as an electron acceptor while dissolved hydrogen serves as an electron donor. Halorespiration occurs as a two-step process, which results in the interspecies hydrogen transfer by two distinct strains of bacteria. In the first step, bacteria ferment organic compounds to produce hydrogen. During primary or secondary fermentation, the organic compounds are transformed to compounds such as acetate, water, carbon dioxide, and dissolved hydrogen. Fermentation substrates are biodegradable, nonchlorinated contaminants or naturally occurring organic carbon.

In the second step, the nonfermenting microbial consortia utilize the hydrogen produced by fermentation for halorespiration. Process of PCE reductive dechlorination is shown in Figure 2.5 to be an example for the explanation of steps involving in dechlorination which PCE is an electron acceptor. The relation between various organic compounds and microorganisms with different function in dechlorination process and cause the transformation of chlorinated compound could be seen (Suthersan, 2001)

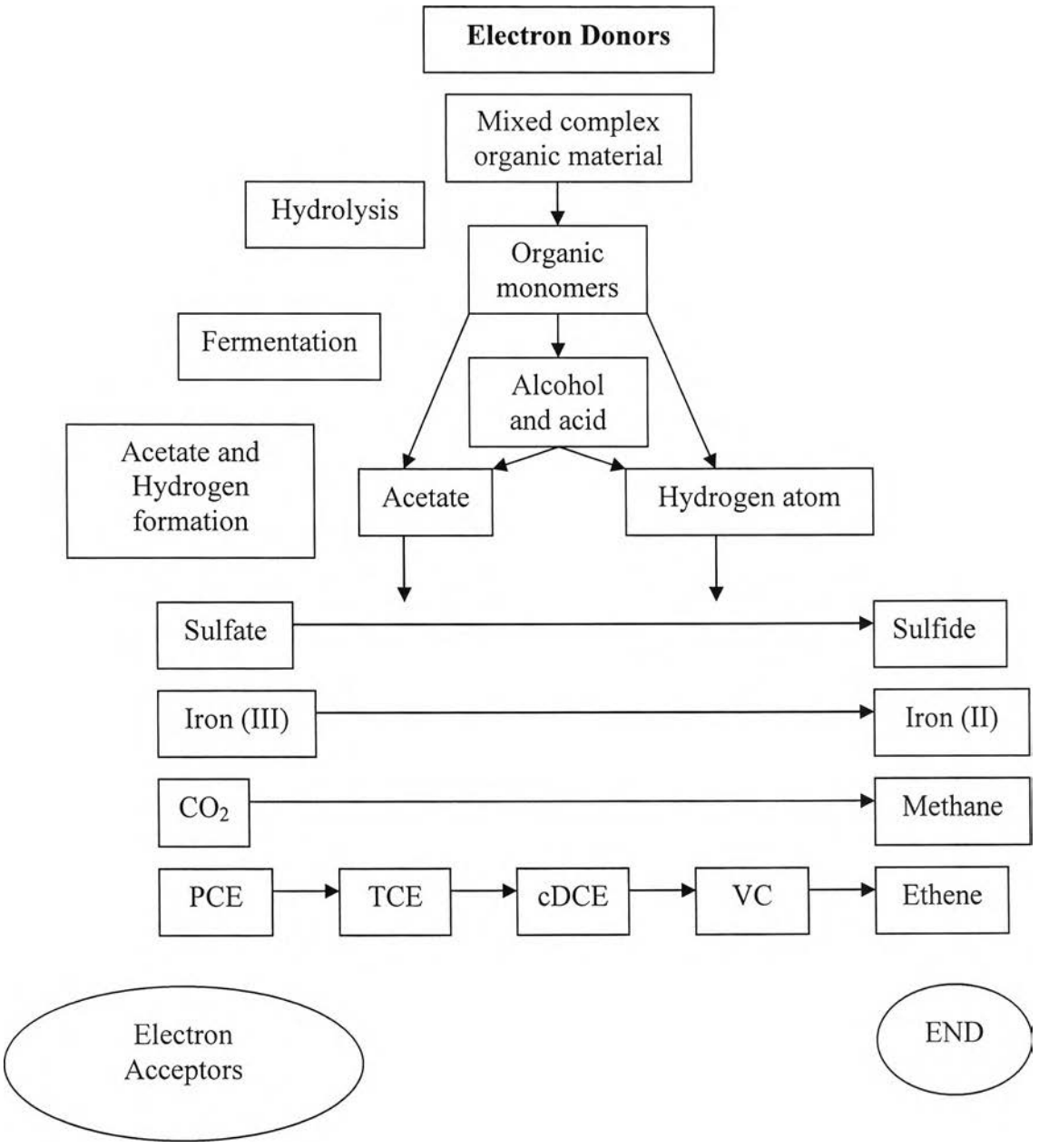


