Treatment trains for the remediation of aquifers polluted with MTBE and other xenobiotic compounds

Tsitonaki, Aikaterini; Bjerg, Poul Løgstrup; Smets, Barth F.; Mosbæk, Hans

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Aikaterini Tsitonaki

Department of Environmental Engineering

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Address:	Department of Environmental Engineering DTU Environment Technical University of Denmark Miljoevej, Building 113 DK-2800 Kgs. Lyngby Denmark
Phone reception:	+45 4525 1600
Phone library:	+45 4525 1610
Fax:	+45 4593 2850
Homepage:	http://www.env.dtu.dk
E-mail:	reception@env.dtu.dk

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PREFACE

This thesis comprises the research carried out for a PhD project from February 2005 until April 2008 at the Technical University of Denmark, Department of Environmental Engineering (DTU Environment). The main supervisor was Professor Poul L. Bjerg and the co-supervisors were Professor Barth F. Smets and Associate Professor Hans Mosbæk, all from DTU Environment. The project was funded through a PhD scholarship by the Technical University of Denmark. This thesis is composed of a summary, a conference paper, three journal papers (1 published, 1 accepted for publication and 1 manuscript), and a technical note.

I. **Tsitonaki, A.**, Mosbaek, H., Bjerg, P.L., 2006. Activated persulfate as a first step in a treatment train. Paper D-77, In: Proceedings of the Fifth International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2006), Battelle Press, Columbus, OH, ISBN 1-57477-157-4.

II. **Tsitonaki, A**., Smets, B.F., Bjerg, P.L. 2008. Effects of heat-activated persulfate oxidation on soil microorganisms. *Water Research* 45 (4-5), 1013-1022.

III. **Tsitonaki, A.**, Petri, B., Crimi, M. Mosbæk, H., Siegrist, R.L., and Bjerg P.L. *In situ* chemical oxidation of contaminated soil and groundwater using persulfate: A review. *Accepted for publication in Critical Reviews in Environmental Science and Technology.*

IV. **Tsitonaki, A.**, Mosbæk, H., Smets, B.F., and Bjerg P.L. Effective treatment of xenobiotic compounds in groundwater by sequential persulfate oxidation and biodegradation. *Manuscript*.

V. **Tsitonaki, A.,** El Azhari, N., Smets. B.F., Real time PCR and RFLP analysis for the quantification of aromatic degraders. *Technical Note.*

The in-text references of the above articles are: Tsitonaki et al., **I**, **II, III, IV,** and **V**.

The papers are not included in this www-version but may be obtained from the Library at the Department of Environmental Engineering, Technical University of Denmark, Miljoevej, Building 113, DK-2800 Kgs. Lyngby, Denmark, email: library@env.dtu.dk.

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I would like to thank post doc researcher *Najoi El Azhari* who guided and helped me through molecular microbiology procedures with patience and expertise. Fellow PhD student *Sanin Musovic* and post doc researchers *Arnaud Dechesne* and *Akihido Terada* offered help and advice on numerous occasions.

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During my visit to Canada I enjoyed the hospitality and benefited from discussions with Professors *Jim Barker, Neil Thomson,* and *Steven Forsey* at the University of Waterloo. In Colorado, Associate Professor *Junko Munakata-Marr* was very generous and hospitable.

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This thesis is dedicated to Henrik; it is his fault I ended up doing a PhD in Denmark.

ABSTRACT

This thesis consists of a summary of the subject "Treatment trains for the remediation of aquifers polluted with MTBE and other xenobiotic compounds" along with 5 papers describing the work carried out during this Ph.D. project.

Contaminated aquifers often present challenges (complex contaminant mixtures, high contaminant levels, hydrogeological heterogeneities) that complicate the remediation process and result in very high remediation costs, if stringent clean-up goals need to be met. Combining different technologies in an integrated strategy can help overcome the limitations of individual technologies and lead to cost-efficient remediation. Such combinations are also known as "treatment trains". This thesis has focused on investigating the combination of *in situ* chemical oxidation (ISCO) and bioremediation.

ISCO is a popular remediation technology that involves the injection of chemical agents in the subsurface for the removal of organic contaminants from soil and groundwater. This project has focused on persulfate, the newest oxidant used for ISCO. Persulfate has shown promising results but knowledge and documentation of field applications are still sparse.

A critical review of the existing scientific literature on the use of persulfate for ISCO was carried out. It was established that persulfate can be effective towards many of the commonly targeted organic contaminants, such as gasoline components, chlorinated solvents, creosote compounds and others. The efficiency of ISCO with persulfate depends on the competition kinetics between contaminants, activation aids and reactive species in the soil and groundwater system. The reviewed literature suggests that heat activation is the most effective activation technology, but when upscaling, heating the aquifer can be a challenge. It was identified that more research should be directed on the interactions of persulfate with soil and groundwater components and on upscaling issues in order to design more successful ISCO systems.

Experimental work investigated the effectiveness of persulfate towards MTBE, Trichloroethylene (TCE) and 1,1,1-Trichloroethane (TCA) and a comparison of different activation methods (heat and iron) in both aqueous and soil-water systems. Heat-activated persulfate oxidation at 40 ^{o}C was the most effective method and achieved 98.6% removal of MTBE, and 89.9% of TCE in the soil-water systems within 24 hours. The ability of activated persulfate to degrade TCA was also confirmed. Heat activation was also the only method to achieve full mineralization of MTBE. A supplementary experiment in finding the optimal activation temperature for persulfate

showed that there is a threshold value, above which increasing temperature leads to unproductive contaminant decomposition without additional contaminant destruction.
The effectiveness of ISCO can be limited by the lack of contact between the oxidant and the contaminated zone; cost efficiency also decreases at lower concentrations. It can therefore be advisable to apply ISCO in highly contaminated zones and follow up with natural or engineered bioremediation for removing the residual contamination. ISCO can also be applied for multi-component contaminations to remove compounds that are resistant to biodegradation under the prevailing aquifer conditions, and followed by bioremediation for removing the biodegradable part of the contamination.

This work investigated the combination of ISCO and bioremediation, with main focus on the use of activated persulfate for ISCO followed by intrinsic bioremediation.

Challenges related to the coupling of ISCO and bioremediation result from the oxidants' toxicity on microorganisms and ISCO induced environmental changes that inhibit microbial processes. The effects of heat-activated persulfate on indigenous microorganisms and microcosms augmented with *Pseudomonas putida* KT2440 were studied in laboratory batch reactors with aquifer material. Microscopic enumeration was used to measure the changes in cell density and acetate consumption was used to evaluate metabolic activity after exposure to activated persulfate. The cell enumerations showed that persulfate concentrations up to 10 g/L did not affect the indigenous microorganisms but were detrimental to *P.putida* survival. Acetate consumption was inhibited at the highest persulfate dose (10 g/L). These results emphasize the necessity of using multiple toxicity assays and indigenous cultures in order to realistically assess the potential effects of ISCO on soil microorganisms.

The combination of ISCO with heat activated persulfate and bioremediation was also studied in a column reactor laboratory set up. In these experiments, the contamination comprised of a mixture of creosote contaminants and MTBE. It was found that preexisting natural biodegradation processes persisted after treatment with persulfate concentrations of up to 30 g/L. Moreover these experiments highlighted the advantages of treatment trains, as MTBE, which was resistant to the natural biodegradation processes, was successfully removed by heat activated persulfate.

A comparison to other studies investigating the coupling of ISCO and bioremediation suggested that ISCO is more compatible with aerobic biodegradation processes, partly due to the generation of oxidized conditions. Also, the effects of activated persulfate on soil microorganisms are less damaging than those of Fenton's reagent and hydrogen peroxide.

To conclude, combining ISCO and bioremediation is a viable option for dealing with complex contaminant mixtures, and high contaminant concentrations where bioremediation alone would not be effective and ISCO alone would not be cost-efficient. In order to optimize the design of such treatment trains further research is needed on a) the effects of different oxidants on aquifer microorganisms under realistic conditions, b) whether the effects of ISCO on aquifer microbial communities favor specific degraders, and c) the duration of the changes in redox conditions and other environmental factors after ISCO.

DANSK SAMMENFATNING

Denne afhandling består af en sammenfatning af emnet "Sekventiel oprensning af grundvandsmagasiner forurenet med MTBE og andre miljøfremmede stoffer" samt 5 artikler, der dækker arbejdet udført under ph.d. projektet.

Komplekse forureningsblandinger og feltforhold kan vanskeliggøre en økonomisk forsvarlig oprensning, hvis stramme grundvandskvalitetkriterier skal overholdes. Kombination af forskellige oprensningsmetoder i en integreret sekventiel oprensningsstrategi, også kaldt "treatment trains", kan imødekomme disse vanskeligheder. Denne afhandling har fokuseret på kombinationen af *in situ* kemisk oxidation (ISCO) og *in situ* biologisk nedbrydning.

ISCO er en populær afværgeteknologi, der omfatter injektion af forskellige oxidationsmidler i undergrunden, hvor de kan fjerne forureningskomponenter. Dette arbejde har fokuseret på persulfat, som er det nyeste oxidationsmiddel for ISCO. Persulfat har vist lovende resultater, men viden og dokumentation af felterfaringer er endnu begrænsede.

En kritisk gennemgang af den videnskabelige litteratur om persulfat er blevet udført som del af dette projekt. Det viste sig at persulfat kan være effektiv overfor mange almindelige forureningsstoffer som benzin-relaterede stoffer, tjære-komponenter, klorerede opløsningsmidler, osv. Effektiviteten afhænger af konkurrerende reaktioner mellem persulfat og forureningen, de aktiverende stoffer og jordens komponenter. Den eksisterende litteratur tyder på at varme-aktivering er den mest effektive aktiveringsmetode af persulfat, selvom denne kan være vanskelig at udføre i felten. Det er blevet konstateret at mere forskning bør rettes mod interaktioner mellem persulfat og jord- og grundvandskomponenter samt opskaleringsproblemstillinger for at forbedre planlægning og implementering af feltanvendelser.

Forsøgsarbejde udført under ph.d. projektet havde til formål at undersøge persulfats effektivitet mod MTBE, TCE og TCA i vand og i jord- og vand-systemer ved brug af forskellige aktiveringsmetoder (varme og jern). Varme-aktiveret persulfat var det mest effektive metode og kunne fjerne 98.6% af MTBE og 89.9% af TCE i jord- og vandsystemet indenfor 24 timer. Det blev også bekræftet at persulfat kan nedbryde TCA. Varme-aktivering var den eneste metode der resulterede i komplet mineralisering af MTBE. Supplerende forsøg blev udført for at finde den optimale aktiveringstemperatur. Konklusionen var at forøgelse af temperaturen til over 45-50 ^{o}C resulterer i kraftigere nedbrydning af persulfat uden at det sker højere fjernelse af forureningsstoffer.

ISCO-effektivitet er ofte begrænset af manglende kontakt mellem oxidationsmidlet og forureningen; og metoden bliver mindre omkostningseffektiv ved lave koncentrationer. Det kunne derfor være hensigtsmæssigt at kombinere kemisk oxidation med efterfølgende naturlig eller stimuleret biologisk nedbrydning. ISCO kan i dette tilfælde blive brugt til at fjerne den mest resistente del af komplekse forureningsblandinger, der ikke kan fjernes biologisk under de eksisterede feltforhold, hvorefter bioremediering kan anvendes til fjernelse af biologisk nedbrydelige komponenter.

Dette ph.d. projekt har undersøgt kombinationen af ISCO og bioremediering. Fokus har ligget på anvendelsen af aktiveret persulfat efterfulgt af naturlig biologisk nedbrydning.

Kombination af ISCO og bioremediering kan blive mindre effektiv pga. oxidationsmidlernes toksicitet på mikroorganismer og de ændringer, ISCO medfører i akviferen (f.eks. iltning, pH sænkning), der kan være hæmmende for biologiske processer. Forsøgsarbejdet under projektet undersøgte effekten af varme-aktiveret persulfat på naturlige jordbakterier og laboratorie-dyrkede *Pseudomonas putida* KT2440 kulturer i laboratorie-mikrokosmer. Ændringer i antal af levende og døde bakterier blev målt ved mikroskopisk tælling og bakteriernes evne at forbruge acetat blev brugt til at evaluere deres aktivitet efter de var blevet udsat for forskellige koncentrationer af varme-aktiveret persufat. Persulfat koncentrationer op til 10 g/L reducerede ikke antallet af levende jordbakterier, men var hæmmende for *P.putida*. Acetat forbrug var også hæmmet i begge typer mikrokosmer. Disse resultater understreger at oxidationsmidlernes toksicitet bør undersøges i forhold til både antal og aktivitet af naturligt forekommende bakterier i stedet for ensartede undersøgelser.

Kombinationen af ISCO med varme-aktiveret persulfat og bioremediering blev undersøgt i kolonneforsøg. I disse forsøg, bestod forureningen af en blanding af MTBE og tjærestoffer. Tjærestofferne kunne fjernes ved naturlig biologisk nedbrydning før kemisk oxidation. Denne biologiske nedbrydning kunne forsætte også efter jorden var blevet behandlet med en høj dosis af persulfat 30 g/L. MTBE var i høj grad resistent overfor biologisk medbrydning men stoffet blev fjernet ved brug af persulfat. Dette understreger fordelen ved at kombinere oprensningsmetoder for at fjerne komplekse forureningsblandinger.

En sammenligning med andre studier om kombinationen af ISCO og bioremediering tyder på at ISCO er mest kompatibel med aerob bionedbrydning, delvist pga. de iltede forhold der opstår efter ISCO. Sammenligningen har også vist at persulfat virker mindre skadende på bakterier end Fentons reagens og hydrogen peroxid.

Konklusionen er at ISCO og bioremediering med fordel kan kombineres for at oprense komplekse eller kraftige forureninger i tilfælde hvor bioremediering alene ikke vil være effektiv, og ISCO alene ikke ville være økonomisk forsvarligt. Med hensyn til videre udvikling og feltanvendelse af sekventiel oprensning med ISCO og bioremediering er det tilrådeligt at undersøge følgende: a) effekter af de forskellige oxidationsmidler på jordbakterier under realistiske (akvifer-lignende) forhold, b) om ISCO medfører nogle ændringer i bakteriernes diversitet der kan være til fordel for specifikke bionedbrydningsprocesser, c) varighed af de geokemiske ændringer, som ISCO medfører i akviferen (f.eks. iltning, pH sænkning).

1. INTRODUCTION

Groundwater is a vital natural resource with a high ecological and economical value because it provides recharge to surface water bodies, and is a source of clean water for drinking, agricultural and industrial uses. Contamination from natural and anthropogenic sources threatens the quality of this resource. A variety of human activities can result in groundwater pollution. For example, gaswork sites where creosote is used for the production of fuel gas often result in groundwater contamination. Creosote is a mixture of hundreds of chemicals some of which are considered genotoxic or mutagenic [1]. Another common groundwater contaminant MTBE (Methyl-tertiary-butyl ether) that has been used as an antiknocking agent to petrol since the late 1970's in USA, and since 1985 in the European Union [2]. MTBE contamination mainly stems from gasoline releases. MTBE is the second most common contaminant in urban groundwater in USA [3] and poses a threat to groundwater quality primarily due to its strong odor and taste threshold.

In order to re-establish groundwater quality at already contaminated sites, engineers are implementing remediation efforts. Groundwater remediation technologies are a developing field, and new technologies or modifications of old technologies emerge continuously. In the last 10 years, *in situ* technologies where the contaminated groundwater is treated in the aquifer have gained popularity, due to their high performance compared to traditional pump and treat systems [4]. Contaminated sites can range from a "simple" case of a limited gasoline release, to mega-sites where large areas are contaminated to a variable extent with several different contaminants. Such conditions complicate the remediation process and can result in very high remediation costs, if stringent clean-up goals need to be met.

Combining different technologies in an integrated strategy can help overcome the limitations of individual technologies and lead to cost-efficient remediation. Such combinations are also known as "treatment trains" [5]. Treatment trains can include the following types: a) different remediation technologies targeting different contaminants, b) a fast and aggressive remediation technology is used to remove the main volume of the contamination, whereafter a low maintenance, cheaper and long term technology deals with the remaining pollution in the same contaminated zone. In this manner, a smaller area is treated by the most expensive technology. The first technology can often pave the way for the following through creating more favorable site conditions. Reuse of equipment can also lead to further cost reduction.

In situ chemical oxidation (ISCO) is a popular remediation technology that involves the injection of chemical agents (oxidants) in the subsurface for the removal of organic

contaminants. ISCO is suitable for a wide spectrum of contaminants even at high concentrations. Common oxidants include catalyzed hydrogen peroxide (Fenton's reagent), ozone and permanganate. Persulfate ($S_2O_8^{2-}$) is the newest ISCO oxidant to receive wide use [6] and has generally shown promising results [7,8]. However, knowledge and experience is still sparse compared to more established oxidants such as potassium permanganate, ozone, and catalyzed hydrogen peroxide.

The effectiveness of ISCO can be limited by the lack of contact between the oxidant and the contaminated zone; cost-efficiency also decreases at lower concentrations. It can therefore be advisable to apply ISCO in highly contaminated zones and follow up with a subsequent natural or engineered bioremediation step for removing the residual contamination. ISCO can also be applied for multi-component contaminations to remove compounds that may be resistant to biodegradation under the prevailing aquifer conditions, and followed by bioremediation for removing the biodegradable part of the contamination. However, the compatibility of ISCO with bioremediation is under question, because oxidants can also act as disinfectants. ISCO results in an increase of dissolved oxygen and redox potential in the aquifer. These conditions are favorable for aerobic biodegradation processes but may inhibit anaerobic degradation processes. Recent studies on the effects of ISCO on subsequent bioremediation efforts have produced conflicting results [9] and showed that the effects on different biodegradation processes can vary based on oxidant, contaminant, and the nature of the process. The effects of Fenton's and permanganate have been studied by several researchers [10-16], while little is known on the effects of persulfate. Most studies have investigated the combination of ISCO and bioremediation in soil systems [10,11,12,17,18]. The few studies in groundwater systems have focused on the inhibitory effects of ISCO an anaerobic biodegradation processes [15,16,19] but very little work has been carried out in groundwater systems.

This PhD study investigates the compatibility of the combination of *in situ* chemical oxidation and bioremediation in groundwater. The focus is on the use of activated persulfate for ISCO followed by intrinsic aerobic bioremediation. Specific objectives of the PhD study were:

1. To evaluate the current knowledge and experiences with the use of persulfate in ISCO through a literature review.
2. To investigate the potential of using activated persulfate against common contaminants and get a better understanding of the different activation methods through laboratory experiments.
3. To identify the impact of heat-activated persulfate on soil microorganisms in terms of microbial density and activity in an aquifer-representative laboratory set-up.

4. To investigate the performance of a treatment train consisting of ISCO with heat-activated persulfate and bioremediation in an aquifer-representative column reactor laboratory set-up in terms of i) contaminant removal for each treatment step, ii) the ability of natural biodegradation processes to resume after persulfate oxidation, and (iii) the effects of persulfate treatment on the abundance and diversity of specific degrading microorganisms using molecular microbiology tools.
5. To compare the effects of persulfate oxidation and bioremediation to that of other oxidants.

The summary part of this thesis provides an overview of *in situ* remediation technologies for common groundwater contaminants. The principal experimental work of this project evaluated the performance of ISCO and bioremediation against a contaminant mixture of MTBE and creosote components. In order to better understand the results of these experiments, MTBE and creosote pollution and remediation are discussed in detail in the summary. A combination of these contaminants may be uncommon at contaminated sites, but the choice was based on the following criteria: a) both MTBE and creosote are widespread contaminants, b) these compounds can be degraded aerobically, which is the expected state after ISCO, and c) the different compounds have very different mobility and susceptibility to natural biodegradation processes, which reflects the conditions at sites with complex contaminant mixtures. The summary also discusses ISCO and bioremediation in detail, and the combined use of these technologies in a treatment train for groundwater remediation. Experiences from soil remediation studies have been included in order to supply the limited knowledge from groundwater systems. Finally, conclusions and recommendations for future research are provided.

2. GROUNDWATER CONTAMINATION

This chapter gives a general brief introduction to groundwater contamination, defining the extent and manifold nature of the problem. Contamination by MTBE and creosote is discussed in detail, in order to give insight to the contaminants used in this project's experimental work.

2.1. Introduction to groundwater contamination

Groundwater is a vital natural resource. It acts as a reservoir from which good quality water can be abstracted for drinking and for use in industry and agriculture. It is also valuable in maintaining wetlands and river flows, acting as a buffer through dry periods. Groundwater accounts for over 95% of the earth's useable fresh-water resources; it is estimated that more than 2 billion people are directly dependant on aquifers for drinking water [20]. Over 75% of the European drinking water supply is obtained from groundwater [20].

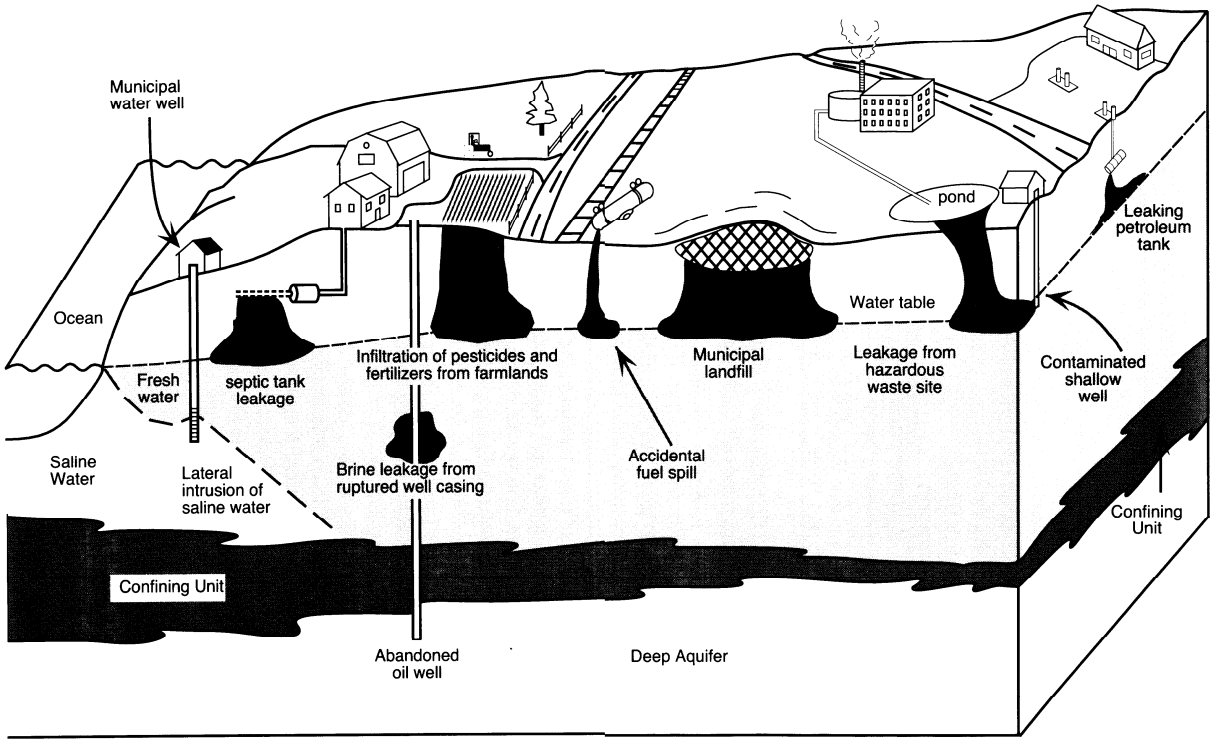


Figure 2.1. Sources and mechanisms of groundwater contamination. Reprinted from Bedient et al., 1997 [21].

