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Comparison of in-situ bioremediation of soil
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Tianyu Qin



Comparison of in-situ bioremediation of soil contaminated with chlorinated hydrocarbons

Tianyu Qin

Master's Thesis Program in Applied Environmental Science

School of business, Engineering and Science, Halmstad University

Supervisor: Sylvia Waara

Associate Professor in Environmental Science

Abstract

In recent years, due to the continuous development of machinery, electronics, leather, chemical companies and dry-cleaning industry, more and more chlorinated hydrocarbons accumulate in the soil, causing serious harm to the environment. The accumulation of chlorinated hydrocarbons and the teratogenic, carcinogenic, and mutagenic hazards seriously threaten human health. Therefore, the remediation of chlorinated hydrocarbons is imminent. Under this premise, in-situ bioremediation has gradually received attention. For in situ bioremediation of soil contaminated with chlorinated hydrocarbons, the most commonly used methods are biostimulation alone, bioaugmentation alone, and a combination with biostimulation and bioaugmentation. The removal rate of trichloroethylene in the case of using biostimulation products alone is significantly lower than that of using bioaugmentation products alone. The removal rate of trichloroethylene by biostimulation products alone does not exceed 60%, and "DCE pause" occurred, but did not occur in the case of using bioaugmentation products. The removal rate of trichloroethylene by bioaugmentation products is generally higher than 98%, and it will promote the degradation of trichloroethylene or tetrachloroethylene to non-toxic ethylene. Therefore, only cases containing bioaugmentation can achieve non-toxic degradation of chlorinated hydrocarbons and take into account the high removal rate of them. In addition, the biostimulation duration is significantly shorter.

Keywords: bioremediation, biostimulation, bioaugmentation, product, chlorinated hydrocarbon

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Acronyms and Abbreviations

bgs	Below ground surface
CA	Chloroethane
DCA	Dichloroethane
DCE	Dichloroethylene
DHC	Dehalococcoides spp
J	Estimated value, below reporting limit.
LEL	Lower Explosive Limit
LPG	Liquefied Petroleum Gas
NA	Not analyzed
ORP	oxidation-reduction potential
PCE	Tetrachloroethylene
ROI	Radius of Influence
scfm	Standard cubic feet per minute
TCA	Trichloroethane
TCE	Trichloroethylene
VC	Vinyl chloride

1. Introduction

In recent years, due to the rapid development of industries such as electronics and chemical industry, the generated solid waste has been piled up or dumped into the soil, and the polluted wastewater also has continuously penetrated into the soil (Lu et al., 2016). Without the establishment of relevant laws and regulations and the treatment mechanism of pollutants, more and more pollutants are accumulated in the soil and penetrate into the groundwater, causing serious harm to the ecological environment and the health of humans, animals and plants (Lu et al., 2016). Among them, chlorinated hydrocarbons, as one of the organic pollutants accumulated in the soil, can take hundreds to thousands of years through natural degradation to harmless levels (O'Connor et al., 2018).

Chlorinated hydrocarbons have been widely used in machinery, electronics, leather, chemical companies and the dry-cleaning industry (Scheutz et al., 2011; Stroo et al., 2003). Due to improper storage and disposal, there are currently a large number of chlorinated hydrocarbon contaminated sites in many countries in the world (Lu et al., 2016). Most chlorinated hydrocarbons are volatile, bioaccumulative and potentially carcinogenic, teratogenic, and mutagenic, which can cause serious impacts on human health and ecological environment (Huang et al., 2014; Meng et al., 2013). Therefore, most of them have been listed as toxic and harmful organic pollutants by many countries (Lei, 2014). In 1976, the US Environmental Protection Agency (USEPA) has listed perchloroethylene (PCE) and trichloroethylene (TCE) as "priority control compounds" and "suspected carcinogens".

Harrad et al. (1994) estimated the amount of chlorinated hydrocarbons contained in the soil, water, sediment and other media in the environment, as well as their source, distribution, migration, fate and other behaviors. It was concluded that the soil is the ultimate destination and that soil constantly receive pollutants in various ways. Therefore, it is of great significance to realize the remediation of chlorinated hydrocarbons in the soil. Studies have shown that biological action in soil and water is the main mechanism of material degradation, and microbes play a leading role in biological degradation (Fanxiang et al., 2007; Qiang et al., 2004). In response to the above problems, the use of microbial products for soil remediation has gradually attracted attention.

This thesis will address three in situ bioremediation methods; biostimulation alone, bioaugmentation alone, and the combination of biostimulation and bioaugmentation. Bioaugmentation of contaminated soil or groundwater, entails the introduction of foreign bacteria to achieve the purpose of degrading pollutants (Shah, 2017).

Biostimulation is different from bioaugmentation. Compared to bioaugmentation the intention with biostimulation is to change the community structure of the microbes at the contaminated site. Biostimulation changes the environment inside the contaminated spot so that bacteria capable of bioremediation can degrade the pollutants. Therefore, it is called "stimulation", and such stimulation can be accomplished by adding an electron donor. The methods of biostimulation and bioaugmentation combine the above two mechanisms to degrade pollutants in the soil.

1.1 Degradation of polychlorinated hydrocarbons

Chlorinated hydrocarbons, especially PCE and TCE can be degraded by aerobic co-metabolism pathway, but they can also be degraded by anaerobic process. Soil and groundwater environments are usually anaerobic conditions (Jian'e et al., 2003; Rittmann & McCarty, 2012), studies have shown that anaerobic reduction dechlorination is the leading biochemical process for high-chlorinated solvents (such as TCE) (Hood et al., 2008; McCarty et al., 2007; Yang & McCarty, 2002).

The reductive dechlorination of tetrachloroethylene (PCE) is the most typical reductive dechlorination of chlorinated hydrocarbons, the complete degradation pathway is shown in Figure 1.

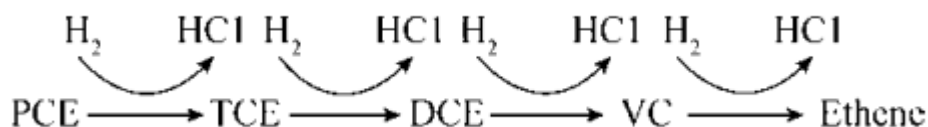


Figure 1. Completely reductive dechlorination degradation of PCE.

