EFFECTS OF FENTON'S REAGENT AND POTASSIUM PERMANGANATE APPLICATIONS ON INDIGENOUS SUBSURFACE MICROBIOTA: A LITERATURE REVIEW

Jonathan P. Waddell and Gregory C. Mayer

AUTHORS: Hydrologists, U.S. Geological Survey, 3039 Amwiler Road, Suite 130, Peachtree Business Center, Atlanta, Georgia 30360-2824.
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Abstract. This review explores existing knowledge pertaining to interactions between chemical oxidants utilized for subsurface remediation (i.e., Fenton's Reagent and potassium permanganate) and subsurface microorganisms. These oxidative processes may negatively affect indigenous microorganisms, and thus the capacity for intrinsic bioattenuation, or provide partially oxidized organics that enhance bioactivity. This synopsis includes discussions of the formation of Fenton's Reagent-derived reactive oxygen species and permanganate ions (MnO_4^-), respective mechanisms of microbial cell damage, laboratory and field studies, and implications for subsurface restoration.

INTRODUCTION

Xenobiotic organic compounds introduced into subsurface environments degrade ground-water quality and threaten human health. In-Situ Chemical Oxidation (ISCO), for remediation of free-phase organic contaminants, may be coupled with monitored natural attenuation (MNA) or enhanced in-situ bioremediation (EISB) to reduce contaminant concentrations to below target levels mandated by the U.S. Environmental Protection Agency, as ISCO alone may be incapable of 100 percent transformation of contaminant mass. Fenton's Reagent and potassium permanganate rely on reactive oxygen species and the electrophilic permanganate ion, respectively, to oxidize target organic contaminants. Due to the inherent nonspecificity of reactive oxygen species and MnO_4^-, other organic compounds in proximity to the injected chemical oxidants may be oxidized, including organic constituents of microbial cells. As microbial involvement is required for both MNA and EISB, chemical oxidants may have immediate or long-term adverse effects on microbial populations and communities responsible for target contaminant biotransformation.

FENTON'S REAGENT AND POTASSIUM PERMANGANATE

Fenton's Reagent and potassium permanganate—on contact with dissolved, aqueous-phase or non-aqueous-phase organic contaminants—facilitate the abiotic oxidation of the contaminants. As a result, the concentration gradient between the source and the dissolved phase increases and mass transfer from the source into the dissolved phase is enhanced. Oxidative reaction end products will depend on parent compound structure and may include partially oxidized organics and/or carbon dioxide.

Fenton's Reagent

Fenton's Reagent is a mixture of hydrogen peroxide (H_2O_2) and ferrous iron salts that reacts to form hydroxyl radicals ($HO\bullet$), ferric iron (Fe^{3+}), hydroperoxyl radicals ($HO_2\bullet$) (Walling, 1975), and/or superoxide radicals (O_2^-) (Smith and others, 2002) according to the following reactions:

(1) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO\bullet$

(2) $HO\bullet + H_2O_2 \rightarrow H_2O + HO_2\bullet$

(3) $HO_2\bullet \rightarrow O_2^- + H^+$ pKa = 4.8

Superoxide, dominant at neutral pH, and hydrogen peroxide also may contribute to hydroxyl radical formation by the overall equation (Halliwell and Gutteridge, 1985):

(4) $H_2O_2 + O_2^- \rightarrow O_2 + HO^- + HO\bullet$

Reactive oxygen species are hypothesized to be responsible for electrophilic attack and/or degradation of organic contaminants (Smith and others, 2002).

Potassium Permanganate

Potassium permanganate ($KMnO_4$), available in purple-colored crystalline form, decomposes to the reactive permanganate ion, and may enhance organic contaminant transformation by mineralization, oxidation to more biodegradable products, and/or adsorption onto hydrous MnO_2 (Walton and others, 1992). Permanganate ion oxidation of organic compounds may involve hydrogen abstraction, electron abstraction, incorporation of oxygen atom into structure, and/or hydride-ion abstraction and is ultimately influenced by pH (Walton and others, 1992).

EFFECTS OF CHEMICAL OXIDANTS ON SUBSURFACE MICROBIOTA

Reactive oxygen species and the permanganate ion are capable of oxidizing macromolecules of the microbial cell including the microbial cell membrane, the cell wall, and nucleic acids. The cell wall separates the more delicate intracellular cell membrane and cytoplasm from harmful extracellular environments, and is composed of lipopolysaccharides, peptidoglycan, lipoproteins, and porins (gram negative) or peptidoglycan, techoic acids, and lipotechoic acids (gram positive) (Madigan and others, 2000). The cellular membrane, composed of phospholipids and proteins, is in effect a permeability barrier and functions as sites for metabolic reactions. Nucleic acids, genes encoding for proteins and enzymes, are located within the cytoplasm and composed of purine or pyrimidine bases attached to deoxyribose or ribose sugars and interconnected by phosphodiester bonds and hydrogen bonds (Madigan and others, 2000).

Fenton's Reagent

Miller and others (1996) studied the effects of Fenton's Reagent treatment of pendimethalin-contaminated soils on microorganisms and observed that direct application of Fenton's Reagent to microorganisms resulted in death. Contact between Fenton's Reagent-pretreated soils and microorganisms, however, resulted in microbial growth and glucose mineralization, increased biodegradability of pendimethalin-derived oxidation products, and decreased microbial diversity. Thus, the potential for conflicting Fenton's Reagent effects, between biocidal and increased biodegradability of organic matter, was suggested. Similar observations were made by Chapelle (2001).