Contamination from natural and anthropogenic sources threatens the quality of groundwater resources. A variety of human activities can result in groundwater pollution (Figure 2.1). They include accidental spills or deliberate disposals at industrial sites, leachates from landfills and surface waste ponds, leakages from

above/underground storage tanks and pipelines, etc. [21]. Diffuse sources such as pesticides and fertilizers from agriculture pose a threat at a regional scale. At local scale, point source pollution can be an intense threat to aquifer quality.

Groundwater contamination is often related to soil contamination of the overlying soil body. According to the European Environmental Agency (EEA) approximately 0.5 million sites in EEA member countries require clean-up and this number will rise by 50% by 2025. Organic xenobiotic compounds such as oil and gasoline compounds comprise the majority of the commonly found contaminants in soil and groundwater (Figure 2.2).

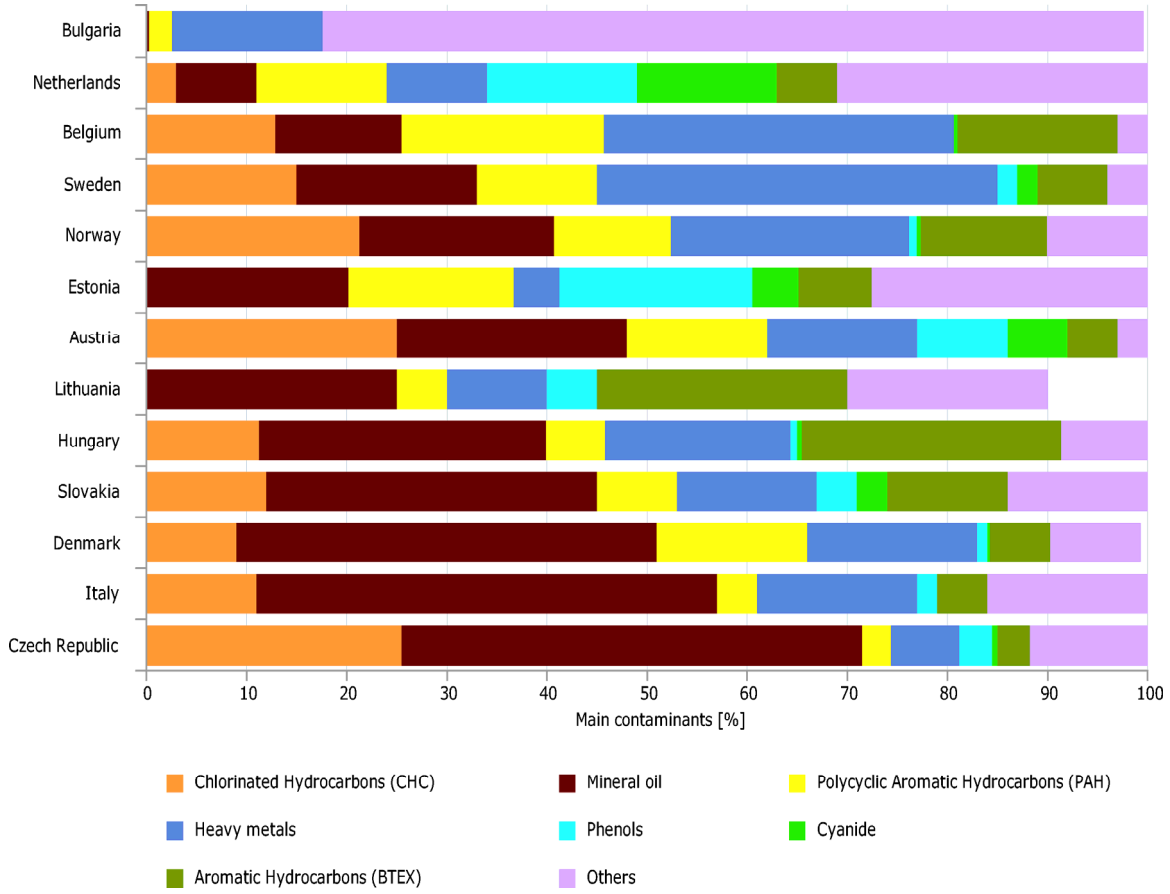


Figure 2.2. Main contaminants affecting soil and groundwater. Percentage of contaminated industrial or commercial sites by country. Data for Belgium refer to the Flanders Region only. Data for Italy refer to the Piemonte Region only. Copyright EEA, Copenhagen, 2006: http://dataservice.eea.europa.eu/atlas/viewdata/viewpub.asp?id=2323.

Today, great attention is devoted to the preservation of groundwater, also reflected in the introduction of the 2006/118/EC directive on the protection of groundwater against pollution and deterioration. However, it may take decades and significant capital to

clean up a legacy of contaminating activities in order to secure usability of the groundwater resources.

2.2. MTBE in groundwater

2.2.1. History and abundance

MTBE (Methyl-tertiary-butyl ether) has been used as an antiknocking agent to petrol since the late 1970's in USA, and since 1985 in the European Union [2]. MTBE in groundwater is typically found in connection with underground storage tanks and pipeline leakages of gasoline. MTBE is the second most common contaminant in urban groundwater in USA [3], and it has been detected in both shallow and deeper aquifers in Denmark [2].

2.2.2. Fate and transport

MTBE is a volatile, colorless liquid (at 20 ^{o}C, 1 atm) with a strong turpentine-like odor [22]. The most important physicochemical properties are summarized in Table 2.1 and the molecular structure is shown in Figure 2.3.

Table 2.1. Physicochemical properties of MTBE. This table is based on information from [22].

Property	Value
Chemical formula	$C_5H_{12}O$
CAS Number	1634-04-4
Molecular weight (g/mole)	88.15
Melting temperature (^{o}C)	-108
Boiling temperature (^{o}C)	55.2-55.3
Density (g/cm^3)	0.741
Vapor pressure (mmHg) @ 20 ^{o}C	245
logK_{ow}	1.06
Water solubility (mg/L) @ 25 ^{o}C	42000

The main release source of MTBE in the groundwater is gasoline spills. As gasoline moves towards the groundwater table, a fraction of MTBE may evaporate and form a gaseous plume. Due to its relatively low Henry's constant and high water solubility, MTBE can easily transfer from air to water or directly from gasoline to water. These properties combined with the low tendency to sorb to soil particles (logK_{ow}=1.06), makes MTBE a particularly mobile compound in the subsurface. Once it reaches the groundwater table, MTBE is mixed with the groundwater and migrates at the same rate, contrary to other benzene components, which are retarded by sorption to the soil [2].

Under normal aquifer conditions MTBE is generally resistant to biodegradation, although some bacterial communities seem to be adapting [23]. Several naturally

occurring microorganisms have been shown to directly or cometabolically degrade MTBE in laboratory experiments [24]. Under anaerobic conditions, MTBE degradation has been observed to a limited extent, at very slow removal rates [24].

2.2.3. Risks related to MTBE in groundwater

At typical environmentally relevant oral exposures, MTBE does not cause adverse health effects to humans with regards to neurological system, reproduction and development [25]. Classification of the carcinogenicity of MTBE is a continuous controversy. The substance has produced borderline results as there were indications of carcinogenicity in two animal species [26]. USEPA has classified MTBE as a potential human carcinogen [27] but EU considers MTBE unclassifiable [26]. MTBE is considered to express low ecotoxicity to freshwater and marine organisms with acute or chronic effects first arising at concentrations above 26 mg/L [28,29]. However, recent studies have shown that chronic exposure to low concentrations of MTBE (0.11mg/L) can cause reproductive dysfunctions in zebrafish [30] and some soil microorganisms e.g. *Streptomyces spp.* [31]. Still, the main risk from MTBE in groundwater is odor and taste nuisances, as individuals can detect MTBE at concentrations as low as 10 μg/L. Neither the EU, nor the USA have set drinking water thresholds for MTBE in groundwater yet. A threshold value of 5 μg/L in drinking water has been set by the environmental ministry in Denmark [32]. This value ensures the protection of the population from potential health effects and is also below the odor and taste detection limit.

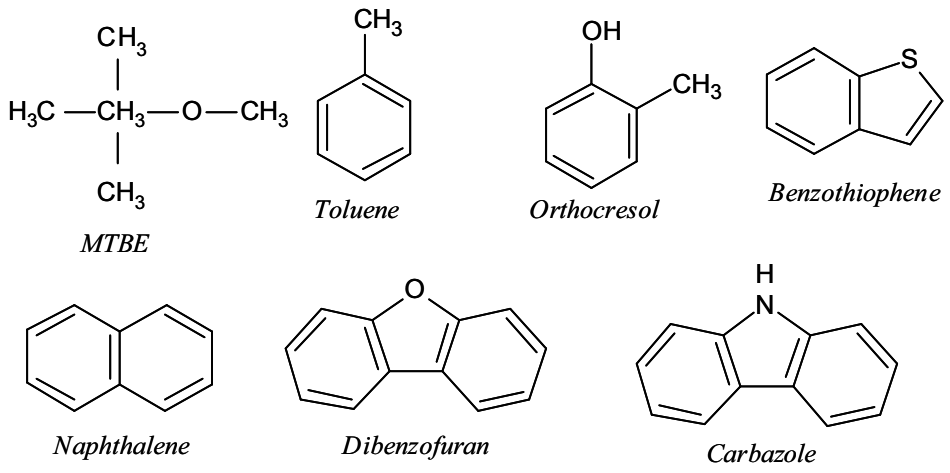


Figure 2.3. Molecular structure of the groundwater contaminants studied in this work.

2.3. Creosote compounds in groundwater

2.3.1. History and abundance

Coal tar creosote is a byproduct of the gasification process, where fuel gas is produced from coal in gaswork plants. At small gaswork sites, creosote was considered a waste product. At large gas plants it was stored and subsequently sold to the industry, where it could be used as a component of asphalt, fungicides and pesticides [33]. Through careless disposal, spills and leaking storage facilities, contamination of the soil and groundwater with creosote near gasworks has occurred. Subsurface pollution with creosote is considered a widespread problem in industrialized countries [34].

Creosote is a mixture of several hundred chemicals but only 20% of those are present in concentrations higher than 1%. The major classes of compounds are mono- and polyaromatic hydrocarbons, phenolics compounds, aromatic amines and NSO heterocyclic compounds [1,35]. Dyreborg [35] reviewed the composition of 5 different creosotes and found that the PAHs fraction comprises 50-90%, the MAHs 3%, and NSO compounds contribute approximately 15%.

Table 2.2. Physicochemical properties of the selected creosote compounds. From [1,36,37]

Name	Toluene	Orthocresol	Benzothiophene	Naphthalene	Dibenzofuran	Carbazole
Chemical Formula	C_7H_8	C_7H_8O	C_8H_6S	$C_{10}H_8$	$C_{12}H_8O$	$C_{12}H_9N$
CAS number	108-88-3	95-48-7	95-15-8	91-20-3	132-64-9	86-74-8
Molecular weight (g/mole)	92.15	108.15	134	128.18	168.2	167.22
Melting point (^oC)	-95	32-33.5	29-32	81-83	81-83	240
Boiling point (^oC)	110.6	191	221	217.7	285	355
Density (g/cm^3)	0.86	1.048	1.149	1.15	1.086	1.1
Vapor pressure (mmHg @ 25 ^oC)	28.7	0.25	0.238	0.03	<0.01	<0.01
logKow	2.69	1.99	3.11	3.35	4.74	3.29
Solubility in water (mg/L) @ 25 ^oC	515	26000	130	31	3.1	1.2

2.3.2. Fate and transport

Creosote spills behave as DNAPL [34,38]. The spill can be trapped in the pores of the vadose zone from where the volatile constituents can evaporate and the soluble compounds leach with infiltrating water towards the water table. The rate of dissolution is controlled by the effective solubility of the individual compounds. Groundwater contamination near gaswork sites is generally comprised of the water soluble fraction of creosote, i.e. MAHs, 2-3 ring PAHs, phenols and certain NSO compounds [33,38,39]. The molecular structure of selected creosote compounds that have been used in this work is shown in Figure 2.3. The physicochemical properties of these compounds are presented in Table 2.2. Biodegradation is an important mechanism for removing soluble

creosote contaminants from groundwater. The degradation of BTEX, phenols, and naphthalene in sandstone aquifers has been documented [40]. The biodegradation of creosote is dependant on mixture related interactions. While most of the soluble creosote compounds are considered biodegradable, inhibitory effects due to competition and toxicity. For example, the presence of NSO compounds can have inhibitory effects on the aerobic degradation of toluene, xylene, benzene and naphthalene [41,42]. Typical NSO compounds such as benzothiophene, thiophene and benzofuran can be degraded cometabolically under aerobic conditions [35]. By contrast, many NSO compounds were found persistent under anaerobic conditions, that are typical in most aquifers [43].

2.3.3. Risks related to creosote in groundwater

As discussed above, the natural attenuation of the individual contaminants may be inhibited by the complex interactions in creosote mixtures and/or oxygen limitations in aquifers. The toxic effects of many creosote compounds intensify this risk, and have brought focus on creosote remediation. According to the USEPA, coal tar creosote is a probable human carcinogen. The World Health Organization states that creosote expresses high genotoxicity and certain components are mutagenic. Degradation products of creosote components e.g. S-compounds can also be mutagenic [38]. Furthermore, creosote has a high ecotoxicity on aquatic organisms [1,38]. The toxicological effects of individual creosote compounds and their metabolites have not yet been evaluated in detail.

3. *IN SITU* REMEDIATION TECHNOLOGIES

3.1. Brief overview of *in situ* remediation technologies

To protect and preserve groundwater resources, scientists and engineers have devised many technologies to remediate contaminated aquifers. The main remediation strategies include: a) the physical removal of the contamination source (i.e. removal of leaking tanks, and other waste deposits by excavation), b) the containment of the source or plume by use of physical or hydraulic barriers, and c) mass reduction of the contamination using physical, chemical or biological treatment, *in situ, on site* or *ex situ*.

This chapter will focus on *in situ* mass reduction technologies for the remediation of groundwater. These technologies emerged around 1993, once it was documented that traditional, pump and treat systems (where groundwater was pumped out and treated *on site* or *ex situ*) failed to clean up groundwater to acceptable water quality levels [21]. This was the result of complicated site conditions, such as the presence of NAPL, or preferential flow pathways. There is a continuously increasing trend for choosing *in situ* over *ex situ* technologies in the last 20 years, especially in USA as illustrated in Figure 3.1 [4]. However, on the European market, despite an increasing effort to develop *in situ* remediation technologies, very few technologies receive recognition, and the vast majority of sites are treated with pump and treat, containment, or excavation [44].

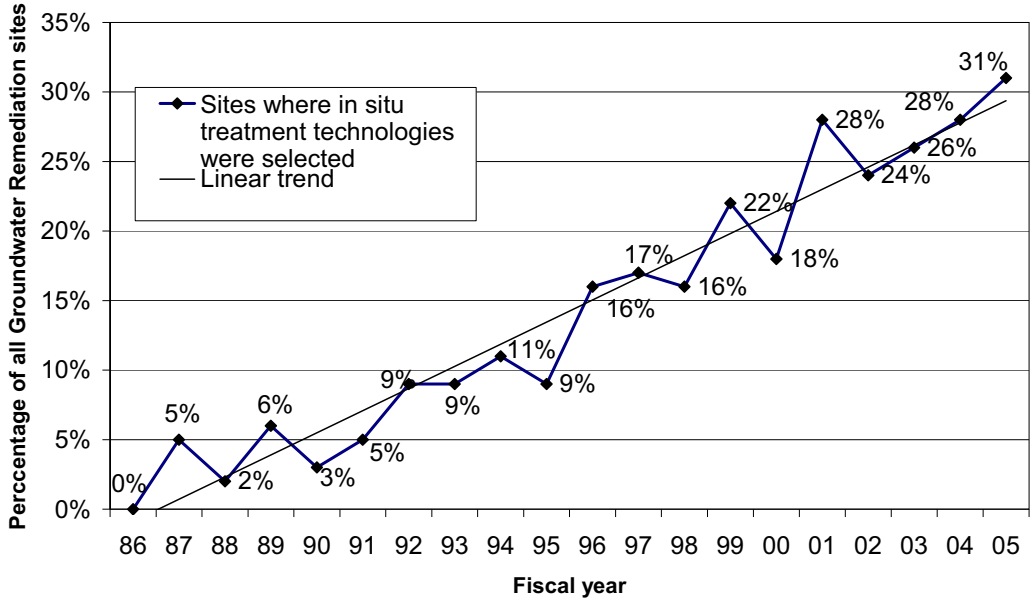


Figure 3.1. The increase in application of *in situ* technologies in groundwater remediation projects in the USA from 1986 to 2005. Redrawn from [4].

Table 3.1. Overview of *in situ* remediation technologies.

Technology	**Description** [4] Unless other is noted	Removal mechanism
***In situ* physical remediation technologies**		
Air sparging	Air or oxygen is injected into the aquifer to create a strip that removes VOCs towards the unsaturated zone from which they are extracted through soil vapor extraction (see below).	Mass transfer through volatilization
Electrical separation	A low density current is applied to mobilize contaminants in the form of charged species. Removal occurs by pumping near the electrode, or attachment of the contaminants to the electrodes.	Electrokinetics
Multiphase extraction	Uses a vacuum system to remove vapors and lower the water table. Contaminants in the newly exposed vadose zone are then accessible to vapor extraction. Once above ground, the extracted vapors or liquid-phase organics and groundwater are separated and treated.	Volatilization, vacuuming, sorption
Vapor extraction	A high vacuum is applied to remove vapors.	Volatilization, vacuuming
Thermal Treatment (Conductive heating Electrical resistant heating Steam injection)	The use of heat to facilitate extraction of VOCs through volatilization. Often combined with soil vapor extraction from the vadose zone.	Volatilization, increase of solubility
Phytoremediation	The use of plants to remove contaminants through uptake and bioaccumulation in plants.	Sorption/plant uptake
In situ flushing	Surfactants or cosolvents are induced in the subsurface to increase the mobility of the contaminants.	Desorption, co-solubility
Permeable reactive barriers	Placement of a barrier on the contaminant path which allows water to flow through but retains or destroys the contaminant by employing physical, biological or chemical treatment.	Sorption, chemical oxidation/reduction, bacterial metabolism
***In situ* biological technologies**		
Intrinsic bioremediation	Involves the detailed investigation and monitoring of natural biodegradation processes that lead to contaminant removal [45].	Metabolic processes of indigenous bacteria
Biostimulation (aeration, nutrient injection, oxygen addition through air sparging (bioventing) or hydrogen peroxide, addition of electron acceptor/donors)	Includes an engineered change of conditions to stimulate microbial growth. Can involve addition of oxygen, addition of a substrate, addition of nutrients, and controlling the temperature or the pH.	Stimulated metabolic processes of indigenous bacteria
Bioaugmentation (Microbial injection)	Includes the addition of specific microorganisms that are known to degrade the contaminants and have been adapted to such contamination.	Metabolic processes of injected bacteria
Phytoremediation	The use of plants to remove contaminants through enhanced rhizosphere biodegradation, uptake and metabolization in plants.	Bacterial/plant metabolism
***In situ* chemical remediation technologies**		
Chemical oxidation	Application of strong oxidizing agents in the subsurface that react with the contaminants.	Chemical destruction through redox reactions
Chemical reduction	Application of reducing agents (e.g. zero valent iron in permeable reactive barriers) in the subsurface that react with the contaminants	Chemical destruction through redox reactions

In situ remediation technologies can be divided in 3 major categories, based on the primary mechanism of removal/mass reduction.

1. **Physical remediation technologies** use the physical properties of the contaminant or the medium to separate or immobilize the contamination.
2. **Biological remediation technologies** convert the contaminants to less hazardous compounds through biological transformations aided by the stimulation of microorganism growth or the addition of exogenous microorganisms or plants. Natural biological processes are also included in this category.
3. **Chemical remediation technologies** convert the contaminants to less hazardous compounds through chemical transformations aided by the addition of a chemical reagent.

Table 3.1 provides an overview of specific mass reduction technologies included in the above categories with a brief explanation of the mechanism behind each technology. Containment or stabilization technologies have not been reviewed. Phytoremediation is presented twice because it employs both physical and biological removal mechanisms.

Monitored natural attenuation (MNA) is a remediation approach that relies on natural attenuation processes to effectively reduce contaminants in the groundwater to clean-up target levels in a time frame comparable to that which could be achieved through active restoration [46]. These processes can be physical, chemical and biological and they include biodegradation, abiotic degradation, stabilization, volatilization, sorption, dispersion and dilution. For organic contaminants, biodegradation is the only natural process that has the potential of leading to complete site remediation [45]. In this thesis, the term intrinsic bioremediation is used to refer to natural attenuation via biological processes.

3.2. *In situ* remediation technologies for common groundwater contaminants

The technologies discussed in Section 3.1 can address a variety of common groundwater contaminants in a variety of settings, including PAHs, BTEX, halogenated and non-halogenated VOCs, and pesticides. To illustrate the versatility of the technologies, the extent to which different technologies have been used for each contaminant group in the projects initiated by the USEPA is shown in Figure 3.2.