Roberts et al. (1996) proved that the main way to degrade chlorinated hydrocarbons is the β elimination route, that is, the main generation is *cis*-1,2-DCE and very few *trans*-1,2-DCE. For example, in case A, the generation of *trans*-1,2-DCE accounts for 1% of the total DCE. Such a small amount of generation of *trans*-1,2-DCE is also common in other cases Therefore, in the following, we will mainly discuss *cis*-1,2-DCE.

1.1.1 Bioaugmentation of polychlorinated hydrocarbons

Bioaugmentation is the inoculation / introduction of microorganisms on the contaminated site / soil to facilitate biodegradation (Shah, 2017). As shown in Figure 1, the end point of PCE non-toxic degradation is ethylene, but a large number of bacteria can only be completed From PCE to DCE. So far, only *Dehalococcoides* spp. has been found to be able to complete the reductive dechlorination of PCE to ethylene (Frascari et al, 2015). Therefore, the current microbial remediation products for remediation of

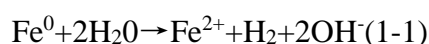
chlorinated hydrocarbon-contaminated soil are mainly rich in *Dehalococcoides* spp (DHC).

DHC is an anaerobic specific organic halide respiring microorganism, and it can only use hydrogen or formate as an electron donor. DHC acts as an electron acceptor for halogenated hydrocarbons through dehalogenation, (Hug et al., 2013) to break the carbon - halogen bond of organic halides, and obtain energy from it to supply its own metabolic activities.

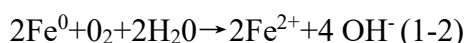
1.1.2 Biostimulation of polychlorinated hydrocarbon

Biostimulation in in-situ soil remediation of chlorinated hydrocarbons often uses substances capable of providing hydrogen as electron donors and indigenous microorganisms as electron acceptors (Shah, 2017). Generally, the hydrogen required in actual processing is mainly produced by fermentation or anaerobic reaction using organic compounds (such as organic acids or alcohols) and zero-valent iron (ZVI). By comparing the degradation rate of PCE, Rosenthal et al. (2004) concluded that the electron donor 's ability to provide electrons as follow: ZVI> H₂> Butyric acid > Lactic acid> Formic acid. For biostimulation, the market mainly uses zero-valent iron (ZVI) or organic compounds.

Zero-valent iron (ZVI) is chemically active, has strong reducibility, and is extremely electronegative. Sweeny (1979) first reported in the 1978 that zero-valent iron can be used to reduce and degrade organic chlorides. Gillham and O'Hannesin (1994) later reported that metal zero-valent iron can be used in contaminated groundwater remediation. Since then, ZVI has been widely used to degrade organic chlorides, such as chlorinated aromatic hydrocarbons, polychlorinated biphenyls, nitroaromatic hydrocarbons, pesticides DDT, herbicide atrazine, etc. (Arnold & Roberts, 2000; Dong et al., 2011; Huang & Zhang, 2005; Xu et al., 2009). In addition, the ZVI has the characteristics of low price and simple reaction conditions, so it can be widely used as a reducing agent to provide electrons for the effective degradation of chlorinated hydrocarbons in groundwater and soil (Fennelly & Roberts, 1998; Ebert et al., 2006). ZVI produces hydrogen required for dechlorination reduction reaction through surface corrosion, and its corrosion reaction is as formula (1-1).



ZVI will also react further, producing OH⁻, raising the pH of the soil environment or groundwater (formula 1-2).



Therefore, the introduction of ZVI can alleviate the situation that the pH continues to fall due to the continuous generation of acid by the dechlorination reaction.

The use of ZVI for remediation in Europe is rare, but it is common in the United States (Mueller et al.,2012).

Organic compounds usually produce hydrogen by fermentation under anaerobic conditions (Liang et al., 2013). The specific reaction is shown in Figure 2. Commonly used organic compounds are methanol, ethanol, glucose, valerate, lactic acid, benzoate, etc., as well as hydrogen releasing compounds (HRCs)

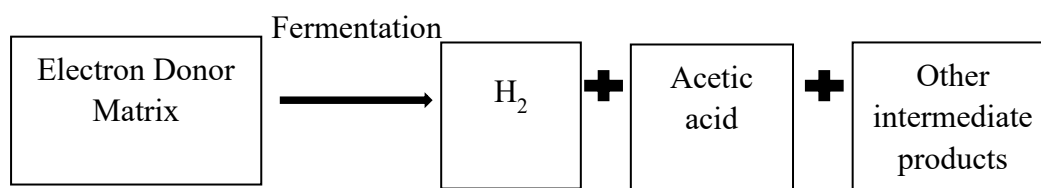


Figure 2. Hydrogen production process of organic mixture fermentation

In addition, the organic mixture can also be used as a carbon source by indigenous microorganisms.

1.2 Aim

The aim of this thesis is to describe methods and products used for in situ bioremediation of chlorinated hydrocarbons with a focus on the use of biostimulation and/or bioaugmentation in the degradation of TCE and PCE to non-toxic end productions. It will include a comparison of treatment efficiency, remediation cycles and the cost of remediation using two case studies in which products on the market has been employed. To fulfill the aim, the following research questions will be addressed:

- What are the end points of different bioremediation methods for remediation of soil contaminated with chlorinated hydrocarbons?
- How does the use of different bioremediation methods affect the removal rate of chlorinated hydrocarbons?
- What is the approximate cost of remediating soil contaminated with chlorinated hydrocarbons using bioremediation?

2. Materials and methods

In this thesis, a total of three databases (US EPA, Web of Science and CNKI) were used to find relevant and useful literature. The search keywords and results are shown in Table 1.