Figure 2.5 Steps in the process of biodegradation of PCE by reductive dechlorination (Suthersan, 2001)

As shown in Figure 2.5, biodegradable organic matter is requied as an electron donor to initiate the process. Different types of microbes are involved at each state. The bottom steps shows that PCE must compete for electrons with sulfate, iron, and

carbon dioxide, meaning that a large amount of organic electron donors may be needed to supply enough electrons.

2.8 PCE Anaerobic Reductive Dechlorination

Under anaerobic condition, PCE is transformed into TCE, dichloroethene (cis-DCE, tran-DCE and 1,1-DCE), vinyl chloride, and non-toxic end products (ethane and ethene) by the reductive dechlorination process as shown in Figure 2.6. Anaerobic reductive dechlorination occurs in a microbe's respiratory processes during food digestion. H^+ is produced in the acidogenesis process and chloride molecules in the structure of chlorinated compounds are replaced with hydrogen molecule until the end of reaction.

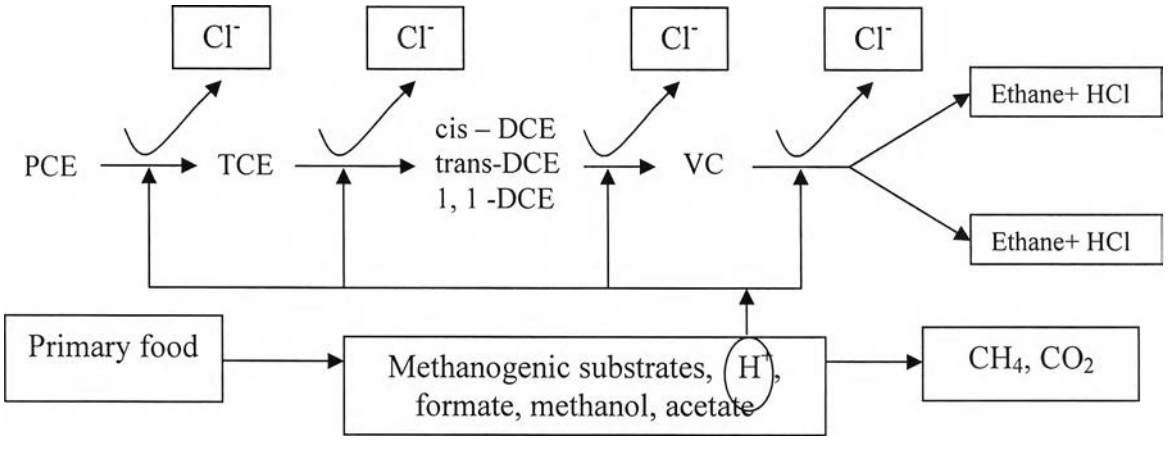


Figure2.6 Anaerobic reductive dechlorination of PCE (Ferguson and Pietari, 1999)