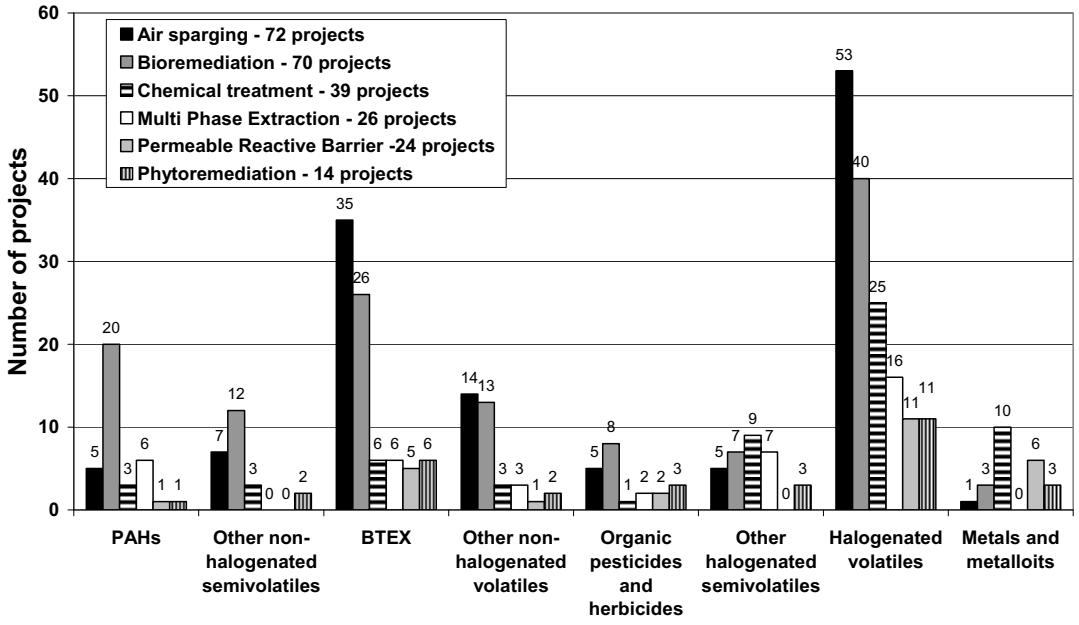


Figure 3.2. Contaminant groups treated by the most common *in situ* technologies in USA from 1985 to 2005. Redrawn from [4].

3.2.1. In situ remediation technologies applicable to MTBE

The most common *in situ* remediation technologies used for the remediation of MTBE contaminated groundwater are air sparging, phytoremediation, ISCO, *in situ* bioremediation, and monitored natural attenuation [47]. Air sparging is a popular technology for MTBE removal as it both results in volatilization and enhances the potential for aerobic biodegradation of MTBE through the addition of oxygen. In a review of air sparging systems [48] contaminant removal was above 97% in all three completed field cases with MTBE and BTEX contamination that were investigated. The potential of phytoremediation has been demonstrated in lab and field studies with hybrid poplar trees [49,50]. In this case MTBE removal occurs through uptake in the plant and volatilization [51]. ISCO is also a popular technology for MTBE removal. The ability of various oxidants to destroy MTBE has been demonstrated in numerous laboratory studies [27,52-55,Tsitonaki et al., **I**]. Finally, successful bioremediation of MTBE has been applied at many field sites through a variety of specific enhancements [56]. These include stimulation with oxygen [57,58] or oxygen releasing compounds [59,60], and bioaugmentation with MTBE degrading cultures [61]. Natural attenuation of MTBE through aerobic biodegradation has also been observed [62] and it may become more widespread in the future, if natural populations adapt to MTBE [23,63].

3.2.2. In situ remediation technologies applicable to creosote

Remediation of creosote contaminated sites is a challenging task due to the complexity of creosote mixtures and the variable properties of creosote components. Conventional

pump and treat systems are not effective, as creosote components include heavy PAHs and NAPL phases. In order to enhance remediation efficiency, cosolvent or surfactant flushing can be applied prior to another remediation technology [64]. An electric current can also be applied in order to enhance the dissolution of the contaminants to the surfactants [65]. The combination of electrokinetics and chemical oxidation was tested in laboratory experiments [66]. The results showed no remarkable improvement compared to oxidation alone, but further optimization of voltage and dosage may lead to enhanced treatment efficiency. *In situ* chemical oxidation is an effective remedy for creosote sites, as it can address both the immobile and the water soluble fraction. It has been used successfully on several occasions [12,67-71]. Bioremediation can also be a viable alternative if contaminant concentrations and site conditions allow it [1,34,35]. Intrinsic bioremediation alone is unlikely to successfully address all the components of creosote, as the biodegradation processes may stall once electron acceptors and the most available substrates are depleted.

4. *IN SITU* CHEMICAL OXIDATION

In this chapter, an overview of ISCO as a remediation technology is provided. Large sections are focused on activated persulfate which has been the oxidant studied in this thesis. ISCO of MTBE and creosote is discussed in more detail.

4.1. Oxidation chemistry and technology overview

In Situ Chemical Oxidation is a remedial process where strong oxidants are introduced into the subsurface to react with the contaminants of concern [72]. Oxidation of organic compounds may include oxygen addition, hydrogen removal and the withdrawal of electrons. When oxidation is complete, the contaminants are oxidized into carbon dioxide, water and remaining ions (e.g. Cl^-) (Eq. 4.1). Figure 4.1 displays a conceptual application of ISCO, where the oxidant is delivered in the subsurface by probe injection and activated by the use of heat.

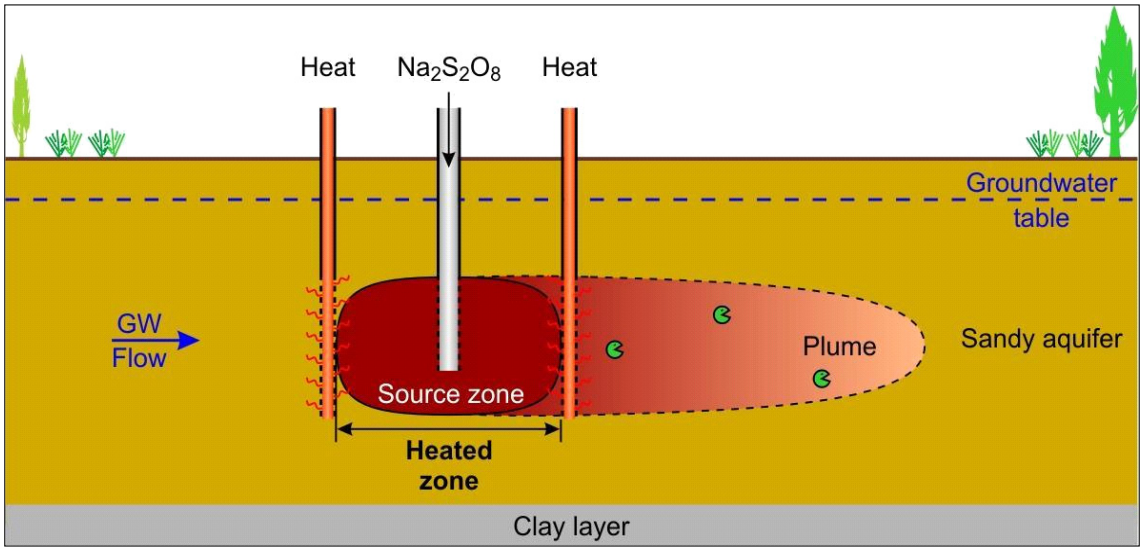


Figure 4.1 Example of an ISCO application in the field. Sodium persulfate ($Na_2S_2O_8$) is used as an oxidant and heat is used as an activation aid.

4.1.1. Oxidants

The most commonly oxidants used for *in situ* chemical oxidation are:

- Hydrogen peroxide and catalyzed hydrogen peroxide (CHP) also referred to as modified Fenton's reagent
- Ozone
- Permanganate
- Persulfate and activated persulfate

Oxidants can generally be grouped to radical and non-radical oxidants depending on whether they propagate the formation of free radicals. Persulfate and hydrogen peroxide

can function both directly and through radical formation. Ozone is primarily used in the unsaturated zone. A brief overview of the properties and reactions of the oxidants that are commonly used for groundwater remediation is given below.

Table 4.1. Oxidant strengths Modified from [73]

Oxidizing species	Standard oxidation potential (Volts)
Hydroxyl radical	2.8
Sulfate radical	2.6.
Ozone	2.1
Sodium persulfate	2.0
Hydrogen peroxide	1.8
Permanganate	1.7

Table 4.1 shows the standard oxidation potential for common oxidants and the most important radical species generated from oxidants. While these values can be used as a general reference for ranking different oxidants, they are of little value for predicting how different oxidants will perform in ISCO applications, where many other variables (e.g. stoichiometry, kinetics, thermodynamics, natural oxidant demand, site conditions, oxidant delivery) play a significant role [73].

Catalyzed Hydrogen Peroxide (CHP)

For *in situ* applications, peroxide is mostly used along with iron salts, to yield hydroxyl radicals ($OH^{\bullet}$ or $HO^{\bullet}$) in a reaction that is commonly known as Fenton oxidation. Pignatello et al. [74] have reviewed Fenton chemistry in detail.

Fenton oxidation is a sequence of reactions proposed to proceed as shown in Eqs. 4.1-4.7 [75-77].

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + OH^{\blacksquare} \quad (4.1)$$

$$Fe(III) + H_2O_2 \rightarrow Fe(II) + H^+ + HO_2^{\blacksquare} \quad (4.2)$$

$$OH^{\blacksquare} + H_2O_2 \rightarrow H_2O + HO_2^{\blacksquare} \quad (4.3)$$

$$Fe(II) + OH^{\blacksquare} \rightarrow Fe(III) + OH^- \quad (4.4)$$

$$Fe(III) + HO_2^{\blacksquare} \rightarrow Fe(II) + O_2H^+ \quad (4.5)$$

$$Fe(II) + HO_2^{\blacksquare} + H^+ \rightarrow Fe(II) + O_2H^+ \quad (4.6)$$

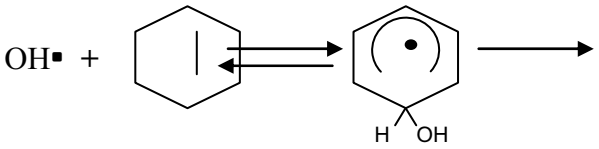
$$2HO_2^{\blacksquare} \rightarrow H_2O_2 + O_2 \quad (4.7)$$

This sequence is produced under acidic conditions. The hydroxyl radical, which is the desired oxidant for contaminant degradation is produced in the first reaction but it can be scavenged in reactions 4.3 and 4.5. For *in situ* applications of Fenton oxidation, new methods of iron activation have been developed which do not require acidification of the aquifer (see Section 4.1.3). The hydroxyl radical is a very versatile agent that reacts

with organic compounds by attacking the C-H, N-H, or O-H and C=C bonds, (Eqs. 4.8-9) or by adding to aromatic rings (Eq. 4.10) [74].

$$OH^{\bullet} + R\text{-}H \rightarrow H_2O + R^{\bullet} \quad (4.8)$$

$$OH^{\bullet} + C{=}C \rightarrow HO\text{-}C\text{-}C^{\bullet} \quad (4.9)$$



(4.10)

In the field, concentrated solutions of hydrogen peroxide (4-20% w/w) are injected in the subsurface followed by ferrous iron solutions. In order to avoid the reaction between Fe(II) and H_2O_2 before the solution is in contact with the contaminant, it is important that peroxide and iron are injected separately.

The use of ***permanganate*** (MnO_4^-) for *in situ* remediation surfaced around the mid 1990s [78]. Potassium and sodium permanganate are the two common forms of permanganate (MnO_4^-) used for *in situ* treatment of contaminated sites [6]. The oxidation process strictly involves direct electron transfer, rather than the free radical processes that characterize the other oxidants [72]. Permanganate is applicable over a wide pH range with the process following three primary redox reactions according to the pH (Eq. 4.11-4.13) [6,72].

$$MnO_4^- + 8\ H^+ + 5\ e^- \rightarrow Mn^{2+} + 4\ H_2O \quad \text{at pH} < 3.5 \quad (4.11)$$

$$MnO_4^- + 2\ H_2O + 3\ e^- \rightarrow MnO_2(s) + 4\ OH^- \quad \text{at } 3.5 < \text{pH} < 12 \quad (4.12)$$

$$MnO_4^- + e^- \rightarrow MnO_4^{2-} \quad \text{at pH} > 12 \quad (4.13)$$

$KMnO_4$ is a crystalline solid from which aqueous MnO_4^- solutions up to 4% w/w can be prepared on site, whereas $NaMnO_4$ is supplied as a concentrated liquid (40%) that is diluted on site and applied at lower concentrations [6,72]. In either case, a permanganate solution is injected on site through injection wells or probes.

Persulfate – Activated persulfate

The use of persulfate for *in situ* chemical oxidation emerged around 2000. The use of persulfate for ISCO has been reviewed in detail by Tsitonaki et al. **[III].** Persulfate usually occurs in the form of sodium, potassium or ammonium salts. The preferred form used in ISCO in groundwater is sodium persulfate because it has the highest water solubility. Persulfate salts dissociate in water to the persulfate anion, which is a strong but relatively stable oxidant (Eq. 4.14).

$$S_2O_8^{2-} + 2e^- \rightarrow 2SO_4^{2-} \quad (4.14)$$

If activated by UV, heat (40-60 ^oC), or low-valent metals, persulfate can initiate a free radical pathway through the formation of the sulfate radical (Eqs. 4.15-4.17).

$$S_2O_8^{2-} \xrightarrow{heat/UV} 2SO_4^{\bullet-} \quad (4.15)$$

$$S_2O_8^{2-} + M^{n+} \rightarrow SO_4^{\bullet-} + SO_4^{2-} + M^{n+1} \quad (4.16)$$

$$SO_4^{\bullet-} + M^{n+} \rightarrow SO_4^{2-} + M^{n+1} \quad (4.17)$$

The term 'activated persulfate' refers to the reactive intermediates that are generated by the use of an activation aid, while 'non-activated persulfate' refers to the use of persulfate ion alone without any aid. Note that during metal activation, the metal initiator is involved in both radical generation and radical scavenging. Once the sulfate radical is generated it can propagate a series of reactions (Eq. 4.18-4.23) involving the formation of other radicals and hydrogen peroxide [79]. The formation of hydroxyl radicals can be advantageous and lead to higher contaminant destruction. Furthermore, hydrogen peroxide can further activate the remaining persulfate and restart the initiation chain.

$$SO_4^{\bullet-} + H_2O \Leftrightarrow HO^{\bullet} + SO_4^{2-} + H^+ \quad (4.18)$$

$$2SO_4^{\bullet-} \rightarrow S_2O_8^{2-} \quad (4.19)$$

$$SO_4^{\bullet-} + HO^{\bullet} \rightarrow HSO_5 \quad (4.20)$$

$$2HO^{\bullet} \rightarrow H_2O_2 \quad (4.21)$$

$$H_2O_2 \rightarrow H_2O + ½O_2 \quad (4.22)$$

$$H_2O_2 + S_2O_8{}^{2-} \rightarrow 2H^+ + 2SO_4^{2-} + O_2 \quad (4.23)$$

Sulfate radicals are highly reactive species with a half-life of several seconds [80], which can oxidize a variety of organic compounds, much like the hydroxyl radicals, although the mechanism for the reaction can be significantly different. For instance the sulfate radicals preferably remove electrons from an organic molecule (Eq. 4.24) whereas the hydroxyl radicals add to C=C bonds or abstract hydrogen from the C-H bond (see Eqs. 4.9-4.10).

$$CH_3CO_2^- + SO_4^{\bullet-} \rightarrow CH_3CO_2^{\bullet} + SO_4^{2-} \quad (4.24)$$

The sulfate radical is an electrophilic reagent, which means that it has a higher reactivity with sites of high electron density. Therefore, electron donating groups such as amino (-

NH_2), hydroxyl (-OH) or alkoxy (-OR) increase the rate of the reaction whereas electron withdrawing groups such as nitro (-NO_2) or carbonyl (C=O) substitutes decrease the rate of the reaction [67,81].

In the field, persulfate is applied similarly to Fenton's, where a solution of persulfate is injected in the subsurface followed by the addition of an activating solution (often some form of ferrous iron). Another practice is to raise the temperature of the aquifer material to above 35 ^{o}C after the persulfate injection. Non-activated persulfate can persist in the subsurface for some weeks and this will allow it to reach the contaminated zones. Heat activation could then be used to initiate radical generation in contact with the contaminant.

Oxidant combinations

Oxidants can be used simultaneously in order to stimulate the generation of reactive species, or sequentially to reduce treatment costs related to natural oxidant demand. The most common combinations are:

1. *Hydrogen peroxide and persulfate*, where H_2O_2 can stimulate the generation of sulfate radicals, thereby activating persulfate. This combination is the most common applied technology for persulfate field applications to date [Tsitonaki et al., **III].** It has been shown effective for the removal of chlorinated ethenes, dichloromethane and BTEX [82-84]. Similarly, H_2O_2 can be used in combination with ozone to generate OH▪ [6].
2. *Hydrogen peroxide and permanganate*, where the contaminated zone is pre-treated with H_2O_2 before the application of MnO_4^-. This aims at reducing the total treatment costs as H_2O_2 is a lot cheaper than MnO_4^-, and its reaction with the aquifer materials and contaminants could lower the oxidant demand for permanganate [6].
3. *Permanganate and persulfate* have also been sequentially or simultaneously applied in some field cases [85,86]. It is suggested that persulfate can target the natural oxidant demand, and permanganate the organic contaminants [87]. This approach assumes that both oxidants react with the same naturally present reduced material, which has not been confirmed yet [Tsitonaki et al., **III]**.

4.1.2. Activation by heat

Sodium persulfate is the only oxidant for which heat activation is applied *in situ*. The aim is to generate sulfate radicals. For field applications, temperatures between 35-60 ^{o}C are sufficient.

As part of this work, the oxidation of MTBE by heat-activated persulfate was studied in aqueous systems in a laboratory batch test. A range of different activation temperatures was used. The initial concentrations in the aqueous phase were 1 mg/L MTBE and 4 g/L $Na_2S_2O_8$. The reaction was monitored for 48 hours. Significant removal of MTBE was observed at all temperatures above 35 ^{o}C (see Figure 4.2). However, the degradation product tert-butyl alcohol (TBA) was detected at 35 ^{o}C, while no intermediates were found after oxidation at 40, 45 and 50 ^{o}C.

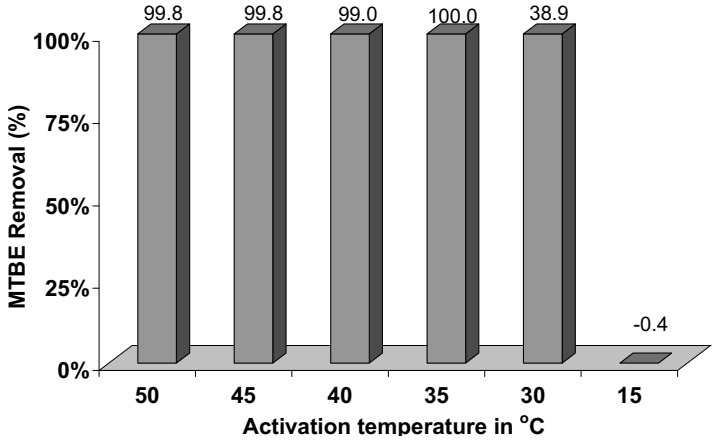


Figure 4.2. Removal of MTBE by heat-activated persulfate oxidation after 48 hrs in laboratory aqueous batch experiments. (Unpublished work)

Persulfate consumption increased and proceeded faster with increasing temperature (Figure 4.3) indicating that the level of activation was higher with increasing temperature. However, this did not result in higher MTBE destruction. It was concluded that the optimal temperature range for the MTBE degradation was 40 to 45 ^{o}C.

This result is in accordance with other studies [55,Tsitonaki et al., **I**] that have shown that there is a temperature threshold above which decomposition of persulfate does not lead to higher contaminant destruction. This is due to the faster, unproductive persulfate depletion at high temperatures. The reactions of persulfate/radicals with potential scavengers (Cl^-) also accelerate at elevated temperatures [88,89]. The reactivity of persulfate at different temperatures obeys the Arrhenius Equation [55,90,91] and thus the degree of impact on the rate of oxidation for each target organic compound depends on thermodynamic properties unique to each compound [Tsitonaki et al., **III**]. Since

persulfate ISCO involves many competing reactions between numerous organic compounds and inorganic species in the subsurface, an 'optimal' activation temperature may be unique to each system and crucial for successful remediation [Tsitonaki et al., **III**].

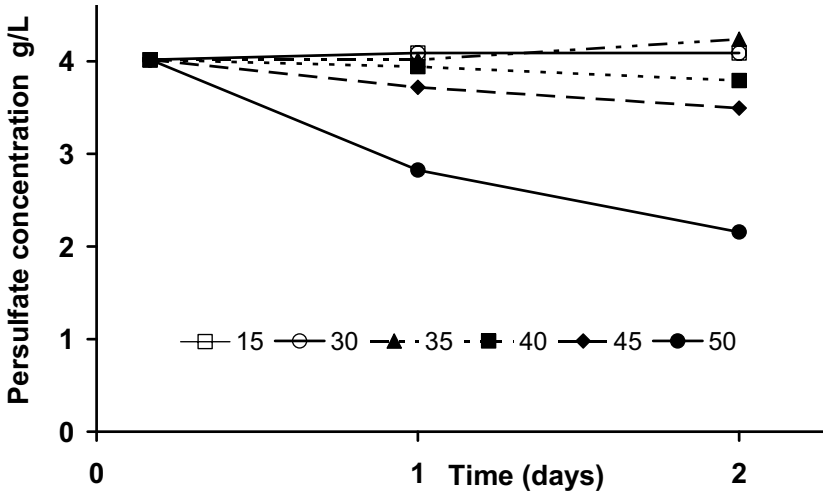


Figure 4.3. Remaining persulfate in laboratory aqueous batch experiments after 2 days with activation at different temperatures. (Unpublished work)

Several thermal treatment technologies can be used to thermally activate persulfate *in situ*. Electrical resistance heating and radio frequency heating seem to be the most appropriate for achieving soil temperatures within the 35-60 ^{o}C range.