Table 1. Keywords and databases used for finding the literature used in this report

Database	Keyword	Limit	Result (no. scientific articles)
US EPA	Soil bioremediation	Resource type: Reports and Assessments	4313
	Soil bioremediation	-	11859
	Soil bioremediation + In-situ	-	1752
	Soil bioremediation+ Chlorinated hydrocarbon	-	120
	Soil bioremediation+ Chlorinated hydrocarbon+ In-situ	-	47
Web of Science	Soil bioaugmentation		1953
	Soil bioaugmentation + Chlorinated hydrocarbon	-	13
	Soil biostimulation	-	982
	Soil biostimulation + Chlorinated hydrocarbon		7
	Dehalococcoides	-	828
	Dehalococcoides + Soil bioremediation	-	54
CNKI	Dehalococcoides	-	2

This thesis explores the use of bioremediation products through case studies to generate new insights and draw conclusions on consumers suggestions for purchasing bioremediation products. In table 2, the main bioremediation products on the market in 2020 are presented.

Table 2. Bioaugmentation and biostimulation products on the market 2020

Company	Products	Note
Ejlskov	Trap&Treat®	Bioaugmentation: Bacteria
Biosorbe	Formula CL	Bioaugmentation: Archaea bacteria
SiREM	KB-1™ KB-1 PLUS™	Bioaugmentation: DHC
Altogen Labs	USPTO application 13/490,508	Bioaugmentation: Natural bacteria
REGENESIS	BDI Plus	Bioaugmentation: DHC

	HRC Primer® HRC-X® HRC®	Biostimulation: hydrogen release compound
	3-D Microemulsion S-MicroZVI®	Biostimulation: lactic acid Biostimulation: ZVI
PeroxyChem	EHC®	Biostimulation: ZVI + Organic carbon
NASA Kennedy Space Center	EZVI	Biostimulation: ZVI

This thesis selected two soil bioremediation cases at Launch Complex 34 in Cape Canaveral Air Force Station, Florida, using bioaugmentation and biostimulation to remediate soil contaminated by chlorinated hydrocarbons. The two demonstration sites are close in distance, so soil pollutants and the soil environment are almost the same. In the two cases, different bioremediation methods were used: the combination with biostimulation and biostimulation (Case A) and biostimulation alone (Case B). Different biostimulation methods are used-organic compounds and zero-valent iron. In the process of bioaugmentation, a representative DHC bacterial agent was injected. Therefore, these two cases are representative to a certain degree.

According to the mechanism of bioremediation, the analysis is mainly carried out through the production of by-products when degrading chlorinated hydrocarbons, the end point of degradation, the degradation duration, and the removal rate of polychlorinated hydrocarbons. General information on the selected cases is presented in Table 3.

Table 3. General information of the bioremediation cases.

Case	A	B
Location	Launch Complex 34, Cape Canaveral Air Force Station, Florida, USA	
Product	Ethanol +KB-1 (DHC)	EZVI (ZVI)
Vendor	SiREM	NASA Kennedy Space Center
Major Pollutant	TCE	
Treatment	Oct 2002-Jun 2003	Jun 2002-Jan 2003
Duration	Nearly 12 months	Nearly 8 months

3.Results

3.1 Case A - Biostimulation & Bioaugmentation in Florida, USA

The polychlorinated hydrocarbon contaminant TCE in case A comes from a chlorinated organic solvent (such as TCE) used to clean the launch engine and other rocket parts. In case A, biostimulation phase started on October 23, 2002 first, and then bioaugmentation was injected inside the plot approximately 14 weeks later (February 6, 2003), and the treatment was ended in June 2003. The entire demonstration time was nearly a year.

During the demonstration, ethanol was used as an electron donor in the biostimulation phase. KB-1™ from SiREM Company was used for bioaugmentation. KB-1™ is rich in Dehalococcoides to reduce the dechlorination of TCE until the production of non-toxic ethylene.

Before the demonstration, the total amount of TCE in the soil was 31.2kg. After the demonstration, the amount of TCE in the soil fell to 0.2kg, and the removal rate of TCE exceeded 98%. Obviously, the combination of electron donor ethanol and KB-1™, which is rich in DHC, can effectively remove TCE accumulated in the soil.

Through the characterization pre-demonstration, the typical exemplary distribution of TCE in groundwater in the test area is drawn, as shown in Figure 3, the central well PA-26 is located in the area with the highest TCE concentration in the test plot. Therefore, by monitoring the data of this well, the extent of reduction and dechlorination of TCE in groundwater by ethanol and KB-1™ can be estimated, and the reduction and dechlorination of TCE in soil can also be determined from the side.