2.9 Hydrogen Release Compound (HRC) for Remediation

HRC is supplied as a various liquid for direct injection into contaminated soil and groundwater. This specially formulated product slowly releases acid upon contact with water. This source of lactic acid is then metabolized by microbes to produce hydrogen which is then used in a natural process know as reductive dechlorination. Being a natural process, reductive dechlorination usually proceeds a very slow, unsustainable rate. HRC offers an efficient, low cost and effective method of treating in-situ, chlorinated compounds. Application of HRC is accomplished inexpensively using push-point or borehole delivery methods. Once in the subsurface, HRC continues to stimulate the biodegradation of contaminants for an extended period of time (up to 18 months) eliminating the need for multiple, more frequent injections. A combination of low-cost application, and extended release profile, no operations and maintenance, minimal site disturbance and a lack of dependence on external power sources give HRC a substantial cost advantage over other treatment technologies. HRC is a sensible, economical solution for treating chlorinated contaminants in saturated soils and ground water.

2.9.1 Plume Wide Remediation

Table 2.1 below illustrates four different size plume and four remediation scenarios, include used of HRC. This comparison assumes a TCE contaminant concentration of 100 ppm.

Table 2.1 HRC a cost effective remediation strategy on plume wide remediation

Treatment	Smaller Site (50′ x 75′)		Larger Site (200′ x 205′)	
	Shallow Aquifer (20′ bgs)	Deeper Aquifer (50′ bgs)	Shallow Aquifer (20′ bgs)	Deeper Aquifer (50′ bgs)
HRC Treatment	$ 130,000	$ 134,000	$ 316,000	$ 324,000
Pump and Treat	$ 595,000	$ 633,000	$ 778,000	$ 876,000
Air Sparging w/SVE	$ 334,000	$ 358,000	$ 639,000	$ 760,000
Chemical Oxidation	$ 320,000	$ 343,000	$ 1,495,000	$ 1,636,000

(Source: http://www.regenesis.com)

Cost comparisons were generated by an independent environmental consulting firm and include costs though project completion, e.g. sampling, monitoring, reporting, etc. All costs are reported as of 2005. A net present value analysis would make HRC treatment appear considerably more favorable.

2.9.2 Plume Cut-Off / Barrier Remediation

Table 2.2 below illustrates four size plume and four typical, cut-off barrier remediation scenarios, include use of HRC. This comparison assumes a TCE contaminant concentration of 100 ppm.

Table 2.2 HRC a cost effective remediation strategy on plume cut-off / barrier remediation

Treatment	Smaller Site (50′ x 75′)		Larger Site (200′ x 205′)	
	Shallow Aquifer (20′ bgs)	Deeper Aquifer (50′ bgs)	Shallow Aquifer (20′ bgs)	Deeper Aquifer (50′ bgs)
HRC Treatment	$ 145,000	$ 149,000	$ 175,000	$ 184,000
Iron Wall Barrier	$ 336,000	$ 394,000	$ 632,000	$ 776,000
Pump and Treat	$ 578,000	$ 615,000	$ 685,000	$ 757,000
Air Sparging w/SVE	$ 350,000	$ 356,000	$ 641,000	$ 675,000

(Source: http:// www.regenesis.com)

Cost comparisons were generated by an independent environmental consulting firm and include all project costs for operating a plume cut-off for a five year period. All costs are reported as of 2005. A net present value analysis would make HRC treatment appear considerably more favorable.

2.10 Wastes for In Situ Anaerobic Bioremediation

Used edible oils such as used lard and used soybean oil are general wastes which normally found in local market. Oil waste from Food Industry and molasses are wastes from domestic wastewater treatment of food industry and sugar industry, respectively. All of materials from above have the following characteristics that make

them good candidates for this type of application: (1) they are rich in carbon, an essential energy source for biodegradation; (2) they have the potential to exhibit sufficient hydrogen bioavailability for reductive dechlorination to occur; and (3) they are relatively inexpensive. Under methanogenic conditions, a portion of the electrons will be transferred to PCE and activate the reductive dechlorination process, resulting in the formation of subsequent intermediates. Wastes storage tank installed at the up gradient area of the plume may be a practical method to enhance PCE biodegradation. Wastes can be injected into remediation wells or permeable trench, and act as the diffusion sources of the primary electron donor for anaerobic reductive dechlorination process.