4.1.3. Activation by other means

Persulfate and peroxide are often activated by ferrous ion in order to produce sulfate and hydroxyl radicals (see reactions 4.16-4.17). There is an optimal ratio of oxidant to available ferrous ion for generating reactive radicals at a rate appropriate for contaminant destruction. If there is an excess of iron it is likely that a large amount of radical species will be generated very fast. As radical to radical reactions and radical to iron reactions proceed faster than radical to contaminant reactions, an ineffective consumption of the radicals may occur. Treatability studies are necessary in order to determine an appropriate oxidant/iron ratio. Gradual addition of iron is a way of controlling the rate of the reaction [85,92]. At *in situ* applications the major challenge is to achieve the oxidant-Fe(II) reaction when the oxidant is in contact with the contaminants. The transport of Fe(II) in the subsurface is also limited by its reactivity (complexation, oxidation and precipitation). To maintain Fe(II) in solution, ligands and chelators are often employed. An excess of chelate is necessary for controlling Fe(II) availability [93,94].

Another interesting approach to achieve *in situ* iron activation would rely on naturally occurring iron, which is often abundant in soil and groundwater systems. Iron minerals

such as goethite, hematite and magnetite should be able to initiate radical generating reactions [74], but these reactions are a lot slower than those with soluble iron [95]. Given the fast decomposition of peroxide, activation by naturally occurring iron will most likely not be cost-efficient. By contrast, the stability of persulfate makes this type of activation more likely to succeed. Laboratory experiments [90,92] and field studies [85] have indicated that this type of activation has a promising potential.

pH manipulation can be used to aid chemical oxidation with CHP or persulfate. In particular, acidic conditions will improve the efficiency of the Fe(II)-H_2O_2 combination, because Fe(II) will remain soluble and the decomposition of H_2O_2 will decrease [74]. However, acidification of an aquifer is difficult to achieve due to the buffering capacity of the soil, and it may result in unwanted mobilization of heavy metals. Manipulation of the pH to alkaline values (pH > 10) is a novel activation aid for persulfate [96]. The activation mechanisms behind this method are unclear but it is likely that both the sulfate and the hydroxyl radical are involved in contaminant destruction (Eqs. 4.25-4.27) [Tsitonaki et al., **III**].

$$SO_4^{\bullet -} + H_2O \rightarrow OH^{\bullet} + SO_4^{2-} + H^+ \quad (4.25)$$

$$SO_4^{\bullet -} + OH \rightarrow OH^{\bullet} + SO_4^{2-} \quad (4.26)$$

$$2OH^{\bullet} \rightarrow H_2O_2 \quad (4.27)$$

Tsitonaki et al. [**III**] reported that alkaline activation has been used with mixed results in the field.

Table 4.2 presents a list of treatability and field studies where different activation aids were compared. In most of these comparative studies, heat and peroxide activation were found more effective than iron or alkaline activation. For example, Tsitonaki et al. [**I**] tested the removal of MTBE, TCE and TCA with non-activated, heat-, and iron-activated persulfate (with chelate or thiosulfate). At the same persulfate dose, heat was the only treatment that could fully mineralize MTBE in aqueous systems within a few hours. Crimi and Taylor [94] found that chelated iron-activated persulfate was more effective than alkaline or peroxide persulfate in the systems they evaluated. The diversity of these findings highlights the need for treatability studies when choosing an appropriate activation aid for a specific site [Tsitonaki et al., **III**].

Table 4.2. Laboratory and field studies comparing different methods for persulfate.
Modified from Tsitonaki et al. [**III**].

Set up	Target Contaminants	Parameters assessed	Results (method with highest removal)	Reference
Alkaline Peroxide Chelated Fe(II) soil slurries	BTEX	Persulfate/Activator /Chelator ratios Porous media types	Up to 99% removal but Generation of by-products from the oxidation reactions (chelated Fe(II))	Crimi and Taylor, 2007 [94]
Alkaline, peroxide chelated Fe(II)/Fe (III) Aq & soil	1,1,1 TCA MTBE, CT Benzene, TCE	Comparison of activation aids	Benzene and TCE > 90% for all methods max MTBE removal >40 % (heat, peroxide and alkaline)	Block and Schreier, 2004 [97]
Non activated Heat 35 ^{o}C Fe(III) EDTA Fe(III) EDTA at 35 ^{o}C Soil slurries	1,1,1- TCA DCA DCE	Comparisons of activation aids	Non activated effective only for DCE. Heat: 85% removal of TCA and 100% for DCA and DCE Fe(III) EDTA: 0% TCA, DCA, 100% for DCE Iron EDTA + heat: TCE 68% (Heat)	Cho et al., 2002 [98]
Heat 40^{o}C Fe(II): Pulse Chelated Thiosulfate Aq. & Soil	MTBE TCE TCA	pH activation aids soil	Non activated persulfate was only effective in aqueous systems, 43-98 % in soil water systems, 66-99% in aq. Systems. Heat was the only method that fully mineralise MTBE (Heat)	Tsitonaki et al., 2006 [I]
Peroxide Fe(II) Soil slurries	Benzene TCE, PCE DCE, DCA Diesel organic compounds	Oxidant stability	All COC s were oxidised within 14 days but not completely. Hydrogen peroxide was consumed in 24 hrs while persulfate lasted for about 7 days	Abranovic et al., 2006 [99]
Heat 40^{o}C Fe(III)-EDTA Aqueous	TNT RDX HMX	pH influence on Fe(III) EDTA persulfate dose activation efficiency	80% destruction with heat Fe(III)-EDTA caused a 4 unit pH drop. Persulfate degradation increased at low pH. Almost no removal with Fe III EDTA (Heat)	Waisner and Hoag, 2006 [100]
Non activated Fe(II)/Fe(II) EDTA Soil	Natural oxidant demand	Oxidant demand for inoganics f_{oc}	Persulfate reacts mostly with the inorganics in the soil, SOD_{7days} 0.98-2.2 g/kg soil (Fe(II)-EDTA had the lowest SOD)	Brown and Robinson, 2004 [101]
Fe(II)-Chelate Peroxide Alkaline Aq. & Soil	Chlorinated BTEX, MTBE PCBs, PAHs Dioxane	Comparison of activation aids	BTEX: non-activated MTBE, chloroethenes, chlorobenzenes: Fe(II). Cl-(m)ethanes: (Heat, alkaline, peroxide)	Block et al., 2004 [97]

4.1.4. Reaction kinetics

Knowledge of reaction kinetics is critical for designing an ISCO application in the field. Here, oxidants will react with the targeted contaminants which most often will be more than one as well as with non-target compounds from the sediment [Tsitonaki et al., **III**]. Such competitive interactions may mean that an increased oxidant concentration is necessary. Contaminants that exhibit fast reaction rates with the oxidants will be

depleted first. By contrast, slow reacting contaminants will remain as residual contamination, if the oxidant is depleted through non-target reactions before it reacts with the contaminants [Tsitonaki et al., **I**].

In general, oxidation and contaminant reactions follow first order kinetics regarding the consumption of oxidant and contaminant [55,79,102,103]. The overall oxidant-contaminant reaction can be described by a second order rate expression (Eq. 4.29)

$$d[\text{contaminant}]/d[t] = -k_2[\text{contaminant}]\,[\text{oxidant}] \quad (4.29),$$

If we assume that the concentration of the oxidant remains stable throughout the reaction, because a significant surplus of oxidant is added, [oxidant] >> [contaminant], the reaction can be described by a pseudo first order reaction rate (Eq. 4.30).

$$d[\text{contaminant}]/d[t] = -k'_1[\text{contaminant}] \quad (4.30),$$

where k'_1 is the pseudo first order reaction rate defined as: $k'_1 = k_2[\text{oxidant}]_{\text{initial}}$ oxidant and contaminant concentrations are expressed in mol/L [102].

For oxidants that can involve more than one active species (e.g. activated persulfate and CHP), the consumption of the contaminant is adequately described by a pseudo first order oxidation rate (Eq. 4.30). This rate is presumed to be the sum of the second order rates for the reactions between the contaminant and each oxidant species (see Eq. 4.31 for activated persulfate) [55,79].

$$k_1 = k''_{SO_4^{-\bullet}} + k''_{OH^{\bullet}} + k''_{other} \quad (4.31),$$

where k′′ represents the second-order rate constants for the reaction of the contaminant with each reactive intermediate. In most cases one of the above species will be dominating the reaction. The role of the individual species in system kinetics has not yet been investigated [79].

4.2. Contaminants amenable to chemical oxidation

In situ chemical oxidation is a very versatile technology that has been used for a variety of contaminants [4,6,73]. The reaction mechanisms between contaminants and oxidants proceed through various pathways. The initial mode of attack depends largely on the active oxidant species and is often a defining factor for contaminant amenability to different oxidants. In particular, the permanganate ion generally attacks C=C bonds through direct electron transfer [104,103]. This renders permanganate suitable for the remediation of ethene contamination, including chlorinated ethenes. In contrast, compounds that have no readily available electron pairs such as alkanes and

chloroethanes are not very reactive to permanganate [105]. Similarly, the stability of the bonds of aromatic compounds results in increased resistance to oxidation, which explains the mediocre performance of permanganate towards PAHs and benzene [6], while substituted carbon atoms increase reactivity; hence, methyl toluene or chlorobenzenes are more amenable to permanganate than benzene [105].

As aforementioned, activated persulfate and catalyzed hydrogen peroxide reactions occur through radical species, mainly the sulfate and hydroxyl radical. These radicals can attack C-H, N-H, or O-H and C=C bonds or add to aromatic rings which makes them effective towards a wider range of contaminants than permanganate. Other active species that are generated from peroxide and persulfate include the superoxide and the perhydroxyl radical. These radicals are nucleophiles, and thus more reactive with sites of lower electron density. This means that these oxidants may be applicable for contaminants such as chlorinated alkanes or nitro-substituted compounds (e.g. nitrobenzene) [6,105,106]. Manipulating the generation of specific active species through adjustment of the activation method would increase the versatility of ISCO but this aspect of the technology has not been developed yet.

4.2.1. Chemical oxidation of MTBE

The ability of various oxidants to destroy MTBE has been demonstrated in numerous laboratory studies. Both CHP [27,52-54], persulfate [Tsitonaki et al., **I],** and activated persulfate [55,Tsitonaki et al., **I, III, IV**] can oxidize MTBE rapidly. The rates of MTBE oxidation with permanganate are very slow [107], thus permanganate is unsuitable for *in situ* applications.

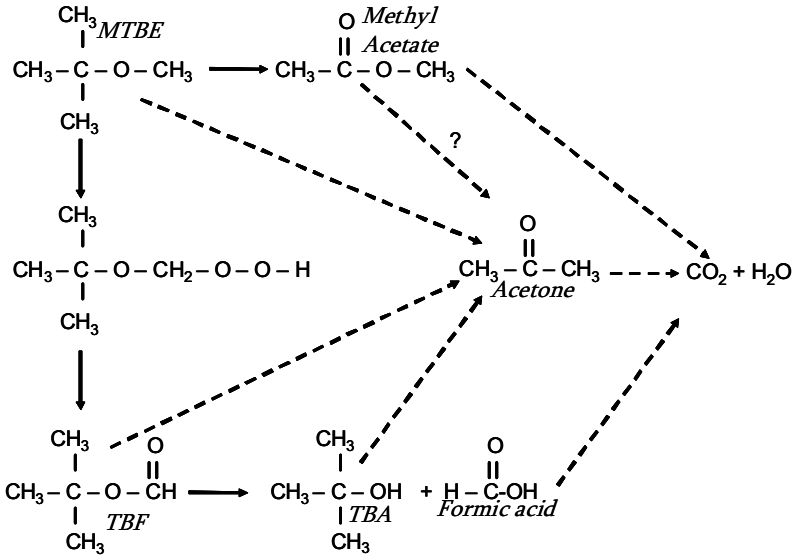


Figure 4.4. Suggested pathways for the oxidation of MTBE by radical oxidants. Drawn based on information from [53,55]. Dashed arrows mean that there may be more than one reaction involved.

Oxidation of MTBE by the hydroxyl or the sulfate radical is a complex process that involves several intermediates including acetic acids and formadehydes [108,109], the most dominant of which are TBA, TBF, acetone and methyl acetate [53,55]. A suggested pathway for the oxidation of MTBE by the hydroxyl or the sulfate radical is shown in Figure 4.4.

4.2.2. Chemical oxidation of creosote

Creosote is a mixture of compounds that have varying degrees of reactivity with different oxidants. For example, permanganate is very reactive towards PAHs (naphthalene, phenanthrene, pyrene) [67,110] but not towards benzene, dibenzofuran and biphenyl [67]. Fenton's and modified Fenton's are effective for many of the PAHs found in creosote [12,68]. Iron-activated persulfate has also been used successfully against creosote compounds such as PAHs [67,69,70] phenolic compounds [67,Tsitonaki et al., **IV**], BTEX [70,71], and the water soluble fraction of heterocyclics [Tsitonaki et al., **IV**]. ISCO can also enhance the availability and dissolution of the sorbed fraction of creosote [10,11]. However, there is a tendency that the low molecular weight compounds are oxidized more extensively, leaving the less biodegradable compounds as a residue [111-113].

Depending on the oxidant and the specific compounds, a variety of chemical pathways are involved in the oxidation of creosote compounds. To date, these pathways are not studied in detail, but there is evidence that when oxidizing complex contaminant mixtures a number of intermediates are generated [7,94]. As many PAH and NSO intermediates can be undesirable in the groundwater, ISCO applications should be designed to account for complete mineralization of all products.

4.3. Challenges and limitations of ISCO

4.3.1. Natural oxidant demand

Natural oxidant demand is an expression of how much oxidant can be consumed by non-target species at a treatment site. Both natural organic (e.g. humic acids) and inorganic matter (oxidizable metals and minerals, radical scavengers) can exert an oxidant demand. Natural oxidant demand is a key issue for dimensioning ISCO applications and affects oxidant stability. NOD is primarily an issue for stable oxidants such as permanganate and non-activated persulfate. CHP and activated persulfate decompose and react at such fast rates that oxidant transport is mainly controlled by their decomposition rather than their reaction with the sediment [6,8]. Most of our knowledge on NOD reactions comes from the study of permanganate, as very few have looked into the NOD for persulfate [Tsitonaki et al., **III**]. Persulfate NOD values for sandy till soil at ambient temperatures range from 0.08-0.24 g $S_2O_8^{2-}$/kg sediment [114] to 1 g $S_2O_8^{2-}$/kg [101] for 10 and 7 days respectively. These values are significantly

lower than the NOD of permanganate for sandy till (1-8 g MnO_4^-/kg) [115]. Brown and Robinson [101] suggested that persulfate reacts primarily with the inorganic constituents of the sediment, as opposed to permanganate which reacts primarily with the organic matter.

4.3.2. Physical site characteristics

Hydrogeological conditions, especially permeability, hydraulic conductivity, and heterogeneities control oxidant distribution and transport capacity in the subsurface and consequently the efficiency of the treatment. The effects of complicated physical site conditions were outside the scope of this work. ISCO in dual porosity media and effects of heterogeneities have been studied by others [116,117].

4.3.3. Chemical site characteristics

The chemistry of the aquifer material and groundwater can have a major impact in ISCO. Site pH can determine the success of CHP or iron activated persulfate as it controls the solubility of ferrous ion. Moreover, the presence of natural iron in the sediment can act as a natural activator [85,90,92] or as radical sink for CHP and persulfate. The natural groundwater temperature can also be important for treatment costs, if heat-activated oxidation is selected for ISCO. Radical oxidants are very reactive with many of the naturally present ions in soils and groundwater including chloride, bicarbonate and carbonate ions, which can exert non-target oxidant consumption [55,79,88,91].

4.3.4. Hydrogeological, geochemical and biological changes after ISCO

Chemical oxidation can cause changes in permeability either through a geochemical process such as cation exchange of Na or K salts with the calcium of clay minerals, or through the generation of gases (CO_2) and precipitates (e.g. MnO_2 from MnO_4^-) from the oxidation reactions [6].

Certain oxidants, such as persulfate, can cause a decrease in pH as shown in both aqueous [7,92] and soil slurry laboratory systems [Tsitonaki et al., **I, II, IV]**. In aquifers with a low buffering capacity, this may be a concern that can also lead to mobilization of heavy metals. Mobilization of reduced metals (especially Cr^{3+}) from the porous media is a concern when oxidants are applied *in situ*. Fortunately, this mobilization seems to be temporary, as such small increases of metal concentrations are expected to be attenuated *in situ* through natural geochemical stabilization processes [72,118,Tsitonaki et al **III**].

Finally, it is anticipated that ISCO can cause a change in redox conditions to a more oxidized level, and possibly to the abundant electron acceptors such as sulfate from

persulfate [Tsitonaki et al., **IV**], or addition of oxygen through activated persulfate or CHP.

4.3.5. Rebound, reaction intermediates and excess oxidants

Failure to deliver the adequate amount of oxidant to the contaminated zones, and challenges connected to permeability reductions can lead to rebounds in contaminant concentrations. Contaminant-oxidant reactions can often terminate at other organic intermediates [7,53,94] instead of the aimed transformation to harmless products. Evaluation of the risk that possible by-products pose to the groundwater and nearby recipients along with supplying sufficient amounts of oxidant can in most situations resolve the problem. Finally, excess amounts of oxidants can pose a risk to sensitive recipients. All these limitations can necessitate further treatment of the contaminated area, possibly by a different remediation technology.

5. INTRINSIC AND ENGINEERED BIOREMEDIATION

Bioremediation is the remediation technology in which microorganisms are employed to transform hazardous contaminants to harmless compounds [119]. Bioremediation takes advantage of the microbial degradation processes through which microorganisms can obtain carbon and energy by decomposing organic contaminants. As presented in Table 3.1 (Chapter 3) bioremediation can involve intrinsic biological processes, the manipulation of environmental conditions, and/or the addition of microorganisms in order to achieve a satisfactory rate and extent of contaminant decomposition.

5.1. Contaminants amenable to bioremediation

Bioremediation applications for contaminated groundwater have been extensively studied and applied. Today, bioremediation can be engineered to address a variety of organic contaminants, as shown in Table 5.1, provided that the conditions favour the desired microbial processes. In general, the rate and extent of biodegradation is related to contaminant structure and concentration and a variety of environmental factors that affect microbial growth and activity.

5.1.1. Bioremediation of MTBE

Bioremediation is a relevant remediation option for MTBE contamination. MTBE-degrading organisms have been found in some contaminated sites but the populations are often too slow-growing and too small to sustain intrinsic biodegradation of MTBE at acceptable rates for remediation goals [120].

The primary and fastest pathway for MTBE degradation occurs under aerobic conditions [23]. There are a few species and consortia [121] that can use MTBE as their sole carbon and energy source in the presence of oxygen [23]. Table 5.2 shows examples of bacteria that can degrade MTBE. Several genera are represented in this table, which means that there is a diversity of microorganisms that can potentially adapt to using MTBE. Anaerobic degradation of MTBE has been observed [122,123] at very slow rates. MTBE degradation via cometabolism has been documented with various substrates including alkanes and ETBE [124]. The presence of co-contaminants such as BTEX at gasoline sites can be inhibitory for MTBE degradation as BTEX is preferentially degraded, often resulting in oxygen limitations [24,60]. A simplified pathway of MTBE biodegradation is shown in Figure 5.1.

The intrinsic bioremediation of MTBE was studied in a long term pilot study for a contamination consisting of gasoline and MTBE [139]. MTBE degradation was slower than BTEX, but only 3% of the initial MTBE mass remained after 6 years. Further

investigations of site microorganisms showed that the presence of MTBE-degrading microorganisms at the site was sporadic, and the factors controlling it could not be identified [140].

Table 5.1. Biodegradation of common organic groundwater contaminants.

Contaminants	Redox conditions	Remarks
Aliphatic hydrocarbons	Preferably aerobic but also possible under anaerobic [125] and methanogenic [126].	Highly branched compounds are more resistant to biodegradation [127].
BTEX	Preferably aerobic [128]. Also possible under anaerobic, but observations of recalcitrance have been reported. [129,130]	Benzene and ethylbenzene are more resistant than toluene and xylenes.
Polyaromatic hydrocarbons	Preferably aerobic. Possible under anaerobic conditions at low rates [131,132].	Biodegradation rates decrease with increasing number of rings [133].
Heterocyclic hydrocarbons	NSO compounds can be degraded cometabolically under aerobic conditions [35] but are typically persistent under anaerobic conditions [43].	N heterocyclics are more biodegradable than S or O heterocyclics [33].
Gasoline additives	Mainly aerobic. Some strains can degrade under anaerobic conditions at very slow rates [120]	The degradation of MTBE and other oxygenates happens through similar pathways [120].
Chlorinated aliphatics	Aerobically mostly through cometabolic processes. The main biodegradation mechanisms is reductive dehalogenation which occurs either cometabolically or as dehalorespiration. Only *Dehaloccocooides Ethenogenes* strain 195 can dechlorinate chlorinated ethanes to ethane [134].	Aerobic biodegradation activity declines with the increasing number of chlorides and chain length . Ethenes are more biodegradable than ethanes [135].
Polychlorinated biphenyls (PCBs)	Degrade under both aerobic and anaerobic conditions. In most cases the end products are chlorobenzoates [136].	Biodegradability increases with increasing number of chlorides under anaerobic conditions and decreases under aerobic [137].
Chlorinated benzenes	Aerobic degradation occurs similarly to benzene while anaerobic pathway is reductive dechlorination [136].	
Pesticides/ herbicides	Degradability varies greatly depending on the specific compound [138].	

Because most field studies have shown that the intrinsic biodegradation potential of MTBE is little or too slow [56], field applications of bioremediation for MTBE involve the addition of MTBE-degrading cultures to the aquifer, and/or the manipulation of site conditions to enhance the growth of MTBE degrading microorganisms. Field applications of bioremediation in the USA have shown concentration reductions of up to 99% [120]. One of the most common amendments is oxygen addition [60,142,143] by injection of oxygen or an oxygen releasing compound. Supply of a co-substrate such as propane or cyclohexane has also been successful [23]. Finally, bioaugmentation with an

MTBE degrading strain or culture has been successfully applied in some sites [144] usually combined with stimulation of site conditions to favor the survival and growth of the added microorganisms [56,120].

Table 5.2. Examples of microorganisms that can degrade MTBE under aerobic conditions. This table is composed using information from [124].