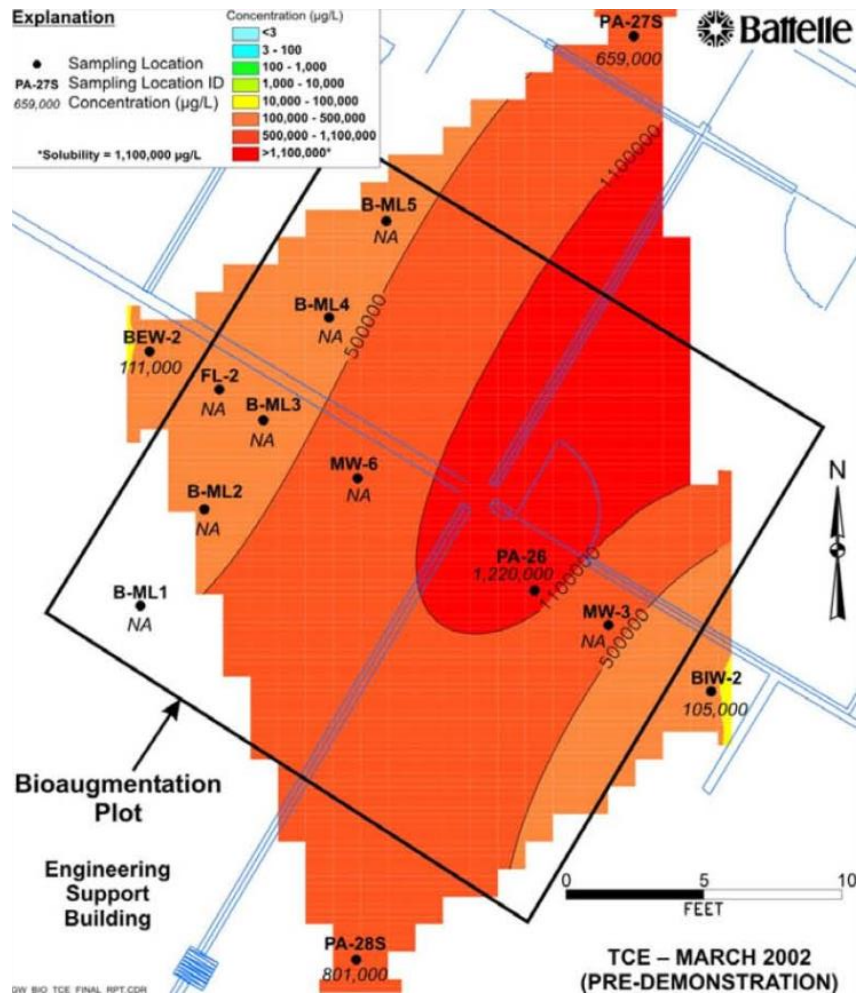


Figure 3. Pre-Demonstration Dissolved TCE Concentrations ($\mu\text{g/L}$) in Shallow Wells in the Treatment Plot (March 2002)

According to the data (Table 4) of chlorinated hydrocarbons and ethylene in the groundwater fed back from Central Well (that is, performance evaluation well), it can be seen that by injecting ethanol for biostimulation, the reduction speed of TCE to *cis*-1,2-DCE and *trans*-1,2-DCE increased.

Table 4. Monitoring Results of the Biostimulation and Bioaugmentation Demonstration in Center Well PA-26

	Pre-Demo	Dec 2002	Mar 2003	Post-Demo	Long Term
	$\mu\text{g/L}$	$\mu\text{g/L}$	$\mu\text{g/L}$	$\mu\text{g/L}$	$\mu\text{g/L}$
TCE	1220000	7460	13800	239	<10
<i>cis</i> -1,2-DCE	31600	94700	19400	780	62.4
<i>trans</i> -1,2-DCE	<1000	350	419	436	143
VC	<1000	3430	103000	8040	161
Ethene	573	30	2310	22900	4380

Pre-demonstration = March 2002; During Biostimulation = December 2002; During Bioaugmentation = March 2003; post-demonstration = June 2003. Long-Term: January 2004

In the biostimulation phase, the TCE concentration in the groundwater of the central well was suddenly reduced from the original 1220000 $\mu\text{g} / \text{L}$ to 7460 $\mu\text{g} / \text{L}$, and the removal rate exceeded 99%. The concentration of *cis*- 1,2-DCE has nearly tripled. This indicates that the injection of ethanol actually accelerates the reduction of TCE. The concentration of VC has also increased, presumably because the injection of a large number of electron donors has made indigenous microorganisms more active, and dechlorinated DCE to generate VC.

After the injection of KB-1 TM, the TCE concentration in the groundwater of the central well eventually decreased to 239 $\mu\text{g} / \text{L}$ after undergoing an increase, and further decreased in the monitoring half a year later, the concentration was less than 10 $\mu\text{g} / \text{L}$. It can be judged initially that the reduction effect of KB-1 TM on TCE in this demonstration is worthy of recognition. The concentration of *cis*-1,2-DCE in groundwater dropped further. At the end of the demonstration, the concentration of *cis*-1,2-DCE in groundwater was only 780 $\mu\text{g} / \text{L}$, and it was also observed that the concentration dropped further in the monitoring half a year later.

The concentration of VC in groundwater increased significantly, from 3430 $\mu\text{g} / \text{L}$ to 103000 $\mu\text{g} / \text{L}$, indicating that the exogenous dechlorination microorganisms injected into the treatment plot can promote the reduction reaction. At the end of the demonstration, the VC concentration decreased significantly, and the ethylene concentration increased rapidly, indicating that a large amount of VC was dechlorinated and reduced to non-toxic ethylene. The purpose of the demonstration was achieved. In addition, the concentration of all TCE by-products was further reduced after half a year of monitoring, indicating that the injected exogenous dechlorination microorganisms still maintain a high activity in the test area.

Table 5 lists the concentration of TCE and its degradation by-products in the groundwater in different wells in the treatment plot. As shown in Figure 1-1, the selected six wells are located in different directions, and the original groundwater TCE concentration is also in three different levels. The central well PA-26 detected the highest TCE concentration in groundwater, followed by PA-27 and PA-28S, and the lowest TCE concentration was BIW-2 and BEW-2.

Table 5. TCE Degradation Byproducts in the Treatment Plot Before, During, and After the Demonstration

Well ID	Pre-Demo	Dec 2002	Mar 2003	Post-Demo	Pre-Demo	Dec 2002	Mar 2003	Post-Demo
	TCE ($\mu\text{g} / \text{L}$)				<i>cis</i> -1,2-DCE ($\mu\text{g} / \text{L}$)			
PA-26	1220000	7460	13800	239	31600	94700	19400	780
PA-27S	659000	347000	379000	168000	67300	16900	186000	219000
PA-28S	801000	69200	68200	67500	28100	95100	162000	136000

BIW-2	105000	117000	93000	<20	45700	30000	54300	11800
BEW-2	111000	5750	79600	227	55600	3360	65400	19800
	<i>trans</i> -1,2-DCE (µg / L)				Vinyl Chloride (µg / L)			
PA-26	1220000	7460	13800	239	<1000	3430	103000	8040
PA-27S	300J	320J	420J	822	520	100J	28700	52800
PA-28S	170J	321	480	360J	<1000	7420	55800	3720
BIW-2	370	139	307	428	161	179	16400	30900
BEW-2	206	24.4	409	464	325	69	17600	44900

Well IDs: S = shallow well (Upper Sand Unit) BIW-2 = injection well; BEW-2 = extraction well.

Pre-demonstration = March 2002; During Biostimulation = December 2002; During Bioaugmentation = March 2003; post-demonstration = June 2003. Long-Term: January 2004

From the change of TCE concentration and degradation by-product concentration in Figures 4, 5, and 6, the trend of TCE changes in different wells is almost the same. That is to say, using the combination of biostimulation and bioaugmentation, the degradability in groundwater with different TCE concentrations is almost the same.