2.11 Properties of Substrate

2.11.1 Fat and Oils

The common feature of these lipids is that they are all esters of moderate to long chain fatty acids. Acid or base-catalyzed hydrolysis yields the component fatty acid, some examples of which are given in the following table, together with the alcohol component of the lipid. These long-chain carboxylic acids are generally referred to by their common names, which in most cases reflect their sources. Natural fatty acids may be saturated or unsaturated, and as the following data indicate, the saturated acids have higher melting points than unsaturated acids of corresponding size. Table 2.3, presented the normal formula, common name and melting point of saturated and unsaturated fatty acid.

Table 2.3 Component of fatty acids

Saturated fatty acid		
Formula	Common Name	Melting Point
$CH_3(CH_2)_{10}CO_2H$	lauric acid	45 °C
$CH_3(CH_2)_{12}CO_2H$	myristic acid	55 °C
$CH_3(CH_2)_{14}CO_2H$	palmitic acid	63 °C
$CH_3(CH_2)_{16}CO_2H$	stearic acid	69 °C
$CH_3(CH_2)_{18}CO_2H$	arachidic acid	76 °C

Unsaturated fatty acid		
Formula	Common Name	Melting Point
$CH_3(CH_2)_5CH=CH(CH_2)_7CO_2H$	palmitoleic acid	0 °C
$CH_3(CH_2)_7CH=CH(CH_2)_7CO_2H$	oleic acid	13 °C
$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7CO_2H$	linoleic acid	-5 °C
$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7CO_2H$	linolenic acid	-11 °C
$CH_3(CH_2)_4(CH=CHCH_2)_4(CH_2)_2CO_2H$	arachidonic acid	-49 °C

(Source: http://www.cem.msu.edu)

The triesters of fatty acids with glycerol (1,2,3-trihydroxypropane) compose the class of lipids known as fats and oils. These triglycerides (or triacylglycerols) are

found in both plants and animals, and compose one of the major food groups of our diet. Triglycerides that are solid or semisolid at room temperature are classified as fats, and occur predominantly in animals. Those triglycerides that are liquid are called oils and originate chiefly in plants, although triglycerides from fish are also largely oils. Some examples of the composition of triglycerides from various sources are given in the following table 2.4.

Table 2.4 General composition of fat and oil

	Saturated Acids (%)					Unsaturated Acids (%)		
Source	C_{10} & less	C_{12} lauric	C_{14} myristic	C_{16} palmitic	C_{18} stearic	C_{18} oleic	C_{18} linoleic	C_{18} unsaturated
Animal Fats								
lard	-	-	1	27	15	48	6	2
human fat	-	1	3	25	8	46	10	3
Plant Oils								
coconut	-	50	18	8	2	6	1	-
corn	-	-	1	10	3	50	34	-
palm	-	-	2	41	5	43	7	-

(Source: http://www.cem.msu.edu)

2.11.2 Soybean Oil

Soybean oil is a triglyceride that typically contains 14% stearic, 23% oleic, 55% linoleic, and 8% linolenic acid (Parreira et at., 2002). Three of these are unsaturated acids: oleic (18:1), linoleic (18:2), and linolenic (18:3) (Figure 2.7).