Microorganism	Primary substrate
Rhodococcus Ruber IFP 2001	ETBE
Rhodococcus zopfii IFP 2005	ETBE
Mycobacterium sp. IFP 2009	ETBE
Pseudomonas putida CAM ATCC 17453	camphor
Pseudomonas putida GPo1	octane
Pseudomonas putida KR1	n-alkanes
Mycobacterium vaccae JOB 5	n-alkanes
Methylibium petroleiphilum PM1	MTBE
Hydrogenophaga flava ENV 735	MTBE
Mycobacterium austroafricanum IFP 2012	MTBE

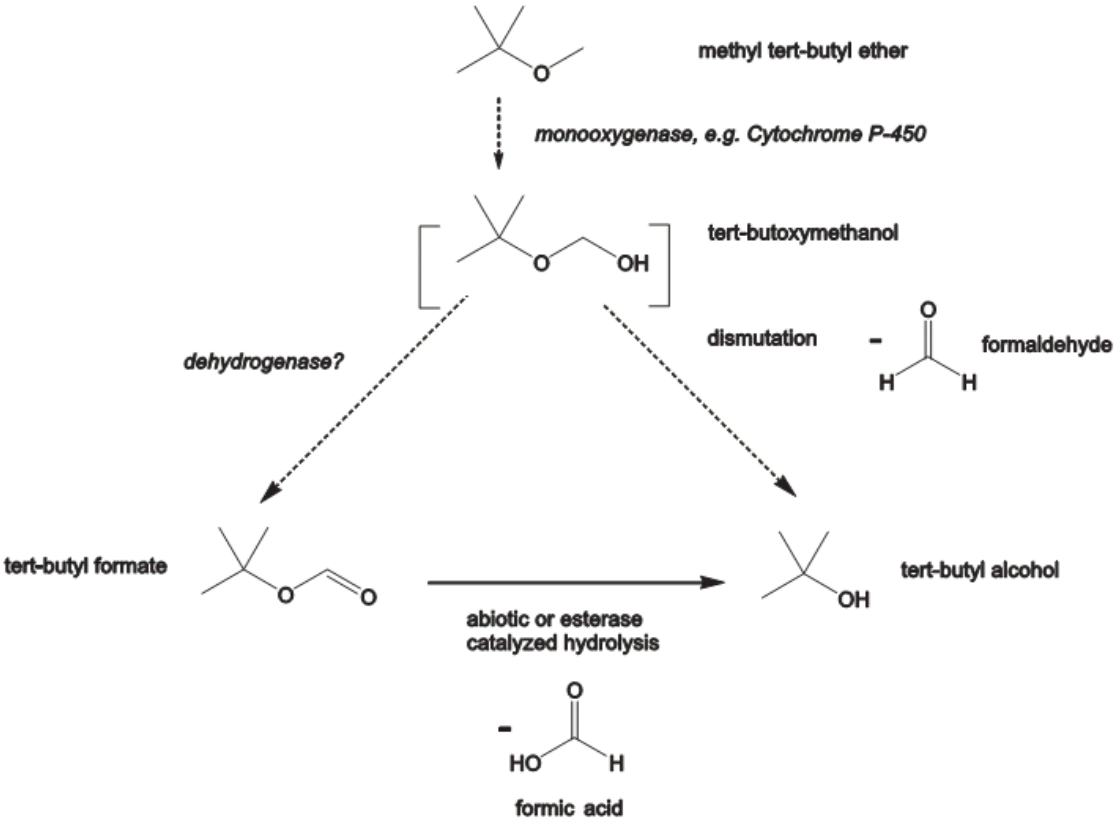


Figure 5.1. Pathway for MTBE degradation under aerobic conditions as proposed by Schmidt et al. [141].

5.1.2. Bioremediation of creosote

As creosote is a complicated mixture of many organic contaminants, the extent of its microbial degradation can vary greatly from site to site. Still, the use bioremediation is possible. Laboratory evidence suggests that creosote compounds are biodegraded even in complex mixtures [1,34,35], although lag periods and half-lives can vary greatly. Field applications of bioremediation have included the addition of nutrients, surfactants, electron acceptors and adapted microorganisms [1]. Intrinsic bioremediation has been studied at Borden aquifer [145], biotransformation of several creosote compounds including carbazole and phenol was observed. The authors concluded that natural attenuation is a removal mechanism to be considered during risk management, as the process may take several years.

Due to the complex nature of creosote, a great variety of strains can be involved in biodegradation. Table 5.3 shows examples of strains that can degrade the creosote compounds used in this study. Degradation pathways are also variable but a common first step is the cleavage the aromatic ring by a mono- or dioxygenase enzyme. The biodegradation pathways of many creosote compounds, such as phenols, converge at protocatechuate which is subsequently transformed to acetyl Coenzyme and succinyl Coenzyme permitting entry into the Krebs cycle [146], a key step of cell respiration for aerobic bacteria. Therefore, the genes encoding for the degradation of protocatechuate are appropriate molecular markers for estimating the density of several aromatic degraders [146, Tsitonaki et al., **V**].

Table 5.3. Examples of microorganisms that can degrade the creosote compounds used in the experiments of Tsitonaki et al. [IV].

Compound	Microorganism	Reference
Benzothiophene	*Pseudomonas putida*	[147]
	Pseudomonas aeroginosa PRG -1	[33]
Carbazole	*Pseudomonas spp.* Ca06 & Ca10	[148]
	Pseudomonas sp. HL7B	[149]
Dibenzofuran	*Sphingomonas Rw1*	[150]
	Pseudomonas sp. HL7B	[149]
Napthalene	*Pseudomonas sp.* HL7B	[149]
	Pseudomonas putida C18	[151]
	Pseudomonas fluoresense	[152]
O-cresol	soil fungi: *Aspergillus, Cladosporium, Fusarium, Monicillium, Penicillium and Phanerochaete*	[153]
	Arthrobacter MTCC 1553	[154]
	Penicillium frequentans Bi 7/2 (ATCC 96048)	[155]
	Pseudomonas sp. CP4	[156]
Toluene	*Pseudomonas putida* X18	[151]
	Pseudomonas putida F1	[157]

5.2. Limitations of bioremediation

The success of bioremediation applications is highly dependant on environmental conditions. Microbial processes are affected by aquifer temperature, pH, availability of electron acceptors and nutrients. Biostimulation or bioaugmentation applications often manipulate the environmental conditions in order to favor the desired microbial processes. Also, intrinsic biodegradation processes are dependant on environmental conditions and may be sensitive to changes in those.

In contaminated aquifers, biodegradation can be limited by hydrogeological heterogeneities and free phase of contaminants, as well as other unfavorable conditions such as: a) too high concentrations of contaminants that can be toxic for microorganisms, b) competition or preferential degradation of some contaminants leading to the depletion of nutrients or electron acceptors, c) unsuitable redox conditions for the specific contaminants d) lack of specific degraders e) lack of nutrients or electron donors, and f) non-biodegradable contaminants.

6. TREATMENT TRAINS

6.1. Definition and concepts

The "treatment train" remediation approach is defined as the sequential or combined use of individual remediation technologies in order to clean up the same volume of contaminated soil and groundwater [5]. The concept of treatment trains is a result of the realization that no remediation technology is a silver bullet. Treatment trains aim to address the following problems:

1. **Complex contaminant mixtures** that include many different compounds with diverse physicochemical properties and amenability to degradation. A combination of technologies is necessary in order to address the different contaminants. For example, industrial landfill sites may be contaminated with a combination of chlorinated solvents and non-chlorinated hydrocarbons (BTEX, PAHs, etc), which degrade under different redox conditions. A combination of sequential anaerobic and aerobic bioremediation can be applied in these cases (see Figure 6.1). This combination can also address the fact that some compounds degrade through an anaerobic pathway to form aerobically degradable daughter products, e.g. the reductive dechlorination of PCE to vinyl chloride.

2. **Sites with significant heterogeneities in contaminant distribution**. In general, hot spot contamination is not amenable to biological remediation technologies, due to the very high contaminant concentrations that and the possible presence of free phase. ISCO and thermal remediation technologies are examples of powerful remediation methods that are effective at hot spots. However, efficiency falls with decreasing contaminant concentrations. As thermal and chemical technologies are generally more costly than biological, it is preferable to use these technologies to remove the major part of the contaminant mass at the hot spots and combine them with a cheaper treatment step for treating the adjacent area and the plume.

3. **Remaining contaminant mass after the first remediation technology**. Although present remediation technologies are capable of removing significant contaminant mass, some contamination usually remains trapped in parts of porous media where treatment did not reach. Treatment train approaches can account for rebound events through the implementation of a low cost and low maintenance polishing step. This approach increases the cost-efficiency of the initial treatment method, if that is designed to accept a higher contaminant

concentration as endpoint (see previous comment on cost-efficiency vs. contaminant concentration).

4. **Unfavorable site conditions for a specific remediation effort.** A treatment train approach can be used in order to prime the site for a subsequent remediation technology. For example, at low permeability media, hydraulic or pneumatic fracturing could be used to enhance the delivery of subsequent treatment agents (oxidants, surfactants, nutrient, bacteria) [158]. Another common example is the active enhancement of the contaminants' mobility and bioavailability. Zoller et al. [159,160] showed that surfactant flushing enhanced mobilization of a NAPL phase, which, in turn, enhanced biodegradation. Thermal and chemical pretreatment can also be used to enhance bioavailability of sorbed or entrapped contaminant phases [161,162].

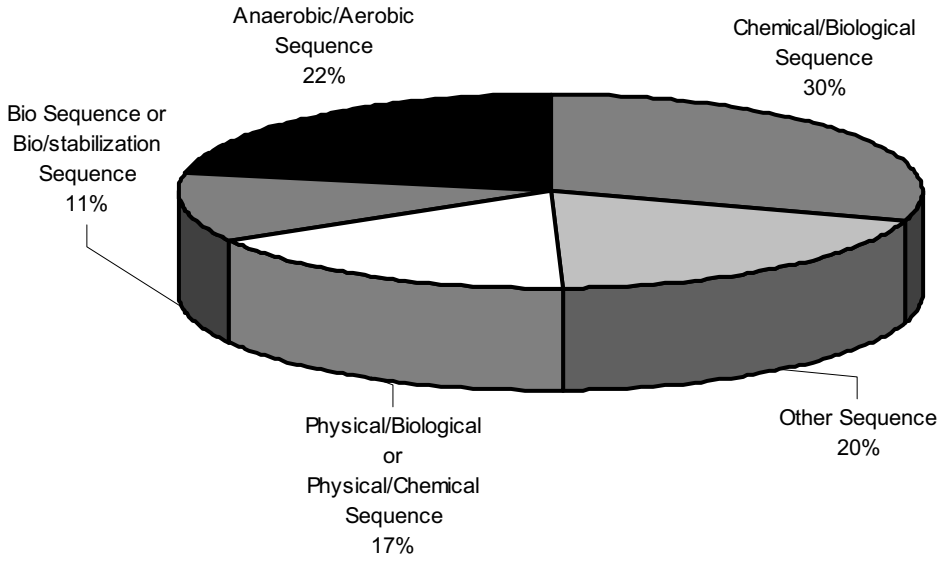


Figure 6.1. Treatment train combinations for soil and groundwater treatment from 42 laboratory and field studies. Redrawn from [5] excluding *ex-situ* applications.

In order to further increase cost-efficiency, system components for treatment trains (e.g. injection wells) can be designed to function for all applied technologies [5]. Information on treatment trains for the remediation of soil and groundwater from 48 case studies was collected in a recent report conducted for USEPA [5]. Figure 6.1 shows the distribution of the different combinations. Although it is not possible to define trends from so few cases, it is clear that in many cases, bioremediation is chosen as the second (usually final) treatment step. This sets focus on the compatibility of different remediation technologies with bioremediation.

6.2. Challenges with combining aggressive mass removal technologies and bioremediation

The compatibility of different remediation technologies is called into question [5]. It has been suggested that aggressive mass removal technologies can have detrimental effects on microorganisms either due to direct toxicity of the reagents on the soil biota, or due to the changes they cause on environmental conditions. Table 6.1 summarizes the effects that two common treatment technologies can have on subsequent microbial processes. The coupling of ISCO and bioremediation will be discussed in detail in the next chapter. Most remediation technologies lead to changes in dissolved oxygen and redox conditions. The importance of these changes depends on whether the subsequent biodegradation is intended to proceed through aerobic or anaerobic conditions. Recent studies on treatment trains show that the inhibitory effects of most treatments are often temporary [19,163,164], but recovery times for microbial activity can vary from several months to a few years. Depending on the clean-up target, and the time frame, the suitability of a specific treatment train can be evaluated.

Table 6.1. Effects of remediation technologies that can influence microbial degradation processes.

Treatment	Effects on redox conditions	Other effects
Surfactant/cosolvent flushing	may decrease dissolved oxygen and redox potential [165]	May remove or add electron donors leading to preferable degradation of remaining surfactants rather than the target contaminants [166]. Surfactants and solvents can have toxic effects on bacteria [167].
Thermal treatment	little effect on redox conditions [168]	Increased organic matter availability[168]. High temperatures have adverse effects on microorganisms, but microbial activity can recover after 8-14 months [169,163].

7. COUPLING ISCO AND BIOREMEDIATION

Treatment trains where ISCO is used prior to *in situ* bioremediation (ISB) are a viable treatment approach for many contaminated sites. There are three primary reasons for the use of an ISCO-ISB treatment train:

1. ISCO is used as a pretreatment in order to increase the bioavailability of immobile, persistent contaminants, such as heavy PAHs from creosote. Table 7.1 presents a list of laboratory- and field-scale studies where chemical oxidants were used to increase contaminant bioavailability. The majority of these studies have been carried out in soil systems. There are cases in which the compounds that result from contaminant oxidation are not biodegradable, or in which chemical oxidation removes only the most bioavailable contaminant fraction, and the persistent, non-biodegradable contaminants still remain [112].
2. ISCO is used as a first step that can prepare the site for a subsequent bioremediation effort by a) removing non biodegradable contaminants, b) removing high contaminant concentrations that are toxic for microorganisms and c) creating aerobic conditions that favor aerobic biodegradation [Tsitonaki et al., **IV**].
3. In order to reduce the total treatment cost, ISCO is only applied to reduce contaminant mass to a certain target point, after which ISB is used as a polishing step [Tsitonaki et al., **IV**,16].

Two or all of the above reasons can often coincide as the motivation for using an ISCO-ISB treatment train.

Coupling an aggressive technology such as ISCO with bioremediation can present several challenges. Oxidants can exert a direct toxicity on microorganisms, which will particularly inhibit intrinsic biodegradation processes. ISCO also causes a variety of environmental changes that can affect the success of bioremediation.

7.1. Toxic effects of oxidants on microorganisms

Oxidation stress has been thoroughly studied at the cellular level [9]. It has been found that it can cause DNA destruction [170-172], as well as damage proteins and lipid membranes [173]. Especially free radicals species (such as the hydroxyl, the superoxide and the sulfate radical) can have detrimental effects on cells. It is, however, important to differentiate between the effects from *in vivo* and *in vitro* exposure to free radicals. While radicals generated inside microbial cells are very destructive, exposure to the same radicals *in vitro* is less damaging. The short life span of the radicals may prevent them from diffusing into the lipid bilayer of the microorganisms [174]. Some

microorganisms have developed mechanisms to overcome oxidative stress, particularly from hydroxyl radicals [9], e.g. by as the production of catalase enzymes [173].

Table 7.1. Laboratory and field studies where chemical oxidation has been used as a pretreatment for increasing contaminant bioavailability.

Oxidant	Scale	Contaminant	Result	Ref.
CHP	Lab-groundwater	Monochlorobenzene	MCB was degraded to more bioavailable products.	[162]
CHP	Lab – soil	Creosote PAHs	Creosote compounds were oxidized to more bioavailable forms.	[11]
CHP	Lab – soil	3-4 ring PAHs	Generated more water-soluble and biodegradable PAHs in sand.	[12]
CHP	Lab-slurry	TCDD	TCDD was oxidized to more biodegradable byproducts.	[17]
CHP	Lab groundwater	Anthracene Benzo(a)pyrene	Enhanced biodegradation.	[18]
CHP	Lab – soil	Benz(a)anthracene	Enhanced the biodegradability of BAA through transforming it to more biodegradable intermediates.	[175]
CHP	Lab – soil	Creosote PAHs	Enhanced biodegradation of 4-5 ring PAHs, inhibition of 2-3 ring PAH degradation.	[68]
Permanganate peroxide, MgO_2	Lab – soil/ groundwater	Jet fuel hydrocarbons	Consumption of the available substrates by the strong oxidants led to inhibition of biodegradation.	[112]
Permanganate	Field-groundwater		Moderate amounts of permanganate may have enhanced biomass (by increasing bioavailability and organic matter).	[176]
Ozone	Lab - soil	Benzo(a)pyrene	Ozonation produced oxygenated intermediates that were more biodegradable.	[177]
Ozone	Lab - soil	Phenanthrene	The products of ozonation were not biodegradable.	[178]
Ozone	Lab - soil	PAHs	Indications that ozonation produced more biodegradable intermediates.	[179]

In the context of ISCO, specific circumstances can mitigate the damaging effects of oxidants on the aquifer microorganisms. These are:

- Exposure to radicals and oxidants happens *in vitro*, which is less damaging than when hydroxyl and superoxide radicals or H_2O_2 are produced inside the cells as byproducts of respiration [180].
- Diverse aquifer communities are likely to be less sensitive to oxidant toxicity compared to pure strain laboratory cultures. This was also demonstrated by Tsitonaki et al. [**II**], who found increased toxicity of heat-activated persulfate on the laboratory strain *Pseudomonas putida* KT2440, compared to indigenous soil microorganisms (see Figure 7.1).

- Microbial communities in aquifers are in stationary phase. Thus, they have lower vulnerability to oxidative and heat stress than the exponentially growing cultures that are often tested in laboratory experiments [173,181,182].
- The physical shelter provided by the sediment particles can protect from oxidant exposure.
- The introduction of new microorganisms from incoming groundwater can help the recovery of the microbial community after oxidant exposure.

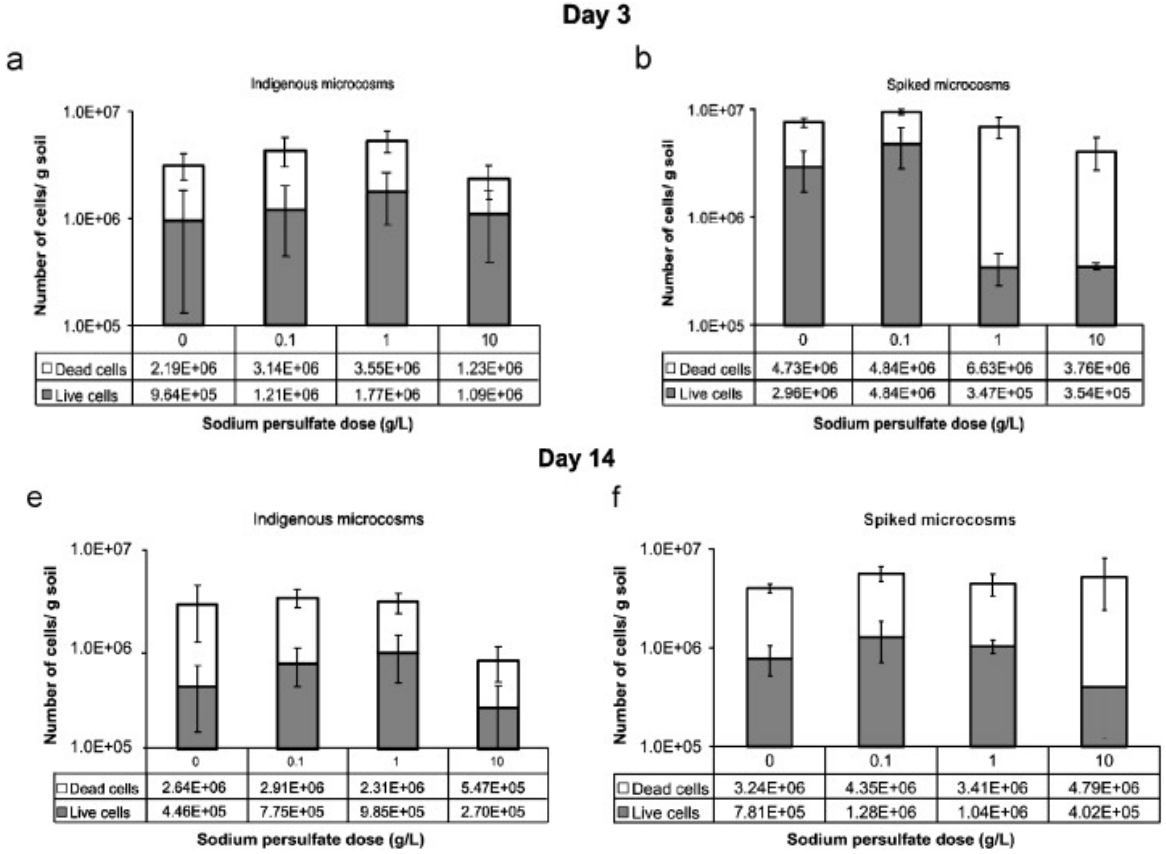


Figure 7.1. Effect of a 2-day exposure to heat-activated persulfate on indigenous microcosms (left) and microcosms spiked with laboratory strain *P.putida* (right). Day 3 shows measurements immediately after a 2-day exposure to heat activated persulfate at 40 ^{o}C, day 14 shows measurements 11 days after the termination of the exposure. This figure has been modified from Tsitonaki et al. **[II]**.

It is difficult to predict the effects of ISCO based on the plethora of oxidant toxicity studies conducted with pure strains in aqueous solutions. Table 7.2 presents a list of studies that were performed under aquifer relevant conditions. To allow a comparison across different oxidants, the oxidant doses are expressed in terms of electron equivalents. The data in Table 7.2 suggest that modified Fenton's reagent and hydrogen peroxide are more damaging to microorganisms than activated persulfate and permanganate. One explanation of why activated persulfate would be less toxic than Fenton's and hydrogen peroxide is that the sulfate radicals produced from the persulfate

reaction are less able to diffuse into the cell membranes due to their larger molecular size.

Another apparent observation from Table 7.2 is that higher oxidant doses result in larger biomass decreases. This implies that low oxidant doses are preferable for ISCO, if ISB will be the subsequent treatment. Lower oxidant doses will also result in less extensive environmental changes.