In terms of the changes of *cis*-1,2-DCE concentration in different wells, the trends of other wells are completely different from the trend of central wells, especially well PA-27S, until the end of treatment, *cis*-1,2-DCE concentration has kept increasing.

Regarding the changes of Vinyl Chloride (VC) concentration in different wells, except for PA-28S, the trends of the other three wells are completely different from that of the central well, and the VC concentration also has kept increasing.

The unusually high probability of the above trends is due to the influence of the flow direction. The direction of groundwater flow in the test plot is from north-northeast to south-southwest. Therefore, the well PA-28S located downstream of the central well is more likely to receive the propagation of electrons and DHC, so that its TCE and its degradation by-product concentration change trends are much closer to that of the central well. The concentration of VC in the other three wells continued to rise, probably because the reductive dechlorination reaction continued. Since the long-term test is limited to the central well, the subsequent degradation in these three wells is unknown.

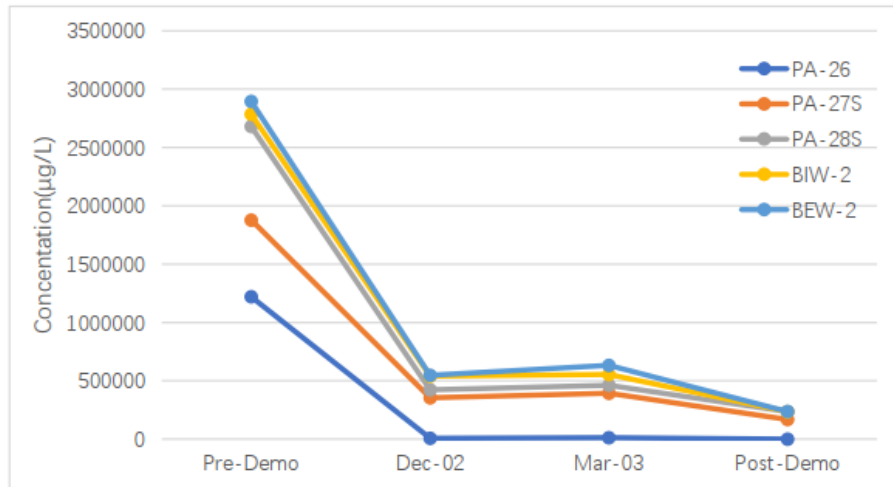


Figure 4. Change of TCE concentration in groundwater (U.S. EPA,2003)

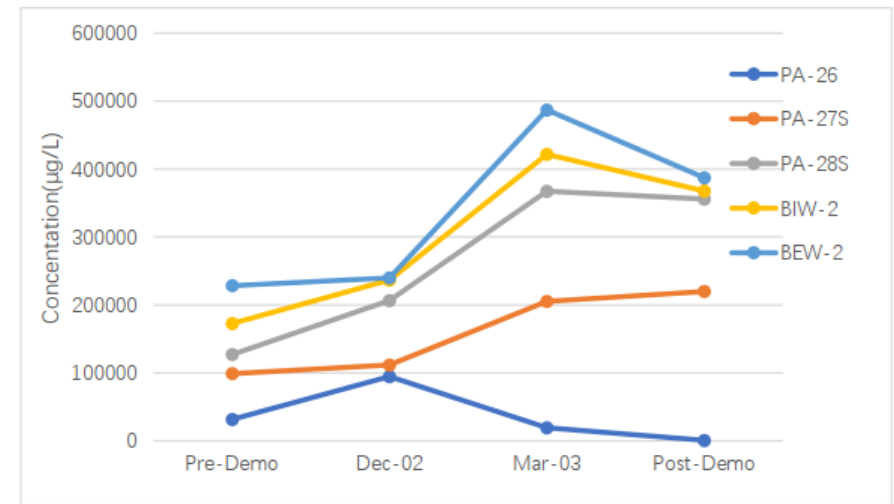


Figure 5. Change of *cis*-1,2-DCE concentration in groundwater (U.S. EPA,2003)

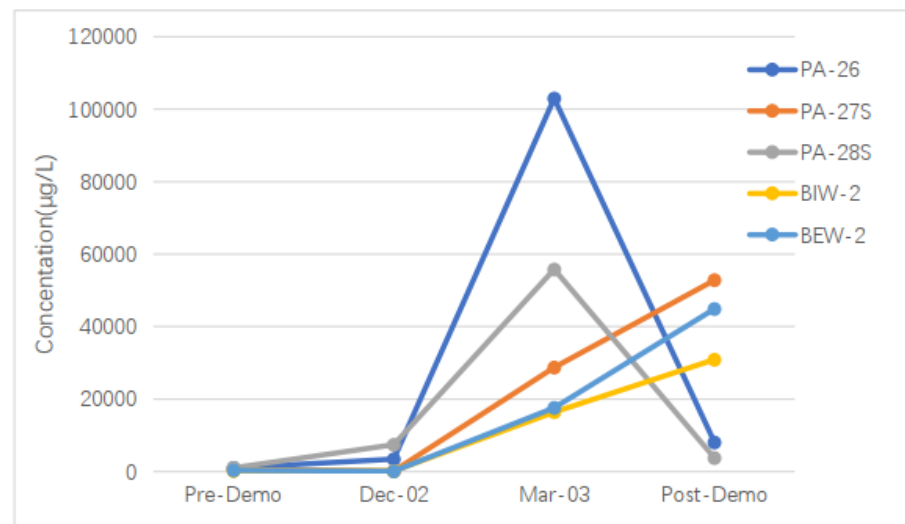


Figure 6. Change of Vinyl Chloride concentration in groundwater (U.S. EPA,2003)

In addition, because the degradation of chlorinated hydrocarbons by DHC theoretically produces a large amount of hydrochloric acid, the change in pH is also worthy of attention. During the demonstration, the pH of the shallow well fluctuated within a relatively narrow range. In the central well PA-26, the pH increased from 6.55 during the pre-demo sampling period to 7.96 in March 2003, and then decreased to 6.5 during the post-demo sampling period. There was no significant decrease.