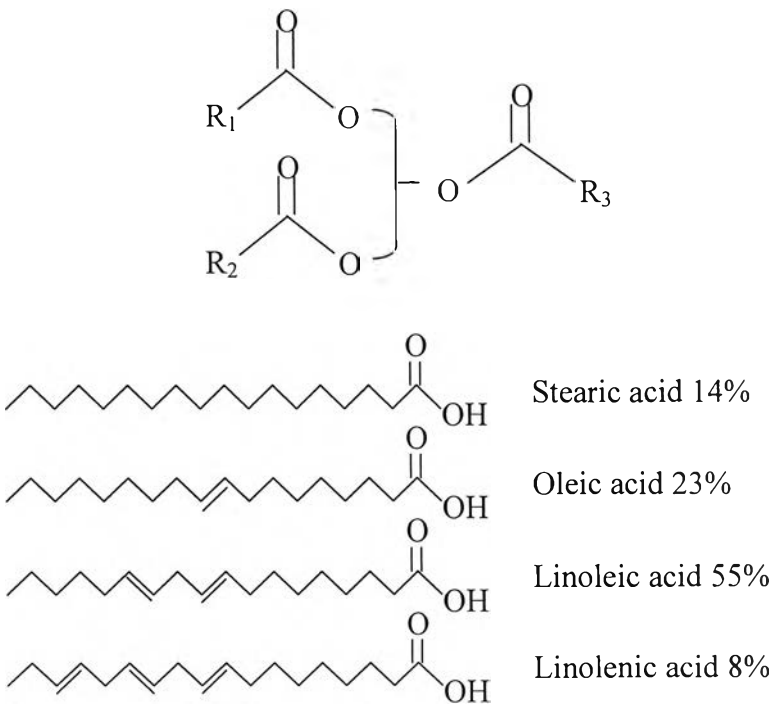


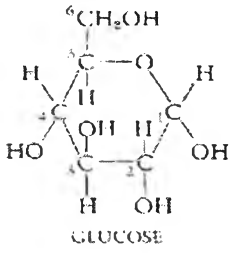
Figure 2.7 Soybean oil structure and its substituted fatty acids

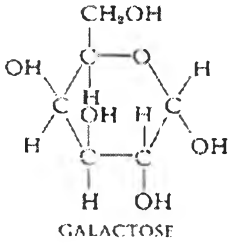
2.11.3 Sugar and Molasses

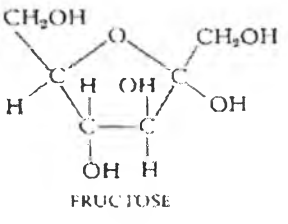
Sugars Composition

Three common sugars share the same molecular formula: $C_6H_{12}O_6$. Because of their six carbon atoms, each is a hexose.

They are:







- glucose, "blood sugar", the immediate source of energy for cellular respiration
- galactose, a sugar in milk (and yogurt), and
- fructose, a sugar found in honey.

Although all three share the same molecular formula ($C_6H_{12}O_6$), the arrangement of atoms differs in each case. Substances such as these three, which have identical molecular formulas but different structural formulas, are known as structural isomers.

Molasses Composition

Properties of Molasses from United State Sugar Cooperation 2003 were shown in Table 2.5.

Table 2.5 Properties of Molasses

Properties	
1. Weight/gallon	11.8-12.0 lbs
2. Nitrogen	1.01%
3. Crude Protein	6.30%
4. Total Sugars	48.3%
5. Density (as fed)	11.8 lbs/gal
6. Dry Matter	76.5%
7. Moisture	23.5%
8. Organic Matter	62.5%
9. Sucrose	35.9%
10. Fructose	5.6%
11. Glucose	2.6%
12. pH	4.9-5.4

(Source: http://www.suga-lik.com/molasses/blackstrap.molasses.html)

From Table 2.5, there were found that the combination percent of sucrose, fructose and glucose was approximately 44.1. Therefore, one molecule of molasses composes with sugar 44.1%.

2.12 Literature Review

2.12.1 Relationship between electron donors and PCE reductive dechlorination

In 1995, Doong and Wu reported that the chlorinated compounds were dechlorinated under anaerobic conditions by supplying various substrates as electron donor. The substrate fed-batches were anaerobically incubated with acetate, glucose, methanol and humic acid as supplemental substrates with concentrations ranging from 10 to 30 mg/l. The sequence of the efficiency of enhancement is consistent with the sequence of the reducing potentials of the dechlorination reaction. In addition the better removal efficiencies of PCE were increased from 61% to 99.9% when the substrate concentrations in the batches were increased from 10 to 30 mg/l.