The effects of oxidants on microorganisms can also be different depending on whether biomass or activity indicators are used in the assessment. For example, in the experiments of Tsitonaki et al. **[II]**, although persulfate concentrations of 10 g/L did not affect the number of live cells in indigenous microcosms, a dramatic inhibition of acetate consumption occurred at that concentration (see Table 7.2). A similar tendency was observed for modified Fenton's reagent (see Table 7.2). This highlights the need for multi-parameter assessment of the effects of chemical oxidants on indigenous communities, as one-sided analyses may yield biased results.

It would be expected that less aggressive oxidants such as permanganate or persulfate would be less toxic than radical generating oxidants. This is partly true; however for ISCO application, the duration of exposure may be critical for the survival of microorganisms [16]. Persulfate and permanganate are more stable in the subsurface and this can have adverse effects in microorganisms. In the study of Tsitonaki et al. [**II**], it was found that although 10 g/L heat-activated persulfate had no immediate effects on biomass density, a significant decrease was observed 14 days later. This could be due to the toxicity of the remaining non-activated persulfate in the microcosms. Macbeth et al. [176] studied the impacts of ISCO with permanganate on indigenous microbial communities and found that both biomass and diversity were negatively affected.

Table 7.2. The effects of different oxidants on microorganisms in laboratory studies. From Tsitonaki et al. [**II**]. For references see Appendix II.

Oxidant	Dose		Type of bacteria	Exposure time	Changes in cell density and diversity		Changes in activity	Ref.
	Mass units	Electron eq (meq)			Before oxidation	After oxidation		
Fenton's reagent	180 g/kg soil[a]	5300/kg soil[a]	Indigenous (soil)	2 d	6 colony types 10^3 CFU *pseudomonas*/mL	3 colony types 10^5 CFU *pseudomonas*/mL	Enhanced biodegradation of pendimethalin	/a/
Modified Fenton's reagent	0.15 g/L[b]	4.4/l[b]	*Xanthobacter flavus*	<6 h	10^7 cells/mL	10^5 cells/mL	Low doses increased catalase activity	/b/
Modified Fenton's reagent	400 g/kg soil[a]	11760/kg soil[a]	Indigenous (soil)	4 d	9×10^9 live cells/g soil	5×10^9 live cells/g soil	Aerobic biodegradation of PAHs continued. Inhibition of extracellular esterase activity	/c/
Modified Fenton's reagent	0.34–1.7 g/L 3.4g/L	10–50/L 100/L 200/L	Indigenous (soil)	10 h	$10^{7.5}$ CFU/g soil $10^{7.5}$ CFU/g soil $10^{7.5}$ CFU/g soil	$10^{7.5}$ CFU/g soil: No effect $10^{6.8}$ CFU/g soil 10^6 CFU/g soil	Temporary inhibition of heterotrophic oxalate assimilation at doses above 100/L	/d/
H_2O_2	0.1 g/L[b]	3/L[b]	*E. coli*	120 min	10^{10} CFU/mL	10^5 CFU/mL	Not reported	/e/
H_2O_2	10 g/kg soil[a]	294/kg soil[a]	Indigenous (soil)	2 d	$10^{7.5}$ CFU/g soil	$10^{5.42}$ CFU/g soil	Not reported	/f/
H_2O_2	0.3 g/L[a]	8.82/L[a]	Indigenous (aquifer)	3 h	10^7 CFU/mL	10^2 CFU/mL	Increased catalase activity	/g/
$MnO_4^?$	10 g/kg soil[a]	252/kg soil[a]	Indigenous (soil)	2 d	$10^{7.5}$ CFU/g soil	$10^{7.12}$ CFU/g soil	Not reported	/f/
[c]$S_2O_8^{2?}$	10 g/kg soil[a]	104/kg soil[a]	Indigenous (soil)	2 d	$10^{7.5}$ CFU/g soil	$10^{7.4}$ CFU/g soil	Not reported	/f/
[d]$S_2O8^{2?}$	0.08–8.06 g/L	0.84–84/L 0.02–2 g/kg[e] 0.21–21/kg soil[e]	Indigenous (aquifer)	2 d	8.19×10^6 live cells/g soil	9.06×10^6 cells/g soil	Dramatic inhibition of acetate consumption at the highest per sulfate dose	This work
[d]$S_2O_8^{2?}$	0.08 g/L 0.8 g/L 8.06 g/L	0.84/L=0.2/kg 8.4/L=2/kg soil 84/L=21/kg soil	*P. putida* spiked	2 d	2.96×10^6 live cells/g soil	4.84×10^6 cells/g soil 3.4×10^5 cells/g soil 3.5×10^5 cells/g soil	Dramatic inhibition of acetate consumption at the highest per sulfate dose	This work

/a/ Miller et al. (1996), /b/ Büyüksönmez et al. (1998), /c/ Palmroth et al. (2006), /d/ Ndjou'ou et al. (2006), /e/ Watts et al. (2003), /f/ Bou-Nasr et al. (2006), /g/ Spain et al. (1989).The reported Fenton's reagent concentrations refer to H_2O_2 concentrations. The oxidant doses in electron equivalents are estimates based on transferred electrons for complete consumption of each oxidant. Partial redox reactions contribute to the following number of electrons per mole of oxidant: $H_2O_2\times1$, $MnO_4^-\times3$ and $S_2O_8^{2-}\times2$.[a] Only one dose tested.[b] A range of doses tested but we choose the most suitable for comparison with this work.[c] Iron-activated. [d] Heat-activated.[e] Recalculated per kg aquifer material.

7.2. Environmental changes from ISCO that affect biological processes

In situ chemical oxidation can also affect biological processes indirectly, because it changes the environmental conditions that control these processes. Several studies that have studied the coupling of ISCO and bioremediation have reported on those changes and the results they had on the desired biodegradation processes. Environmental factors that are affected by ISCO and are important for bioremediation are: pH, temperature, redox conditions and electron donors.

7.2.1. Changes in water chemistry

ISCO can cause a decrease in pH [Tsitonaki et al., **II**,**IV**] which may be inhibitory for biodegradation processes. Acidification may cause the solubilization of heavy metals that may be toxic for microorganisms. Atagana et al. [183] studied the influence of different pH values on the biodegradation of creosote compounds. The microorganisms involved could utilize creosote at pH 5.5 to 8 and kept reproducing but maximum activity was observed at neutral pH 6.5-7.

Chemical oxidation often results in an increase in dissolved oxygen and the redox potential of the aquifer [19,165]. Particularly when ISB is used as a polishing step after ISCO, the redox conditions can be very important for the establishment of the desired microbial processes as they control the availability of different electron acceptors. The application of persulfate has been observed to cause increased sulfate levels in both field and laboratory scale experiments ([86,Tsitonaki et al., **IV**].

ISCO is also likely to release organic matter bound to soil minerals [184], which can act as an electron donor for some processes. Nutrients associated to soil minerals may also be released. So far, there are no studies investigating these changes from ISCO and what effects they could have on bioremediation.

7.2.2. Changes in temperature

ISCO with CHP or activated persulfate may cause increases in temperature because oxidation reactions are exothermic. Heat-activated persulfate employs heating to enhance the efficiency of oxidation. Microbial degradation processes are sensitive to temperature changes. Many of the biodegrading bacteria belong in the mesophilic group, with an optimal growth temperature between 20 and 40 ^{o}C. Thus, elevating the aquifer temperature from 15 to 40 ^{o}C, as is the case with ISCO, could enhance biodegradation, as observed in studies of reductive dechlorination [185]. The increased temperatures also enhance the bioavailability of the contaminants by increasing their solubility. Kosegi et al. [186] studied the effect of temperature on DNAPL biodegradation using modeling software. Their results showed that by increasing the temperature from 15 to 35 ^{o}C the amount of DNAPL removal increased by 94% and

biomass counts showed a 70% rise, but the calculated biodegradation rate dropped dramatically above 35 ^{o}C. By contrast, incubation of microcosms at temperatures from 15-50 ^{o}C in another laboratory batch experiment did not affect biomass density or microbial activity as measured by acetate consumption [Tsitonaki et al., **II**].

7.3. Effects of ISCO on specific biodegradation processes

The nature and extent of the effects that ISCO can have on biodegradation depends strongly on the nature of these processes. Specifically, aerobic processes will benefit from the oxidized conditions, while anaerobic processes will most likely be inhibited.

Most of the existing work on coupling ISCO and bioremediation has been conducted in soil systems, where ISCO was used as a pretreatment to aerobic bioremediation of primarily PAHs (Table 7.1). Very few have looked into the combination of ISCO and aerobic bioremediation in an aquifer environment. Bittkau et al. [187] found that the application of Fenton's reagent provided oxygen, which enhanced the microbial degradation of monochlorobenzene.

Tsitonaki et al. **[IV]** investigated the potential of combining with heat-activated persulfate oxidation with intrinsic aerobic bioremediation in an aquifer-representative set- up in laboratory column reactors. The target contaminants were MTBE (9 mg/L) and creosote components Heat-activated persulfate was applied at 30 g/L. In the preoxidation phase, MTBE was persistent, but then completely removed by the repeated injections of heat activated persulfate (data shown in Appendix IV). Figure 7.2 shows the concentration profiles for the creosote compounds. In the pre-oxidation phase both carbazole and orthocresol were only partly removed by intrinsic biodegradation processes, while the rest of the contaminants were removed to below detection levels. The persistence of orthocresol is attributed to the high feeding concentrations. Chemical oxidation by persulfate resulted in complete removal of all creosote compounds. In the post-oxidation phase the columns were flushed with low concentrations of a creosote mixture (~10mg/L) in order to simulate rebound of contamination in a potential field application. All of the PAHs were removed in the post-oxidation phase. Hence, persulfate oxidation clearly did not destroy the biodegradation potential of the aquifer material. On the contrary, the removal of the high orthocresol concentrations by persulfate oxidation may have enhanced the potential for carbazole biodegradation. The application of persulfate caused temporary changes in water chemistry (reduction of the pH and increased sulfate concentration) but levels returned to normal after flushing with sterile groundwater medium. This reflects the effects incoming groundwater would have in an *in situ* application. The presence of degrading microorganisms in the post oxidation phase was confirmed by PCR for the *pcah* gene [Tsitonaki et al., **V]**, which encodes for a critical part of the aromatic degradation pathway [146].

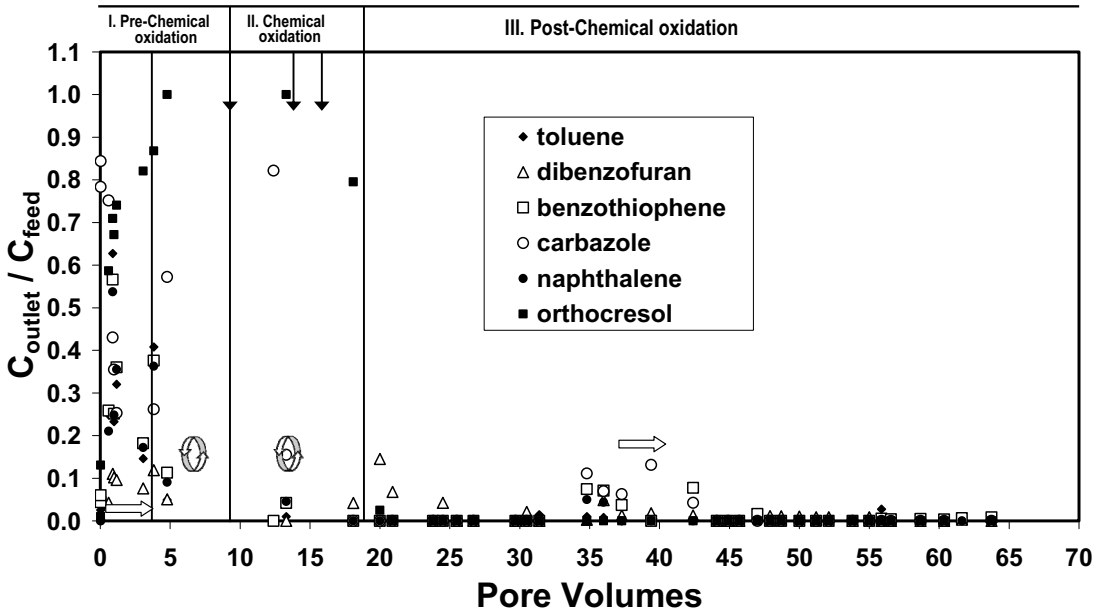


Figure 7.2. Profiles of creosote compounds for a sequential treatment of chemical oxidation with persulfate and intrinsic bioremediation in laboratory column reactors. Modified from Tsitonaki et al. [**IV**].

Other studies on the coupling of ISCO and bioremediation in groundwater systems have focused on the negative impacts ISCO can have on anaerobic biodegradation. Hrapovic et al. [15] showed that after treatment with permanganate in laboratory soil packed reactors, re-establishment of reduced conditions was necessary for TCE degradation to occur, even though the soil was amended with a dechlorinating culture. They recommended that bioaugmentation should be applied after reduced conditions are re-established to avoid impairment of the dechlorinating culture by oxidized conditions. Sahl et al. [16] observed that PCE dechlorination was inhibited following permanganate oxidation, but it rebounded after flushing with sterile nutrient medium. In an *in situ* application of Fenton's reagent [19], the efficiency of dechlorination was decreased as the redox conditions shifted from sulfate- to iron-reducing in the source zone. However, dechlorination did not cease, and it rebounded once the aquifer returned to its original state (<6 months). It seems that despite the initial inhibition of anaerobic biodegradation processes, these processes can rebound once the aquifer conditions returned to reduced.

The above findings show that ISCO and bioremediation are compatible. If this combination is part of an integrated treatment train, certain measures can be taken in order to ensure the success of the treatment:

1. The selection of oxidant should be considered. The effects of ISCO on biodegradation processes are oxidant specific and certain oxidants seem to exert higher toxicity than others [Tsitonaki et al., **II**].
2. The selection of oxidant dose is critical. There is general consensus on the use of lower dose for more cost-efficient use of the oxidant. Higher oxidant doses will cause a higher inhibition on the microbial community and longer lasting environmental changes.
3. If anaerobic bioremediation is desired after ISCO treatment, the recovery time may be longer than for aerobic. Certain amendments such as carbon substrates can stimulate anaerobic conditions. Bioaugmentation with anaerobic cultures is recommended only after the site conditions have returned to reduced.
4. It is preferable to combine ISCO with aerobic bioremediation in order to take advantage of the elevated dissolved oxygen levels and redox potential after ISCO.

8. CONCLUSION

Soil and groundwater contamination is a widespread problem that occupies environmental engineers, politicians and the public, as we are increasingly concerned about the quality and suffice of drinking water resources and the protection of sensitive ecosystems.

Combining individual remediation technologies in an integrated strategy can help overcome the limitations of individual technologies and lead to cost-efficient remediation. Such combinations are also known as "treatment trains". This work investigated the compatibility of *in situ* chemical oxidation and *in situ* bioremediation for treating a complex contamination consisting of MTBE and a mixture of creosote compounds. Particular focus was on persulfate, the newest agent for ISCO. The main findings from the reviewed literature and the conducted experiments are:

Regarding ISCO with persulfate

- *In situ* chemical oxidation with persulfate can be effective towards many of the commonly targeted organic contaminants in soil and groundwater systems. These include: BTEX, chlorinated solvents, chlorinated benzenes, PAHs, and MTBE.
- Heat activation is the most effective activation technology. However, when upscaling, heating the aquifer can be a challenge. Heat activation can allow persulfate to transport/diffuse into the contaminated zone and then activated when in contact with the contaminant, achieving maximum oxidant efficiency. Other feasible means of activating persulfate, once it is in contact with the contaminant, can be probe injections of other activators (peroxide, base or iron). When choosing an activation aid, contaminant type, environmental conditions, availability of equipment, and costs, must be taken into consideration.
- Moderate heating to 40 ^{o}C is recommended in order to achieve high contaminant destruction and limit unproductive persulfate decomposition.
- Information regarding the interactions of persulfate with soil and groundwater components is limited, as only few types of aquifer material have been studied.
- There is also very little information on upscaling persulfate oxidation to field scale.

Regarding the combination ISCO with persulfate and bioremediation

- In this work, indigenous aquifer microorganisms showed a high robustness to persulfate in terms of cellular integrity, but substrate utilization ability was negatively affected at high dosage. This highlights the need for multi-parameter assessment of the effects of chemical oxidants on indigenous communities.

- Early findings suggest that persulfate may be less damaging to microorganisms than catalyzed hydrogen peroxide. In this work, natural biodegradation processes persisted after treatment with persulfate concentrations of up to 30 g/L.

Regarding the combination of ISCO and ISB

- There are very few studies where the coupling of ISCO and bioremediation has been investigated using microbial populations that were actually exposed to the oxidative treatment. In most of the existing studies, microorganisms were added after ISCO was completed. Furthermore, studies in soil systems are dominating, very few have looked into the coupling of ISCO and ISB in groundwater.
- Challenges related to the coupling of ISCO and bioremediation result from the oxidants' toxicity on microorganisms and ISCO-induced environmental changes that inhibit microbial processes. To avoid the inhibition of biodegradation processes, the oxidant dose should be carefully chosen instead of the common practice of overdosing.
- The effects of ISCO agents on soil and groundwater microorganisms can be very diverse depending on the applied oxidant and the exposed microorganisms. Dramatic decreases in bacterial abundance are often observed immediately after ISCO, but the microbial density usually recovers with time.
- The toxicity of oxidants on pure-strain microorganisms in laboratory experiments seems to overestimate the effects that ISCO would have on indigenous populations in an aquifer.
- Combining ISCO and bioremediation is a viable alternative for dealing with complex contaminant mixtures, and high contaminant concentrations where bioremediation alone would not be effective.
- ISCO is more compatible with aerobic biodegradation processes, partly due to the generation of oxidized conditions. However, studies suggest that anaerobic activity can resume after ISCO, if reduced conditions are re-established.

9. SUGGESTIONS FOR FUTURE RESEARCH

This work investigated the compatibility of *in situ* chemical oxidation with persulfate with *in situ* bioremediation (ISB). In order to further develop the use of this combination for in situ applications further research is necessary on improving persulfate technology and on issues associated with the coupling of ISCO and ISB.

Regarding persulfate there is a need further research on:

- Activation methods for persulfate, including activation by naturally occurring iron. More research is needed in terms of which types of iron and iron minerals persulfate reacts with.
- The interactions of persulfate with the porous media in relation to interactions with the contaminants and the consumption of persulfate due to activation. Further study of persulfate application in different soil types of variable clay and organic carbon content, inorganic composition, and redox status is needed.
- Field/pilot scale applications of ISCO with persulfate that are well-documented and shed light into upscaling issues, such as effective oxidant distribution, and achieving high and timely activation efficiency.

Regarding the combination of ISCO and bioremediation

This work has contributed with information on the effects of persulfate on microorganisms and biodegradation processes in a laboratory set-up. Further research is suggested on the following:

- The compatibility of different persulfate activation methods with bioremediation.
- Further study on the effects of aquifer heating on subsequent bioremediation efforts is also of interest, including the duration of the elevated temperatures in an aquifer set up.
- The effects of different oxidants on indigenous microorganisms under aquifer representative conditions or in field scale. Molecular microbiology tools could be used to assess the changes ISCO causes on the composition of microbial aquifer communities and whether it favors specific degraders.
- The effects of ISCO on subsequent biodegradation processes are also likely to be media specific. Further research in different aquifer materials (clay, chalk, dual porosity media) is required for optimizing the ISCO-ISB treatment approach.
- Well documented pilot and field scale applications of ISCO-ISB that investigate a) the effects of oxidants on microorganisms b) the duration of the changes in redox conditions and other environmental factors after ISCO. Such investigations could create the foundation for a decision-support tool for the design and implementation of effective and cost-efficient treatment trains.

REFERENCES

[1] C. Melber, J. Kielhorn, I. Mangelsdorf. Coal Tar Creosote. Geneva, World Health Organization. ISBN 92 4 153062 6, Geneva, 2004.

[2] L. Andersen, J. B. Hansen. Evaluation of MTBE and alternative additives-experiences, environmental assessment and energy security (in Danish). Technology development program for soil and groundwater pollution. Environmental project nr. 1086, Danish Environmental Protection Agency. Copenhagen, Denmark 2006.

[3] R. Johnson, J. F. Pankow, D. Bender, D. Price, J. S. Zogorski. MTBE- to what extent will past releases contaminate community water supply wells? Environmental Science & Technology 32, 3194-3199, 2000.

[4] USEPA. Treatment technologies for site cleanup - Annual status report (12th edition). EPA-542-R-07-012, 2007.

[5] D. S. Roote. Technology Status Report: Treatment trains for remediation of soil and groundwater. TS-03-012003. Groundwater Remediation Technologies Analysis Center. GWRTAC Series.

[6] S. G. Huling, B. E. Pivetz. In-Situ Chemical Oxidation. EPA/600/R-06/072, Cincinnati, OH, United States Environmental Protection Agency, 2006.

[7] K. C. Huang, Z. Q. Zhao, G. E. Hoag, A. Dahmani, P. A. Block. Degradation of volatile organic compounds with thermally activated persulfate oxidation. Chemosphere 61, 551-560, 2005.

[8] R. J. Watts, A. L. Teel. Treatment of contaminated soils and groundwater using ISCO. Practice Periodical of Hazardous, Toxic and Radioactive Waste Management, 2-9, 2006.

[9] J. W. Sahl, J. Munakata-Marr. The effects of in situ chemical oxidation on microbiological processes: A review. Remediation 16, 57-70, 2006.

[10] S. H. Lee, J. B. Carberry. Biodegradation of PCP Enhanced by Chemical Oxidation Pretreatment. Water Environment Research 64, 682-690, 1992.

[11] R. Piskonen, M. Itavaara. Evaluation of chemical pretreatment of contaminated soil for improved PAH bioremediation. Applied Microbiology and Biotechnology 65, 627-634, 2004.

[12] N. Kulik, A. Goi, M. Trapido, T. Tuhkanen. Degradation of polycyclic aromatic hydrocarbons by combined chemical pre-oxidation and bioremediation in creosote contaminated soil. Journal of Environmental Management 78, 382-391, 2006.

[13] J. Klens, D. Pohlmann, S. Scarborough, D. Graves. The effects of permanganate oxidation on microbial populations. A. Leeson, M. E. Kelley, H. S. Rifai, V. S. Magar. In: Proceedigns of The Sixth International In Situ and On Site Bioremediation Symposium, San Diego, California. Natural Attenuation of Environmental Contaminants. Columbus Richland, Battelle Press. 2001.