3.2 Case B- Biostimulation alone in Florida, USA

In case B, Emulsified Zero-Valent Iron (EZVI) was injected into the treatment plot. The goal was set before the demonstration. The goal was to remove 50% of the TCE in upper sand unit. Before the demonstration, the total amount of TCE in the soil was 28kg. After the demonstration, the amount of TCE in the soil fell to 11.7g, and the removal rate of TCE was 58%. Therefore, the demonstration reached the expected goal, and the removal rate of TCE in the upper sand unit exceeded 50%.

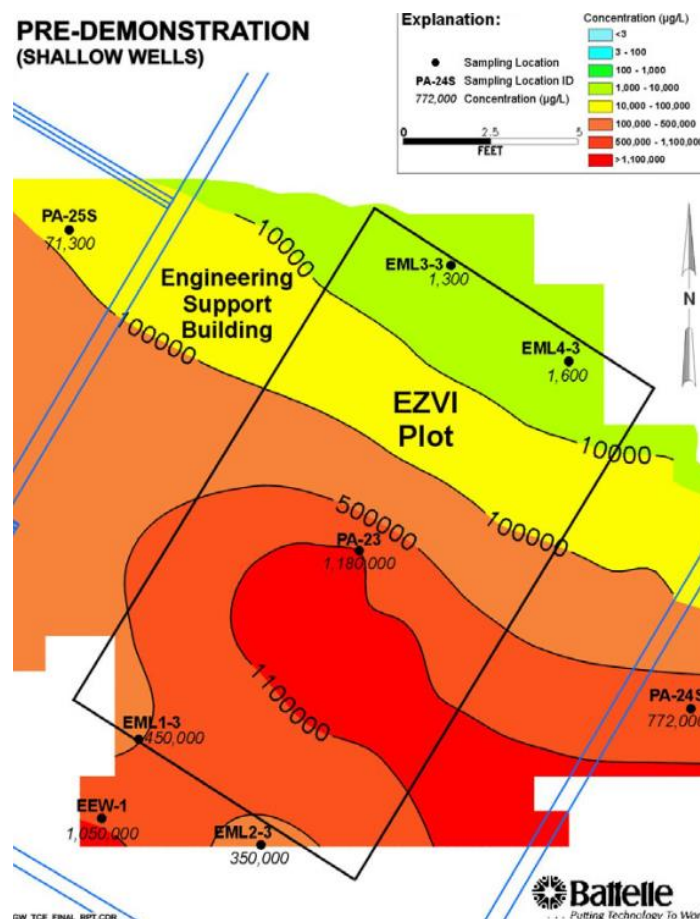


Figure 7. Pre-Demonstration Dissolved TCE Concentrations (µg/L) in Shallow Wells in the Treatment Plot (March 2002)

Through the characterization pre-demonstration, the typical exemplary distribution of TCE in groundwater in the test area is drawn, as shown in Figure 7, the central well

PA-23 is located in the area with the highest TCE concentration in the test plot. Therefore, by monitoring the data of this well, the reduction and dechlorination of TCE in groundwater after injection of EZVI can be determined, and the reduction and dechlorination of TCE in soil can also be determined from the side.

Table 6 shows the changes in the concentration of TCE and its degradation by-products in groundwater in the central well pre, during and post demonstration. Five months after the EZVI injection, the TCE concentration decreased significantly, from 1,180,000 µg / L to 92100 µg / L, the TCE reduction exceeded 90%, and *cis*-1,2-DCE increased slightly.

Table 6. Monitoring Results in Groundwater Before, During and After the Demonstration in Center Well PA-23

	Pre-Demo µg / L	During-Demo µg / L	Post-Demo µg / L	Long-term µg / L
TCE	1180000	92100	8790	2 J
<i>cis</i> -1,2-DCE	16900	17,900	169,000	870
<i>trans</i> -1,2-DCE	<1000	68J	245	NA
VC	<1000	53J	21600	<1000
Ethene	76	10	1680	9280

Pre-demonstration = March 2002; during the demonstration = August 2002; post-demonstration = November 2002 Long-term = March 2004.

NA=Not analyzed.

After the demonstration, the TCE concentration was further reduced to 8,790 µg / L, which was about 99% lower than the TCE concentration in the groundwater before the demonstration. The concentration of *cis*-1,2-DCE in groundwater has increased by nearly ten times and the concentration of VC has also increased significantly, so it can be concluded that EZVI can effectively improve the TCE of degraded water. Therefore, it is very difficult to use ZVI to dechlorinate and reduce TCE or PCE to obtain completely non-toxic products. In addition, due to the presence of DHC in the soil, it is speculated that after a long period of time, VC and *cis*-1,2-DCE can be further dechlorinated and reduced to harmless ethylene.

Table 7 lists the concentrations of TCE and its degradation byproducts in the groundwater in different wells in the treatment area. Because the groundwater conditions of Injection and Extraction Wells were not measured during the demonstration, only two wells were selected for comparison with the central well PA-23.

Table 7. TCE and its degradation by-products pre, during and post the demonstration in EZVI Test Plot

Well ID	Pre-Demo	During-Demo	Post-Demo	Long-term	Pre-Demo	During-Demo	Post-Demo	Long-term
TCE (µg/L)					<i>cis</i> -1,2-DCE (µg/L)			
PA-23	1180000	92100	8790	2 J	16900	17900	169000	870
PA-24S	772000	474000	12100	501	47400	15800	31700	63100
PA-25S	71300	69600	129000	<5	69200	9320	42800	<5
VC (µg/L)					Ethene (µg/L)			
PA-23	<1000	<1000	<1000	<1000	79.3	10	1680	9280
PA-24S	<1000	<1000	<1000	<1000	NA	NA	NA	NA
PA-25S	<1000	<1000	<1000	<1000	NA	NA	NA	NA

Well IDs: S = shallow well (Upper Sand Unit)

Pre-demonstration = March 2002; during the demonstration = August 2002; post-demonstration = November 2002, Long-term = March 2004.