Gao et al (1997) investigated the separate effects of several electron donors on tetrachloroethylene (PCE) dechlorination activity. The substrates tested were methanol, lactate, acetate and sucrose. Various levels of sulfate-reducing, acetogenic, fermentative, and methanogenic activity were observed in all sediment. Lactate was the best condition for dechlorination activity in these experiments and at least 40% of the added PCE was converted to TCE and cis-DCE. Trichloroethylene was the primary product; however small amounts of cis-1,2-dichloroethylene and vinyl chloride were also detected.

There were studies on the effect of the organic carbon content on the sequential reductive dehalogenation of tetrachloroethylene (PCE) in landfill leachates using anaerobic microcosms (Leahy and Shreve, 1999). The results showed that organic carbon is able to serve as an electron donor and its may be the most important factor influencing the rate of chloroaliphatic (CAH) removal via dehalogenation. The significant degradation of PCE occurred within 14 days and all of the PCE, TCE, cis-1,2-DCE was transformed in 30 days. All subsequent laboratory manipulations were conducted in an anaerobic glovebox with an 80% N_2: 20% C_2O atmosphere. Moreover, supplementation with exogenous sources of organic carbon appears to be a viable method for enhancing the rate of CAH degradation in carbon-limited landfills

In 1999, Ferguson and Pietari studied the bioremediation of chlorinated solvents. Chlorinated aliphatic compounds, notably the chlorinated solvents, are common contaminant in soil and ground water at hazardous waste sites. While these compounds are often recalcitrant, under favorable conditions they can be transformed and degrade through microbial mediated process. A laboratory study, using anaerobic sludge that had been fed with chlorinated compounds, showed that mixed anaerobic consortia with several dehalogenating bacteria transformed PCE to ethene. Laboratory studies took 20 days to remove PCE and its by products. In addition, they have demonstrated that reductive dechlorination supports growth of the novel bacteria that carry out the reactions. Hydrogen has been shown to be an electron donor for the bacteria dehalogenation reactions.

Kao et al (2003) reported that cane molasses can use as the primary substrate under methanogenic conditions to clean up PCE contaminated aquifer. The lab scale column system was operated for 65 days in the dark at the ambient groundwater temperature (~ 20°C) and supplied with 90% N_2: 10% C_2O to deplete the residual oxygen. They reported that concentration of PCE and its by products in all four columns reached a quasi-steady state and remained constant after 25 operating days.

2.12.2 An effectiveness of microbial consortium for anaerobic reductive dechlorination

Chang et al. (1998) have investigated the biodegradation of tetrachloroethylene (PCE) and halogenated aliphatic compound by using anaerobic mixed cultivation. The mixed culture degraded PCE at concentrations of up to 150 mg/l in 40 days via trichloroethylene to cis-1,2-dichloroethylene. Small amounts of vinyl chloride and CO_2 were also detected. Acetate was the most effective electron donor for dechlorination, although formate, glucose and lactate were also effective, but to a lesser extent. The mixed culture degraded PCE in the temperature range of 25 to 43°C, with an optimum between 30 and 37°C, and a pH range of 6.2 to 11.0.

Wu et al. (1998) studied the effectiveness of the microbial consortium in the anaerobic dechlorination of PCE in soil, using different inoculum levels (3 or 60 mg VSS/kg soil) and initial PCE concentrations of 10 or 200 mg/kg dry soil. The initial dechlorination reductions were more rapid in the microcosms with a higher inoculum level. However, the dechlorination results were similar after three months,

irrespective of the inoculum level. This suggests that a large amount of inoculum may not be necessary for bioremediation if organisms can survive in the soil. The PCE level had an influence on methane production. A higher efficiency of electron use for dechlorination was observed when a higher level of PCE was added in the microcosms.