[14] T. W. Macbeth, L. N. Peterson, R. C. Starr, K. S. Sorenson, R. Goehlert, K. S. Moor. ISCO impacts on indigenous microbes in a PCE-DNAPL contaminated aquifer. In: Proceedings of the Eighth International In Situ and On-Site Bioremediation Symposium, Baltimore, MA. Battelle Press. 2005.

[15] L. Hrapovic, B. E. Sleep, D. J. Major, E. D. Hood. Laboratory study of treatment of trichloroethene by chemical oxidation followed by bioremediation. Environmental Science & Technology 39, 2888-2897, 2005.

[16] J. W. Sahl, J. Munakata-Marr, M. L. Crimi, R. L. Siegrist. Coupling permanganate oxidation with microbial dechlorination of tetrachloroethene. Water Environment Research 79, 5-12, 2007.

[17] C. M. Kao, S. C. Chen, J. Y. Wang, Y. L. Chen, S. Z. Lee. Remediation of PCE-contaminated aquifer by an in situ two-layer biobarrier: laboratory batch and column studies. Water Research 37, 27-38, 2003.

[18] N. Nadarajah, J. Van Hamme, J. Pannu, A. Singh, O. Ward. Enhanced transformation of polycyclic aromatic hydrocarbons using a combined Fenton's reagent, microbial treatment and surfactants. Applied Microbiology and Biotechnology 59, 540-544, 2002.

[19] F. H. Chapelle, P. M. Bradley, C. C. Casey. Behavior of a chlorinated ethene plume following source-area treatment with Fenton's reagent. Ground Water Monitoring and Remediation 25, 131-141, 2005.

[20] B. L. Morris, A. R. L. Lawrence, P. J. C. Chilton, B. Adams, R. C. Calow, B. A. Klinck. Groundwater and its Susceptibility to Degradation: A Global Assessment of the Problem and Options for Management. Early Warning and Assessment Report Series. United Nations Environment Programme, Nairobi Kenya, 2003.

[21] P. B. Bedient, H. S. Rifai, C. J. Newell. Groundwater contamination: Transport and Remediation, second edition, New Jersey, Prentice Hall, 1997.

[22] EINECS. Risk Assessment: Tert-butyl methyl ether. No 216-653-1, 2001.

[23] L. C. Davis, L. E. Erickson. A review of bioremediation and natural attenuation of MTBE. Environmental Progress 23, 243-252, 2004.

[24] C. K. Waul. Biodegradation of MTBE in reactors. PhD Thesis, Institute of Environment & Resources, Technical University of Denmark, 2007.

[25] D. McGregor. Methyl tertiary-butyl ether: Studies for potential human health hazards. Critical Reviews in Toxicology 36, 319-358, 2006.

[26] M. Krayer von Krauss, P. Harremoës. MTBE in petrol as a substitute for lead. In: Late lessons from early warnings: the precautionary principle 1896-2000, P. Harremoës, D. Gee, M. MacGervin, A. Stirling, J. Keys, B. Wynne, S. Guedes Vaz., Office for Official Publications of the European Communities, European Environment Agency, Luxembourg, 2001.

[27] Burbano, D. Dionysiou, M. Suidan, T. Richardson. Chemical destruction of MTBE using Fenton's Reagent: effect of ferrous iron/hydrogen peroxide ratio. Water Science and Technology 47, 165-171, 2003.

[28] D. C. L. Wong, W. R. Arnold, G. A. Rausina, E. R. Mancini, A. E. Steen. Development of a freshwater aquatic toxicity database for ambient water quality criteria for methyl tertiary-butyl ether. Environmental Toxicology and Chemistry 20, 1125-1132, 2001.

[29] G. A. Rausina, D. C. L. Wong, W. R. Arnold, E. R. Mancini, A. E. Steen. Toxicity of methyl tert-butyl ether to marine organisms: ambient water quality criteria calculation. Chemosphere 47, 525-534, 2002.

[30] D. Moreels, K. Van Cauwenberghe, B. Debaere, E. Rurangwa, N. Vromant, L. Bastiaens, L. Diels, D. Springael, R. Merckx, F. Ollevier. Long-term exposure to environmentally relevant doses of methyl-tert-butyl ether causes significant reproductive dysfunction in the zebrafish (Danio rerio). Environmental Toxicology and Chemistry 25, 2388-2393, 2006.

[31] G. H. S. Bonjar. Potential ecotoxicological implication of methyl tert-butyl ether (MTBE) spills in the environment. Ecotoxicology 13, 631-635, 2004.

[32] Danish EPA. Assessment of risks of MTBE contamination of groundwater aquifers (in Danish). Miljøprojekt, 7852003. Copenhagen, Denmark.

[33] S. Dyreborg. Microbial Degradation of water-soluble creosote compounds.PhD Thesis, Department of Environmental Science and Engineering, Technical University of Denmark, 1996.

[34] M. M. Broholm. Retardation and degradation of coal-ter compounds in fractured geologic media. 1998. Department of Environmental Science and Engineering, Technical University of Denmark.

[35] S. Dyreborg, E. Arvin, K. Broholm, J. Christensen. Biodegradation of thiophene, benzothiophene, and benzofuran with eight different primary substrates. Environmental Toxicology and Chemistry 15, 2290-2292, 1996.

[36] Technical University of Denmark, University of Copenhagen. Kemibrug: Chemical portal. www.kemibrug.dk 2008 .

[37] M. M. Broholm, K. Broholm, E. Arvin. Sorption of heterocyclic compounds from a complex mixture of coal-tar compounds on natural clayey till. Journal of Contaminant Hydrology 39, 201-226, 1999.

[38] S. S. Johansen. Heteroaromatic compounds and their products in creosote contaminated groundwater. PhD Thesis, Department of Environmental Science and Engineering, Technical University of Denmark, 1996.

[39] M. W. G. King, J. F. Barker. Migration and natural fate of a coal tar creosote plume 1. Overview and plume development. Journal of Contaminant Hydrology 39, 249-279, 1999.

[40] M. M. Broholm, E. Arvin. Biodegradation of phenols in a sandstone aquifer under aerobic conditions and mixed nitrate and iron reducing conditions. Journal of Contaminant Hydrology 44, 239-273, 2000.

[41] S. Dyreborg, E. Arvin, K. Broholm. Effects of creosote compounds on the aerobic bio-degradation of benzene. Biodegradation 7, 191-201, 1996.

[42] S. Dyreborg, E. Arvin, K. Broholm. The influence of creosote compounds on the aerobic degradation of toluene. Biodegradation 7, 97-107, 1996.

[43] S. Dyreborg, E. Arvin, K. Broholm. Biodegradation of NSO-compounds under different redox-conditions. Journal of Contaminant Hydrology 25, 177-197, 1997.

[44] Y. Spira, J. Henstock, P. Nathanail, D. Müller, D. Edwards. A European approach to increase innovative soil and groundwater remediation technology applications. Remediation , 81-96, 2006.

[45] L. A. Reitzel. Quantification of Natural Attenuation using Analytical-Chemical Tools. PhD Thesis, Environment & Resources DTU, Technical University of Denmark, 2005.

[46] USEPA. Monitored natural attenuation: USEPA Research Program - An EPA Science Advisory Board Review, EPA-SAB-EEC-01-004, Washington, DC, 2001.

[47] ITRC. Overview of groundwater remediation technologies for MTBE and TBA. Washington D.C., ITRC (Interstate Technology & Regulatory Council), 2005.

[48] D. H. Bass, N. A. Hastings, R. A. Brown. Performance of air sparging systems: a review of case studies. Journal of Hazardous Materials 72, 101-119, 2000.

[49] M. S. Hong, W. F. Farmayan, I. J. Dortch, C. Y. Chiang, S. K. McMillan, J. L. Schnoor. Phytoremediation of MTBE from a groundwater plume. Environmental Science & Technology 35, 1231-1239, 2001.

[50] E. Rubin, A. Ramaswami. The potential for phytoremediation of MTBE. Water Research 35, 1348-1353, 2001.

[51] X. M. Ma, A. R. Richter, S. Albers, J. G. Burken. Phytoremediation of MTBE with hybrid poplar trees. International Journal of Phytoremediation 6, 157-167, 2004.

[52] A. Burbano, D. D. Dionysiou, T. L. Richardson, M. T. Suidan. MTBE remediation in contaminated groundwater: The role of selected process conditions on the degradation of MTBE and reaction intermediate products via reactions involving hydroxyl radicals generated by the Fenton's reagent. Abstracts of Papers of the American Chemical Society 222, U423, 2001.

[53] A. Burbano, D. D. Dionysiou, T. L. Richardson, M. T. Suidan. Degradation of MTBE intermediates using Fenton's reagent. Journal of Environmental Engineering-ASCE 128, 799-805, 2002.

[54] A. Burbano, D. D. Dionysiou, M. T. Suidan, T. L. Richardson. Oxidation kinetics and effect of pH on the degradation of MTBE with Fenton reagent. Water Research 39, 107-118, 2005.

[55] K. C. Huang, R. A. Couttenye, G. E. Hoag. Kinetics of heat-assisted persulfate oxidation of methyl tert-butyl ether (MTBE). Chemosphere 49, 413-420, 2002.

[56] S. Fiorenza, M. P. Suarez, H. S. Rifai. MTBE in groundwater: Status and remediation. Journal of Environmental Engineering-ASCE 128, 773-781, 2002.

[57] R. D. Wilson, D. M. Mackay, K. M. Scow. *In situ* MTBE biodegradation supported by diffusive oxygen release. Environmental Science & Technology 36, 190-199, 2002.

[58] P. Hicks, J. Olinger. *In situ* aerobic bioremediation of MTBE via oxygen gas injection. A. R. Gavaskar,A. S. C. Chen. In: Proceedings of the 4th International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA) Columbus, OH, Battelle Press, 2004.

[59] M. A. Hansen, S. L. Drugan, J. Peters, T. Sizemore. Full scale aerobic bioremediation of MTBE at a bulk terminal in California. A. R. Gavaskar,A. S. C. Chen. In: Proceedings of the 4th International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA) Columbus, OH, Battelle Press, 2004.

[60] S. Koenigsberg. Use of ORC in the bioremediation of MTBE. Abstracts of Papers of the American Chemical Society 219, U655, 2000.

[61] J. P. Salanitro, P. C. Johnson, G. E. Spinnler, P. M. Maner, H. L. Wisniewski, C. Bruce. Field scale demonstration of enhanced MTBE bioremediation through aquifer bioaugmentation and oxygenation. Environmental Science & Technology 34, 4152-4162, 2000.

[62] K. F. Chen, C. M. Kao, J. Y. Wang, T. Y. Chen, C. C. Chien. Natural attenuation of MTBE at two petroleum-hydrocarbon spill sites. Journal of Hazardous Materials 125, 10-16, 2005.

[63] J. T. Wilson, R. Kolhatkar. Role of natural attenuation in life cycle of MTBE plumes. Journal of Environmental Engineering-ASCE 128, 876-882, 2002.

[64] P. S. C. Rao, M. D. Annable, R. K. Sillan, D. P. Dai, K. Hatfield, W. D. Graham, A. L. Wood, C. G. Enfield. Field-scale evaluation of in situ cosolvent flushing for enhanced aquifer remediation. Water Resources Research 33, 2673-2686, 1997.

[65] R. E. Saichek, K. R. Reddy. Electrokinetically enhanced remediation of hydrophobic organic compounds in soils: A review. Critical Reviews in Environmental Science and Technology 35, 115-192, 2005.

[66] P. Isosaari, R. Piskonen, P. Ojala, S. Voipio, K. Eilola, E. Lehmus, M. Itavaara. Integration of electrokinetics and chemical oxidation for the remediation of creosote-contaminated clay. Journal of Hazardous Materials 144, 538-548, 2007.

[67] S. P. Forsey. *In Situ* Chemical Oxidation of Creosote/Coal Tar Residuals: Experimental and Numerical Investigation. PhD Thesis, Department of Earth Sciences, University of Waterloo, Canada. 2004.

[68] K. Nam, W. Rodriguez, J. J. Kukor. Enhanced degradation of polycyclic aromatic hydrocarbons by biodegradation combined with a modified Fenton reaction. Chemosphere 45, 11-20, 2001.

[69] F. Nadim, K. C. Huang, A. M. Dahmani. Remediation of Soil and Ground Water Contaminated with PAH using Heat and Fe(II)-EDTA Catalyzed Persulfate Oxidation. Water, Air, & Soil Pollution: Focus 6, 227-232.

[70] P. F. Killian, C. J. Bruell, C. J. Liang, M. C. Marley. Iron(II) Activated Persulfate Oxidation of MGP Contaminated Soil. Soil & Sediment Contamination 16, 523-537, 2007.

[71] M. L. Crimi, R. L. Siegrist. Geochemical effects on metals following permanganate oxidation of DNAPLs. Ground Water 41, 458-469, 2003.

[72] R. L. Siegrist, M. A. Urynowicz, O. R. West, M. L. Crimi, K. S. Lowe. Principles and practices of in situ chemical oxidation using permanganate. Columbus, Ohio, Battelle Press. 2001.

[73] ITRC. Technical and Regulatory Guidance for In Situ Chemical Oxidation of Contaminated Soil and Groundwater, 2nd ed. ISCO-2. Interstate Technology & Regulatory Council, Washington D.C., 2005.

[74] J. J. Pignatello, E. Oliveros, A. MacKay. Advanced oxidation processes for organic contaminant destruction based on the Fenton reaction and related

chemistry. Critical Reviews in Environmental Science and Technology 36, 1-84, 2006.

[75] W. G. Barb, J. H. Baxendale, P. George, K. R. Hargrave. Reactions of ferrous and ferric ions with hydrogen peroxide. 2. The ferric ion reaction. Transactions of the Faraday Society 47, 591-616, 1951.

[76] W. G. Barb, J. H. Baxendale, P. George, K. R. Hargrave. Reactions of Ferrous and ferric ions with hydrogen peroxide.1. The ferrous ion reaction. Transactions of the Faraday Society 47, 462-500, 1951.

[77] W. G. Barb, J. H. Baxendale, P. George, K. R. Hargrave. Reactions of ferrous and ferric ions with hydrogen peroxide. Nature 163, 692-694, 1949.

[78] J. Hønning. Use of in situ chemical oxidation with permanganate in PCE contaminated clayey till with sand lenses. PhD Thesis, Institute of Environment & Resources, Technical University of Denmark, 2007.

[79] R. H. Waldemer, P. G. Tratnyek, R. L. Johnson, J. T. Nurmi. Oxidation of chlorinated ethenes by heat-activated persulfate: Kinetics and products. Environmental Science & Technology 41, 1010-1015, 2007.

[80] M. Banerjee, R. S. Konar. Polymerization of Acrylonitrile Initiated by $K_2S_2O_8$-Fe(II) Redox System - Comment. Journal of Polymer Science Part A-Polymer Chemistry 22, 1193-1195, 1984.

[81] P. Neta, V. Madhavan, H. Zemel, R. W. Fessenden. Rate Constants and Mechanism of Reaction of So4.- with Aromatic-Compounds. Journal of the American Chemical Society, 99, 163-164, 1977.

[82] FMC, L. Specialty Earth Sciences. Catalysed persulfate oxidation treatment. Fort Belvoir military Garrison. Klozur's Resource Center, 2007 .

[83] G. Cronk, R. Cartwright. Optimization of a chemical oxidation treatment train process for groundwater remediation. In: Proceedings of the 5th International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA) Columbus, OH, Battelle Press. 2006.

[84] S. Thompson, J. Riggenbach, R. A. Brown, J. Hines, J. Haselow. Catalyzed persulfate remediation of chlorinated and recalcitrant compounds in soil. In: Proceedings of the 5th International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA) Columbus, OH, Battelle Press. 2006.

[85] K. L. Sperry, M. C. Marley, C. J. Bruell, C. J. Liang, J. P. G. Hochreiter. Iron Catalysed persulfate oxidation of chlorinated solvents. A. R. Gavaskar, A. S. C. Chen. In: Proceedings of the 3rd International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey CA, Columbus OH, Battelle Press. 2002.

[86] E. X. Droste, M. C. Marley, J. M. Parikh, A. M. Lee, P. M. Dinardo, B. A. Woody, G. E. Hoag, P. Chedda. Observed enhanced reductive dechlorination after in situ chemical oxidation pilot test. A. R. Gavaskar,A. S. C. Chen. In: Proceedings of the 3rd International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey CA, Columbus OH, Battelle Press. 2002.

[87] G. E. Hoag, P. V. Chheda, B. A. Woody, G. M. Dobbs. Chemical Oxidation of Volatile Organic Compounds. United Technologies Corporation. Patent No 73049 United States. 5-5-1998. 2000

[88] J. Liang, Z. S. Wang, N. Mohanty. Influences of carbonate and chloride ions on persulfate oxidation of trichloroethylene at 20 degrees C. Science of the Total Environment 370, 271-277, 2006.

[89] G. Aiken. Chloride Interference in the Analysis of dissolved organic carbon by the wet oxidation method. Environ.Science and Technology 26, 2435-2439, 1992.

[90] J. Liang, C. J. Bruell, M. C. Marley, K. L. Sperry. Thermally activated persulfate oxidation of trichloroethylene (TCE) and 1,1,1-trichloroethane (TCA) in aqueous systems and soil slurries. Soil & Sediment Contamination 12, 207-228, 2003.

[91] J. Liang, Z. S. Wang, C. J. Bruell. Influence of pH on persulfate oxidation of TCE at ambient temperatures. Chemosphere 66, 106-113, 2007.

[92] J. Liang, C. J. Bruell, M. C. Marley, K. L. Sperry. Persulfate oxidation for in situ remediation of TCE. I. Activated by ferrous ion with and without a persulfate-thiosulfate redox couple. Chemosphere 55, 1213-1223, 2004.

[93] J. Liang, C. J. Bruell, M. C. Marley, K. L. Sperry. Persulfate oxidation for in situ remediation of TCE. II. Activated by chelated ferrous ion. Chemosphere 55, 1225-1233, 2004.

[94] M. L. Crimi, J. Taylor. Experimental evaluation of catalyzed hydrogen peroxide and sodium persulfate for destruction of BTEX contaminants. Soil & Sediment Contamination 16, 29-45, 2007.

[95] L. Teel, C. R. Warberg, D. A. Atkinson, R. J. Watts. Comparison of mineral and soluble iron Fenton's catalysts for the treatment of trichloroethylene. Water Research 35, 977-984, 2001.

[96] P. A. Block, R. A. Brown, D. Robinson. Novel activation technologies for sodium persulfate in situ chemical oxidation. A. R. Gavaskar,A. S. C. Chen. In: Proceedings of the 4th International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA) Columbus, OH, Battelle Press. 2004.

[97] P. A. Block, C. G. Schreier. Comparison of activators for destruction of organic compounds of concern by sodium persulfate. In: Proceedings of the 3rd International Conference on Oxidation and Reduction Technologies for In-Situ Treatment of Soil and Groundwater (ORT-3), San Diego, California, USA, Redox Technologies, Inc. 2004.

[98] J. Cho, R. J. Fiacco, A. Brown, G. Skladany. Evaluation of technologies for in situ remediationof 1,1,1-trichloroethane. A. R. Gavaskar,A. S. C. Chen. In: Proceedings of the 3rd International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA) Columbus, OH, Battelle Press. 2002.

[99] J. Abranovic, D. Brown, A. Chemburkar. Persulfate stability is limiting factor for ISCO in fine-graines, iron-rich media. In: Proceedings of the 5th International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA) Columbus, OH, Battelle Press. 2006.

[100] S. Waisner, G. E. Hoag. Fe(III)-EDTA-activated persulfate destruction of explosives and pH dependence of chemistry. In: Proceedings of the 5th International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA) Columbus, OH, Battelle Press. 2006.

[101] R. A. Brown, D. Robinson. Response to naturally occuring organic material: Permanganate vs. persulfate. A. R. Gavaskar,A. S. C. Chen. In: Proceedings of the 4th International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA) Columbus, OH, Battelle Press. 2004.

[102] M. A. Urynowicz, R. L. Siegrist. Interphase mass transfer during chemical oxidation of TCE DNAPL in an aqueous system. Journal of Contaminant Hydrology 80, 93-106, 2005.

[103] R. H. Waldemer, P. G. Tratnyek. Kinetics of contaminant degradation by permanganate. Environmental Science & Technology 40, 1055-1061, 2006.

[104] Y. E. Yan, F. W. Schwartz. Oxidative degradation and kinetics of chlorinated ethylenes by potassium permanganate. Journal of Contaminant Hydrology 37, 343-365, 1999.

[105] I. T. Osgerby. ISCO Technology Overview: Do you really understand the chemistry? E. J. Calabrese, P. T. Kostecki, J. Dragun. In: Contaminated Soils, Sediments and Water, Volume 10: Successes and Challenges, 287-308, Springer. 2005

[106] B. A. Smith, A. L. Teel, R. J. Watts. Mechanism for the destruction of carbon tetrachloride and chloroform DNAPLs by modified Fenton's reagent. Journal of Contaminant Hydrology 85, 229-246, 2006.

[107] J. H. Damm, C. Hardacre, R. M. Kalin, K. P. Walsh. Kinetics of the oxidation of methyl tert-butyl ether (MTBE) by potassium permanganate. Water Research 36, 3638-3646, 2002.

[108] J. W. Kang, M. R. Hoffmann. Kinetics and mechanism of the sonolytic destruction of methyl tert-butyl ether by ultrasonic irradiation in the presence of ozone. Environmental Science & Technology 32, 3194-3199, 1998.

[109] M. I. Stefan, J. Mack, J. R. Bolton. Degradation pathways during the treatment of methyl tert-butyl ether by the UV/H_2O_2 process. Environmental Science & Technology 34, 650-658, 2000.

[110] Ferrarese, G. Andreottola, I. A. Oprea. Remediation of PAH-contaminated sediments by chemical oxidation. Journal of Hazardous Materials 152, 128-139, 2008.

[111] C. Cuypers, T. Grotenhuis, J. Joziasse, W. Rulkens. Rapid persulfate oxidation predicts PAH bioavailability in soils and sediments. Environmental Science & Technology 34, 2057-2063, 2000.

[112] B. Xie, M. J. Barcelona. Sequential chemical oxidation and aerobic biodegradation of equivalent carbon number-based hydrocarbon fractions in jet fuel. Environmental Science & Technology 37, 4751-4760, 2003.