Combined with Figure 7, the selected three wells are located in different directions, and the original groundwater TCE concentration is also in three different levels. In the central well PA-23, the highest TCE concentration was detected in groundwater, followed by PA-24S, and the lowest TCE concentration was PA-25S.

It can be seen from Figures 8 and 9 that the TCE and *cis*-1,2-DCE concentration change trends of the other two wells are different from the trend in the central well. As far as the concentration of TCE is concerned, the trends of the three wells are generally similar. Thirteen months after the end of treatment, the TCE concentration in groundwater was much lower than pre-demonstration. In my opinion that ZVI has a long-term effect on the stimulation of dechlorinable microorganisms in groundwater.

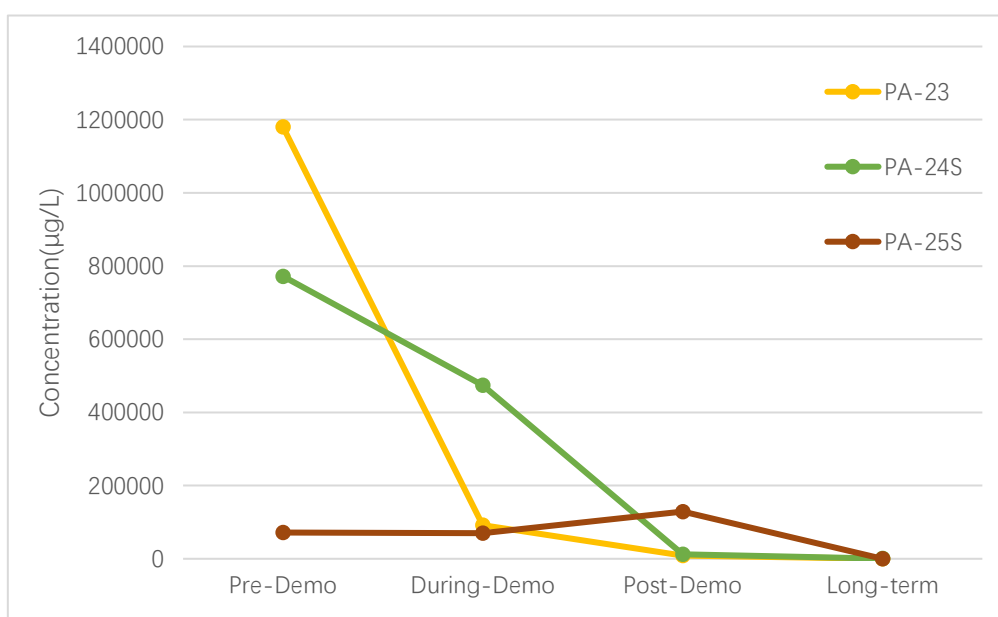


Figure 8. Change of TCE concentration in groundwater (U.S. EPA,2004)

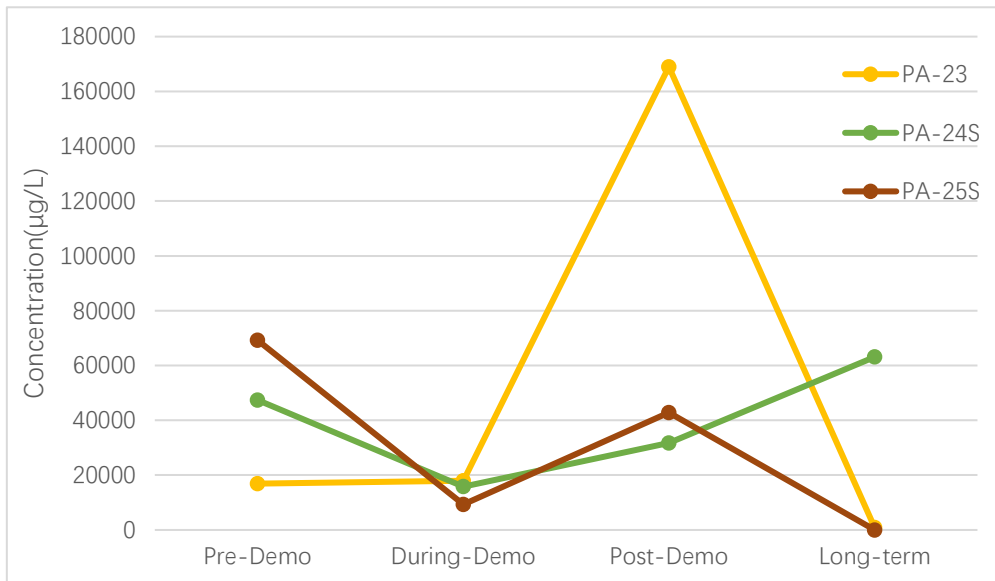


Figure 9. Change of *cis*-1,2-DCE concentration in groundwater (U.S. EPA,2004)

At the end of the demonstration, except for the well PA-24S, *cis*-1,2-DCE in the groundwater in other wells reached a peak. In the thirteenth month after the test, the concentration of *cis*-1,2-DCE decreased significantly, much lower than before the demonstration. However, in the long-term test, the concentration of *cis*-1,2-DCE in the groundwater of well PA-24S continued to rise. It can be seen from Figure 2-1 that the position of each well shows that the possibility of water flow affecting the well PA-24S receiving the electron donor is relatively small. Since the test did not continue since then, the specific situation is unknown. It can be seen from Table 7 that the concentration of VC does not change much, so it can be concluded that the stimulation of indigenous microorganisms by EZVI injection will generally stay in the *cis*-1,2-DCE stage.

The pH value of groundwater in shallow wells rose from 6.4 to 6.6 before the demonstration and rose to 7.0 to 7.2 during the demonstration. The increase in pH is the result of the production of OH⁻ due to the corrosion of ZVI in water. However, the observed increase in pH is much smaller. Generally, the addition of ZVI will increase pH up to 10 or 11. In addition, a large increase in DHC was detected in the treatment plot. Hydrochloric acid produced by the reductive dechlorination of TCE by DHC neutralizes OH⁻ produced by EZVI, which makes the pH rise only slightly. From this, it can be concluded that the use of EZVI as a biostimulant is clearly significant.