Tungmee (2002) investigated HCB dechlorinating ability of unacclimated microorganism in sludge from an anaerobic reactor. Two types of sludge to soil ratios (20:80 and 50:50) were studied in order to investigate the dechlorinating ability and the effective ratio was 50:50. The study used various substrates such as glucose, ethanol, formate, lactate and the combination of acetic acid, butyric and propionic (with COD concentration 3000 mg/l each). The unacclimated sludge is capable to degraded HCB at a concentration of 10 ppm HCB in the dark at room temperature, in the pH range of 6.5-7.5. 80% N_2: 20% C_2O was supplied into reactor in order to keep anaerobic condition. Glucose was the most effective electron donor for HCB dechlorination.

2.12.3 Using Edible oil for enhance anaerobic reductive dechlorination

The studies by Hinchee (2002) have found that adding a food grade substrate such as edible oils, especially hydrogenated soy bean oil due to its hydrogen content act as the electron donor/ carbon rate driven to replace chloride ion in chlorinated compound. When reductive dechlorination process can be maintained, the chlorinated compounds were transformed into harmless. This process stimulated the indigenous

bacteria by injection of one or more substrates (soybean oil) and was referred as biostimulation.

Borden (2003) reported that edible oil slowly dissolves over several years thus it can be provide a carbon and energy source to accelerate the anaerobic biodegradation of the chlorinated solvents. Consequently, the edible oil can be added to the treatment zone through conventional wells or the use of direct pushes technology to reduce costs. In addition, the edible oil substrate process can be used to enhance chlorinated solvent degradation in a variety of situations.

Lee (2003) has reported that groundwater in USA were contaminated by 1TCA, cDCE and their degrade product. Although, an acclimated anaerobic microbial population was present at the site, however it was limited by the availability of carbon. Emulsified edible oil was injected into the ground surface to supply the carbon source. After the substrate addition, decreases in the parent compounds and increases in the daughter products were found. The average concentration of the parent compound has decreased by 96%. There was indication that emulsified edible oil enhanced the dechlorination process.

From the literature review, there is much information about using various food grade or purified substrate such as glucose, acetate, edible-oil as electron donor at varying concentrations. However, there is no information about using wastes as substrate. It would be very useful for economic and technical reason if the types and concentration of waste, which could be added into reactor to enhance the

dechlorination process, are known. Moreover, the efficiency of various wastes and the use of unacclimated sludge as inoculation have never been studied with regard to the reductive dechlorination of biomass in Thailand.

As various carbon sources that serve as electron donors stimulate different parts of an anaerobic population (Doong and Wu, 1995; Gao et al., 1997; Chang et al., 1998; Tungmee, 2002), several materials should be tested in order to enhance efficiency of dechlorinating activity. The type and concentration of waste that provide the best result from this study would be obtained and may be applied for future PCE remediation. In our study unacclimated homogenized granular sludge was utilized as seed; however most of researches had been done using acclimated sludge. Acclimated of sludge seed with PCE is not quite practical for real application because of the high expense for reactor construction, operation, maintenance and transportation. On the other hand, unacclimated sludge can be added directly to the contaminated site and we could leave it for a certain time. This unacclimated sludge will be able to acclimate after exposed to a pollutant at a point of contact.

From the literature review, 24 days could be used as an appropriate incubation time period (Ferguson and Pietari, 1999; Leahy and Shreve, 1999; Kao et al., 2003) for PCE dechlorination. 80% N_2: 20% C_2O was supplied in the reactor in order to deplete oxygen in system by many researchers (Leahy and Shreve, 1999; Tungmee, 2002; Kao et al, 2003). Unacclimated sludge from a UASB wastewater treatment plant can be used as seed to enhance the effectiveness of PCE dechlorination

(Tungmee, 2002). In many studies, anaerobic dechlorination reactors were kept in dark at room temperature (Tungmee, 2002; Kao et al, 2003).