[113] M. R. T. Palmroth, J. H. Langwaldt, T. A. Aunola, A. Goi, U. Munster, J. A. Puhakka, A. Tuhkanen. Effect of modified Fenton's reaction on microbial activity and removal of PAHs in creosote oil contaminated soil. Biodegradation 17, 131-141, 2006.

[114] J. Hønning, M. M. Broholm, P. L. Bjerg. Role of diffusion in chemical oxidation of PCE in a dual permeability system. Environmental Science & Technology 41, 8426-8432, 2007.

[115] Y. Seol, H. Zhang, F. W. Schwartz. A review of in situ chemical oxidation and heterogeneity. Environmental & Engineering Geoscience 9, 37-49, 2003.

[116] M. A. Dahmani, K. Huang, G. E. Hoag. Sodium Persulfate Oxidation for the Remediation of Chlorinated Solvents, Water, Air, & Soil Pollution: Focus 6, 127-141.

[117] J. Hønning, M. M. Broholm, P. L. Bjerg. Quantification of potassium permanganate consumption and PCE oxidation in subsurface materials. Journal of Contaminant Hydrology 90, 221-239, 2007.

[118] R. A. Crother, J. Shipley, R. A. Vogl. Changes in water quality due to potassium permanganate injection. In Chemical Oxidation and Reactive Barriers: In: Proceedings of the 3rd International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey CA, Columbus OH, Battelle Press. 2002.

[119] S. C. Wilson, K. C. Jones. Bioremediation of Soil Contaminated with Polynuclear Aromatic-Hydrocarbons (PAHs) - A Review. Environmental Pollution 81, 229-249, 1993.

[120] USEPA. Technologies For Treating MtBE and Other Fuel Oxygenates. EPA 542-R-04-009, Office of Superfund Remediation and Technology Innovation, Technology Innovation Program. 2004

[121] J. P. Salanitro, L. A. Diaz, M. P. Williams, H. L. Wisniewski. Isolation of A Bacterial Culture That Degrades Methyl T-Butyl Ether. Applied and Environmental Microbiology 60, 2593-2596, 1994.

[122] K. T. Finneran, D. R. Lovley. Anaerobic degradation of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA). Environmental Science & Technology 35, 1785-1790, 2001.

[123] P. M. Bradley, F. H. Chapelle, J. E. Landmeyer. Effect of redox conditions on MTBE biodegradation in surface water sediments. Environmental Science & Technology 35, 4643-4647, 2001.

[124] N. L. Ferreira, C. Malandain, F. Fayolle-Guichard. Enzymes and genes involved in the aerobic biodegradation of methyl tert-butyl ether (MTBE). Applied Microbiology and Biotechnology 72, 252-262, 2006.

[125] Wentzel, T. E. Ellingsen, H. K. Kotlar, S. B. Zotchev, M. Throne-Holst. Bacterial metabolism of long-chain n-alkanes. Applied Microbiology and Biotechnology 76, 1209-1221, 2007.

[126] M. So, L. Y. Young. Anaerobic biodegradation of alkanes by enriched consortia under four different reducing conditions. Environmental Toxicology and Chemistry 20, 473-478, 2001.

[127] P. Morgan, R. J. Watkinson. Biodegradation of components of petroleum. In: C. Ratledge. Biochemistry of microbial degradation, 1994, 1-31. Kluwer Academic Publishers.

[128] J. F. Barker, G. C. Patrick, D. Major. Natural attenuation of aromatic hydrocarbons in a shallow sand aquifer. Ground Water Monitoring and Remediation 7, 64-71, 1987.

[129] J. Heider, G. Fuchs. Anaerobic metabolism of aromatic compounds. European Journal of Biochemistry 243, 577-596, 1997.

[130] Ledin, L. A. Reitzel, P. L. Bjerg. Quantitative determination of toluene, ethylbenzene, and xylene degradation products in contaminated groundwater by solid-phase extraction and in-vial derivatization. International Journal of Environmental Analytical Chemistry 85, 1075-1087, 2005.

[131] W. Yang, Z. P. Jiang, S. Q. Shi. Aromatic compounds biodegradation under anaerobic conditions and their QSBR models. Science of the Total Environment 358, 265-276, 2006.

[132] Widdel, R. Rabus. Anaerobic biodegradation of saturated and aromatic hydrocarbons. Current Opinion in Biotechnology 12, 259-276, 2001.

[133] Mrozik, Z. Piotrowska-Seget, S. Labuzek. Bacterial degradation and bioremediation of polycyclic aromatic hydrocarbons. Polish Journal of Environmental Studies 12, 15-25, 2003.

[134] K. Friis. The potential for reductive dechlorination after thermal treatment of TCE-contaminated aquifers. PhD Thesis Institute of Environment & Resources, Technical University of Denmark. 2006.

[135] J. H. Slater. Microbial dehalogenation of haloaliphatic compounds. In: C. Ratledge. Biochemistry of microbial degradation, Kluwer Academic Publishers. 1994.

[136] H. Chapelle. Ground-water microbiology and Geochemistry. 1st edition, John Wiley & Sons, Inc. 1993.

[137] L. C. M. Commandeur, J. R. Parsons. Biodegradation of halogenated aromatic compounds. In: C. Ratledge. Biochemistry of microbial degradation, 423-458 Kluwer Academic Publishers. 1994.

[138] N. Tuxen. *In situ* bioremediation of groundwater contaminated by herbicides from point sources. PhD Thesis, Environment & Resources DTU, Technical University of Denmark, 2002.

[139] M. Schirmer, J. F. Barker. A study of long-term MTBE attenuation in the Borden aquifer, Ontario, Canada. Ground Water Monitoring and Remediation 18, 113-122, 1998.

[140] M. Schirmer, B. J. Butler, C. D. Church, J. F. Barker, N. Nadarajah. Laboratory evidence of MTBE biodegradation in Borden aquifer material. Journal of Contaminant Hydrology 60, 229-249, 2003.

[141] T. C. Schmidt, S. B. Haderlein, L. Zwank, M. Berg, R. P. Schwarzenbach. identification of in situ MTBE degradation pathways in a contaminated aquifer using carbon and hydrogen stable isotope signatures. In Proceedings of the Second European Conference on MTBE, Barcelona, Spain, 2004.

[142] J. E. Landmeyer, F. H. Chapelle, P. M. Bradley, J. F. Pankow, C. D. Church, P. G. Tratnyek. Fate of MTBE relative to benzene in a gasoline-contaminated aquifer (1993-98). Ground Water Monitoring and Remediation 18, 93-102, 1998.

[143] D. Mackay, R. Wilson, G. Durrant, K. Scow, A. Smith, M. Einarson, B. Fowler. Field tests of enhanced intrinsic remediation of an MTBE plume. Abstracts of Papers of the American Chemical Society 219, U655, 2000.

[144] S. Fiorenza, H. S. Rifai. Review of MTBE Biodegradation and Bioremediation. Bioremediation Journal 7, 1-35, 2003.

[145] M. W. G. King, J. F. Barker, J. F. Devlin, B. J. Butler. Migration and natural fate of a coal tar creosote plume 2. Mass balance and biodegradation indicators. Journal of Contaminant Hydrology 39, 281-307, 1999.

[146] N. El Azhari, S. Chabaud, A. Percept, D. Bru, F. Martin-Laurent. pcaH, a molecular marker for estimating the diversity of the protocatechuate-degrading bacterial community in the soil environment. Pest Management Science 63, 459-467, 2007.

[147] J. K. Fredrickson, F. J. Brockman, D. J. Workman, S. W. Li, T. O. Stevens. Isolation and characterization of a subsurface bacterium capable of growth on toluene, naphthalene, and other aromatic-compounds. Applied and Environmental Microbiology 57, 796-803, 1991.

[148] N. Ouchiyama, Y. Zhang, T. Omori, T. Kodama. Biodegradation of carbazole by *Pseudomonas* Spp Ca06 and Ca10. Bioscience Biotechnology and Biochemistry 57, 455-460, 1993.

[149] J. M. Foght, D. W. S. Westlake. Degradation of polycyclic aromatic-hydrocarbons and aromatic heterocycles by a *Pseudomonas* species. Canadian Journal of Microbiology 34, 1135-1141, 1988.

[150] D. C. Bressler, P. M. Fedorak. Bacterial metabolism of fluorene, dibenzofuran, dibenzothiophene, and carbazole. Canadian Journal of Microbiology 46, 397-409, 2000.

[151] S. A. Denome, D. C. Stanley, E. S. Olson, K. D. Young. Metabolism of dibenzothiophene and naphthalene in pseudomonas strains - Complete DNA-sequence of an upper naphthalene catabolic pathway. Journal of Bacteriology 175, 6890-6901, 1993.

[152] J. D. Leblond, T. W. Schultz, G. S. Sayler. Observations on the preferential biodegradation of selected components of polyaromatic hydrocarbon mixtures. Chemosphere 42, 333-343, 2001.

[153] I. Atagana. Biodegradation of phenol, o-cresol, m-cresol and p-cresol by indigenous soil fungi in soil contaminated with creosote. World Journal of Microbiology & Biotechnology 20, 851-858, 2004.

[154] S. Kar, T. Swaminathan, A. Baradarajan. Biodegradation of phenol and cresol isomer mixtures by Arthrobacter. World Journal of Microbiology & Biotechnology 13, 659-663, 1997.

[155] M. Hofrichter, F. Bublitz, W. Fritsche. Cometabolic degradation of o-Cresol and 2,6-dimethylphenol by *Penicillium-frequentans* Bi-7/2. Journal of Basic Microbiology 35, 303-313, 1995.

[156] P. Y. A. Ahamad, A. A. M. Kunhi, S. Divakar. New metabolic pathway for o-cresol degradation by *Pseudomonas* sp CP4 as evidenced by H-1 NMR spectroscopic studies. World Journal of Microbiology & Biotechnology 17, 371-377, 2001.

[157] S. M. N. Chadhain, R. S. Norman, K. V. Pesce, J. J. Kukor, G. J. Zylstra. Microbial dioxygenase gene population shifts during polycyclic aromatic hydrocarbon biodegradation. Applied and Environmental Microbiology 4078-4087, 2006.

[158] M. Christiansen, C. Riis, S. B. Christensen, M. M. Broholm, A. G. Christensen, K. E. S. Klint, J. S. A. Wood, P. Bauer-Gottwein, P. L. Bjerg. Characterization and quantification of pneumatic fracturing effects at a clay till site. Environmental Science & Technology 42, 570-576, 2008.

[159] U. Zoller, A. Reznik. In-situ surfactant/surfactant-nutrient mix-enhanced bioremediation of NAPL (fuel)-contaminated sandy soil aquifers. Environmental Science and Pollution Research 13, 392-397, 2006.

[160] K. Acuna-Askar, M. V. Gracia-Lozano, J. F. Villarreal-Chiu, J. G. Marmolejo, M. T. Garza-Gonzalez, B. Chavez-Gomez. Effect of soil and a nonionic surfactant on BTE-oX and MTBE biodegradation kinetics. Water Science and Technology 52, 107-115, 2005.

[161] J. M. Kosegi, B. S. Minsker, D. E. Dougherty. Feasibility study of thermal in situ bioremediation. Journal of Environmental Engineering-ASCE 126, 601-610, 2000.

[162] Bittkau, R. Geyer, M. Bhatt, D. Schlosser. Enhancement of the biodegradability of aromatic groundwater contaminants. Toxicology 205, 201-210, 2004.

[163] K. Friis, G. Heron, H. J. Albrechtsen, K. S. Udell, P. L. Bjerg. Anaerobic dechlorination and redox activities after full-scale Electrical Resistance Heating (ERH) of a TCE-contaminated aquifer. Journal of Contaminant Hydrology 88, 219-234, 2006.

[164] S. C. Mravik, R. K. Sillan, A. L. Wood, G. W. Sewell. Field evaluation of the solvent extraction residual biotreatment technology. Environmental Science & Technology 37, 5040-5049, 2003.

[165] R. D. Norris, J. D. Wilson, D. E. Ellis, R. L. Siegrist. Consideration of the effects of remediationtechnologies on natural attenuation. B. C. Alleman,A. Leeson. Proceedigns of The Fith International In Situ and On Site

Bioremediation Symposium, San Diego, California. Natural Attenuation of Environmental Contaminants. Columbus Richland, Battelle Press. 1999.

[166] I. Atagana, R. J. Haynes, F. M. Wallis. The use of surfactants as possible enhancers in bioremediation of creosote contaminated soil. Water Air and Soil Pollution 142, 137-149, 2003.

[167] T. L. Cort, M. S. Song, A. R. Bielefeldt. Nonionic surfactant effects on pentachlorophenol biodegradation. Water Research 36, 1253-1261, 2002.

[168] K. Friis, H. J. Albrechtsen, G. Heron, P. L. Bjerg. Redox processes and release of organic matter after thermal treatment of a TCE-Contaminated aquifer. Environmental Science & Technology 39, 5787-5795, 2005.

[169] R. E. Richardson, C. A. James, V. K. Bhupathiraju, L. Alvarez-Cohen. Microbial activity in soils following steam treatment. Biodegradation 13, 285-295, 2002.

[170] T. Bui, R. G. H. Cotton. Comparative study of permanganate oxidation reactions of nucleotide bases by spectroscopy. Bioorganic Chemistry 30, 133-137, 2002.

[171] A. Imlay, S. Linn. DNA damage and oxygen radical toxicity. Science 240, 1302-1309, 1988.

[172] A. Imlay, S. M. Chin, S. Linn. Toxic DNA damage by hydrogen-peroxide through the Fenton reaction *in vivo* and *in vitro*. Science 240, 640-642, 1988.

[173] S. Izawa, Y. Inoue, A. Kimura. Importance of catalase in the adaptive response to hydrogen peroxide: Analysis of acatalasaemic *Saccharomyces cerevisiae*. Biochemistry Journal 320, 61-67, 1996.

[174] R. J. Watts, D. Washington, J. Howsawkeng, F. J. Loge, A. L. Teel. Comparative toxicity of hydrogen peroxide, hydroxyl radicals, and superoxide anion to *Escherichia coli*. Advances in Environmental Research 7, 961-968, 2003.

[175] D. Lee, M. Hosomi. Clean-up of benz(a)anthracene-contaminated soils by fenton oxidation-microbial treatment. Kagaku Kogaku Ronbunshu 27, 411-415, 2001.

[176] T. W. Macbeth, L. N. Peterson, R. C. Starr, K. S. Sorenson, R. Goehlert, K. S. Moor. ISCO impacts on indigenous Microbes in a PCE-DNAPL contaminated aquifer. Proceedings of the Eighth International In Situ and On-Site Bioremediation Symposium,Baltimore, MA, Battelle Press. 2005.

[177] Y. Zeng, P. K. A. Hong, D. A. Wavrek. Integrated chemical-biological treatment of benzo[a]pyrene. Environmental Science & Technology 34, 854-862, 2000.

[178] Stehr, T. Muller, K. Svensson, C. Kamnerdpetch, T. Scheper. Basic examinations on chemical pre-oxidation by ozone for enhancing bioremediation of phenanthrene contaminated soils. Applied Microbiology and Biotechnology 57, 803-809, 2001.

[179] Nam, J. J. Kukor. Combined ozonation and biodegradation for remediation of mixtures of polycyclic aromatic hydrocarbons in soil. Biodegradation 11, 1-9, 2000.

[180] T. Madigan, J. M. Martinko, T. D. Brock. Brock biology of microorganisms. 11th edition, Upper Saddle River, NJ, Pearson Prentice Hall. 2006.

[181] P. Vattanaviboon, W. Praituan, S. Mongkolsuk. Growth-phase dependent resistance to oxidative stress in a phytopathogen *Xanthomonas-Oryzae Pv Oryzae*. Can.J.Microbiol. 41, 1043-1047, 1995.

[182] Diaz-Acosta, M. L. Sandoval, L. Delgado-Olivares, J. Membrillo-Hernandez. Effect of anaerobic and stationary phase growth conditions on the heat shock and oxidative stress responses in *Escherichia coli* K-12. Archives of Microbiology 185, 429-438, 2006.

[183] I. Atagana, R. J. Haynes, F. M. Wallis. Optimization of soil physical and chemical conditions for the bioremediation of creosote-contaminated soil. Biodegradation 14, 297-307, 2003.

[184] M. Miller, R. L. Valentine, M. E. Roehl, P. J. J. Alvarez. Chemical and microbiological assessment of pendimethalin-contaminated soil after treatment with Fenton's reagent. Water Research 30, 2579-2586, 1996.

[185] K. Friis, A. C. Heimann, R. Jakobsen, H. J. Albrechtsen, E. Cox, P. L. Bjerg. Temperature dependence of anaerobic TCE-dechlorination in a highly enriched Dehalococcoides-containing culture. Water Research 41, 355-364, 2007.

[186] M. Kosegi, B. S. Minsker, D. E. Dougherty. Feasibility study of thermal in situ bioremediation. Journal of Environmental Engineering 126, 601-610, 2000.

[187] Bittkau, R. Geyer, M. Bhatt, D. Schlosser. Enhancement of the biodegradability of aromatic groundwater contaminants. Toxicology 205, 201-210, 2004.

APPENDICES

I. **Tsitonaki, A.**, Mosbaek, H., Bjerg, P.L., 2006. Activated persulfate as a first step in a treatment train. Paper D-77, In: Proceedings of the Fifth International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2006), Battelle Press, Columbus, OH, ISBN 1-57477-157-4.

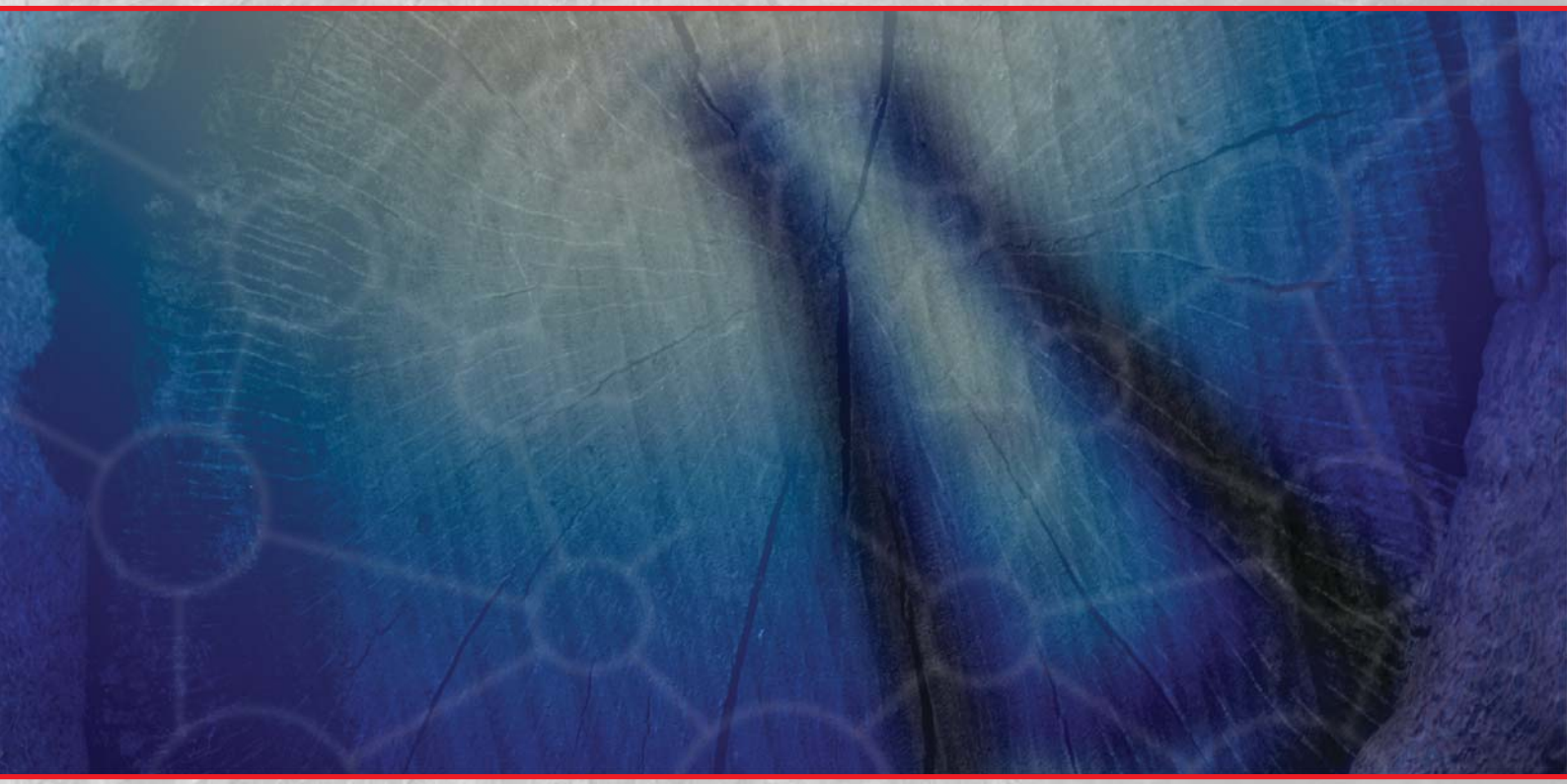
II. **Tsitonaki, A**., Smets, B.F., Bjerg, P.L. 2008. Effects of heat-activated persulfate oxidation on soil microorganisms. *Water Research* 45 (4-5), 1013-1022.

III. **Tsitonaki, A.**, Petri, B., Crimi, M. Mosbæk, H., Siegrist, R.L., and Bjerg P.L. *In situ* chemical oxidation of contaminated soil and groundwater using persulfate: A review. *Accepted for publication in Critical Reviews in Environmental Science and Technology.*

IV. **Tsitonaki, A.**, Mosbæk, H., Smets, B.F., and Bjerg P.L. Effective treatment of xenobiotic compounds in groundwater by sequential persulfate oxidation and biodegradation. *Manuscript*.

V. **Tsitonaki, A.,** El Azhari, N., Smets. B.F., Real time PCR and RFLP analysis for the quantification of aromatic degraders. *Technical Note.*

The papers are not included in this www-version but may be obtained from the Library at the Department of Environmental Engineering, Technical University of Denmark Miljoevej, Building 113, DK-2800 Kgs. Lyngby, Denmark, email: library@env.dtu.dk.



Department of Environmental Engineering
DTU Environment
Technical University of Denmark
Miljoevej, Building 113
DK-2800 Kgs. Lyngby
Denmark

Phone: +45 4525 1600
Fax: +45 4593 2850
e-mail: reception@env.dtu.dk

Please visit our website www.env.dtu.dk

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