From the perspective of cost structure, only EZVI products cost \$ 25,000, accounting for 7% of the total cost. Design and Installation of Recirculation System and wells (accounting for 21%) and Performance monitoring and post treatment characterization (accounting for 21%), followed by Data evaluation and reporting (accounting for 18%) and Injection Method Evaluation / Testing (17%).

In case A and case B, ethanol (organic substrate) and EZVI (zero-valent iron) were used as biostimulants, respectively. Adding biostimulants can effectively accelerate the degradation rate of chlorinated hydrocarbons, and the removal rate of chlorinated hydrocarbons can basically exceed 50%.

In order to further compare the impact of biostimulation on the degradation of chlorinated hydrocarbons, since hydrogen provides electrons at a rate second only to zero-valent iron, the case of using hydrogen for biostimulation was selected. For convenience of discussion, this case is simply referred to as case C.

3.3. Summary of cases

Table 8 summarizes the results from the two cases in this thesis, including bioremediation methods, bioremediation products used, major pollutants, mass or concentration of major pollutants before and after remediation, pollutant removal rate, changes in DHC abundance, and some project costs.

Table 8. A Brief Summary of Results of Cases

Case No	A	B
Major Pollutant	TCE	
Methods	BS+ BA	BS
Removal rate	>98%	58%
Non-toxic degradation	Y	N
DHC Abundance	Increased	Increased
Cost	\$392226 49(\$/c.f.)	\$352000 94(\$/c.f.)

Note: BA= Bioaugmentation, BS= Biostimulation

Y=Yes, N=No

4. Discussion

Both of the above cases are from the US EPA's in-situ soil bioremediation demonstration of soil contaminated with chlorinated hydrocarbons. Remediate the soil contaminated with chlorinated hydrocarbons using biostimulation + bioaugmentation (Case A) and biostimulation (Case B) methods, respectively. The removal rate of TCE were >98% and 58%, respectively. It is important to emphasize that only two cases cannot fully reflect the application of in-situ bioremediation products in practice, but by comparing the situation of the two, it is helpful for the initial understanding of its actual use.

Using bioaugmentation to remediate the soil will reduce the probability of by-products remaining in the soil and generate a large amount of non-toxic and harmless ethylene, so that the entire remediation reaches the non-toxic end point. The soil remediation company REGENESIS (2015) uses DHC-rich product BDI Plus to remediate soil contaminated with PCE. In the last year of treatment, the total amount of PCE has been greatly reduced, the removal rate of PCE has reached 92%, and a large amount of ethylene is produced at the end of the treatment, so that the remediate ends with non-toxicity. This result is similar to case A. The CDM Smith Environmental Processability Laboratory in Bellevue, Washington also reached a similar conclusion. In the miniature experiment, the use of bioaugmentation can reach the non-toxic end point, especially when both biostimulation and bioaugmentation are used to remove TCE in soil. The removal rate of TCE is greater than 99% (Charles, 2012).

Using only ZVI to remediate soil contaminated by TCE has a very low removal rate compared to bioaugmentation. And there is a high probability of "DCE pause ", that is, the degradation of TCE is suspended in the by-product DCE, it is difficult to continue to degrade DCE to VC, let alone produce non-toxic ethylene. Both DCE and VC, which are by-products generated during the degradation of polychlorinated hydrocarbons, are highly biotoxic (Zhou et al., 2005). Newell et al. (2013) injected mixed gas containing hydrogen (biostimulation) to remediate soil contaminated by TCE, reducing TCE mass by 56%, increasing DCE mass by 24% and the amount of VC did not change significantly which means "DCE pause " also occurred.

Therefore, only cases that use bioaugmentation to remove chlorinated hydrocarbons can achieve non-toxic degradation, and the removal rate of chlorinated hydrocarbons exceeds 90%. While cases that use only biostimulation have a removal rate of chlorinated hydrocarbons of no more than 60%, and a "DCE pause" has occurred, which is difficult complete non-toxic degradation.

Table 8 shows the cost cases A and B. The costs are \$392226 and \$352000, respectively. The remediate cost per cubic yard is \$49 and \$94, respectively. Generally speaking, the cost of bioremediation is higher. The cost of Case B is relatively high, and its cost per cubic yard is almost twice that of Case A. Combined with the processing results, the cost of DHC-rich products has good processing effect, medium price, and long-term effect. Combined with the treatment results, the treatment effect of bioaugmentation which use DHC-rich products is better than biostimulation, the price is much lower than biostimulation, and it has a long-term effect. Therefore, bioaugmentation should be the priority for remediation of chlorinated hydrocarbon pollution. The use of bioaugmentation can not only reach the non-toxic end point, protect the environment and human health, but also save costs and produce long-term effects. To a certain extent, it can prevent the continuous pollution of chlorinated hydrocarbons.

5. Conclusion

This thesis has investigated the application of two different ways of in-situ soil bioremediation (biostimulation alone and the combination of biostimulation and bioaugmentation). Based on the results, the following concluding remarks are made.

First, in terms of the ability to degrade chlorinated hydrocarbons. The removal rate of TCE by biostimulation alone is not high, not more than 60%, and usually occurs “DCE pause”. The removal rate of TCE by bioaugmentation is significantly higher than that of biostimulation, up to over 80%. Furthermore, the processing capacity of bioaugmentation is higher than biostimulation. Under the action of biostimulation and bioaugmentation, the degradation effect of polychlorinated hydrocarbon can reach more than 98%.

Secondly, for the degradation cycle of chlorinated hydrocarbons, the treatment duration of biostimulation is generally six months, and the treatment duration of bioaugmentation is longer than that for biostimulation, but it generally does not exceed one year. This may be due to the complete degradation of chlorinated hydrocarbons as shown in Figure 1 under bioaugmentation.

Thirdly, the degradation of PCE or TCE can produce non-toxic ethylene only under bioaugmentation and realize non-toxic degradation. In terms of costs, bioremediation costs are significantly high, especially for biostimulation.

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Tianyu Qin



PO Box 823, SE-301 18 Halmstad
Phone: +35 46 16 71 00
E-mail: registrator@hh.se
www.hh.se