Damage to cell components. Cell membrane lipid peroxidation by a hydroxyl radical involves abstraction of a hydrogen atom from a phospholipid carbon-carbon double bond, creation of a carbon radical, and a chain reaction that results in abstraction of hydrogen atoms from other lipids until the propagation reaction contacts and/or damages an integral membrane protein (Halliwell and Gutteridge, 1985). A radical chain reaction also may occur within a protein that may include alterations to the amino acid side chains, generation of cross-linking, and/or alterations to tertiary structure resulting in protein unfolding causing an increased rate of cellular destruction (Davies, 1997). Loss of one enzyme may promote activation or inactivation of other enzymes (Wolff and others, 1986). This chain reaction may proceed until membrane function and integrity has been lost resulting in cell lysis (Halliwell and Gutteridge, 1985).

As evidenced in plant cell wall oxidation, damage to cell wall polysaccharides by hydroxyl radicals may adhere to the following pathway: (1) cleavage of glycosidic linkages between saccharides, (2) depolymerization of polysaccharides, and (3) release of low molecular weight products (Miller, 1986).

A "crypto-hydroxyl radical" complexed to iron (chelated to phosphodiester backbone of nucleic acid), resistant to hydroxyl scavengers free in solution, is hypothesized to be responsible for DNA oxidation/nicking (Imlay and others, 1988). The mechanism of radical attack on cellular nucleic acids may involve hydrogen abstraction from the ribose sugar and/or oxidation of the nucleotide base (Imlay and Linn, 1988).

Microbial Modes of Protection against Reactive Oxygen Species. Many microorganisms have superoxide dismutase enzymes that scavenge excess superoxide and catalase enzymes that decompose hydrogen peroxide, preventing the formation of hydroxyl radicals and/or intracellular transport of free iron (preventing Fenton's like reaction between H_2O_2/O_2 and free iron). Obligate anaerobic microorganisms, in comparison to aerobic microorganisms, may not contain such enzymes (Fridovich, 1978) and thus are more susceptible to attack by reactive oxygen species.

Laboratory and Field Evidence. Hydrogen peroxide is toxic to microorganisms and is commonly used as a topical antiseptic in medical practice. Peterson and others (1995) observed that H_2O_2 toxicity, dependent on concentration, resulted in low dissolved organic carbon release due to cell wall damage. H_2O_2 was thus described as a chemical oxidant that causes physiological toxicity but releases minor amounts of cellular organic components.

The effects of reactive oxygen species on subsurface microorganisms have also been examined. Stokley

and others (1997), studying the impact of Fenton's Reagent application on polycyclic aromatic hydrocarbon bioremediation in laboratory microcosms, found that prior treatment with Fenton's Reagent slightly reduced numbers of heterotrophic microorganisms. Treatment of a soil undergoing bioremediation, however, resulted in substantial reductions in the number of heterotrophic microorganisms. Büyüksönmez and others (1998, 1999) studied the effects of hydrogen peroxide decomposition products on H_2O_2-acclimated populations of *Xanthobacter flavus* FB71. Second-order equations were developed to describe cell survival percentage and optimal abiotic/biotic tetrachloroethene transformation. Cell survival percentages decreased with increasing H_2O_2/Fe(II) concentrations and increases in the initial cell number (cells/milliliter), and tetrachloroethene oxidation was enhanced given the presence of degrading microbiota. Kastner and others (2000) investigated the effects of Fenton's Reagent application on ground-water and soil methanotrophs and cometabolic biotransformation of trichloroethene in microcosms. Decreases in pH, due to Fenton's Reagent application, were observed to inhibit methanotrophic activity and growth rates, enhance growth of eukaryotes, and have a significant effect on microbial community dynamics and cometabolic TCE degradation. Addition of a carbon or phosphate source could promote microbial re-growth and cometabolic TCE degradation at low pH, which led Kastner and others to observe that Fenton's Reagent application reduces microbial numbers, viability, and diversity, but does not entirely remove them from the applied zone or reduce the potential for cometabolic TCE degradation.

Potassium Permanganate

Wang (1992) showed that permanganate pretreatment of phenolic compounds formed easily biodegradable products for a subsequently inoculated phenol-enriched consortium, but was toxic during early incubation phases. Bowers and others (1992) made similar observations in laboratory experiments with various organic compounds. Thus, $KMnO_4$ oxidation of organic contaminants also may induce biodegradability and/or toxicity to microorganisms.

Damage to Cell Components. Phospholipids within the cell membrane, containing unsaturated fatty acids, may be susceptible to MnO_4^- oxidation at carbon-carbon double bonds. pH dependant oxidative reactions at the carbon-carbon double bond may result in formation of α-hydroxyketones, diols (Walton and others, 1992), and/or epoxides (Stewart, 1965). Such reactions may induce loss in membrane function and cell death. Polysaccharides composing the peptidoglycan cell wall may be oxidized and removed resulting in loss of cell wall stability and structure.

Bui and Cotton (2002), who studied oxidation reactions and reaction rates for MnO_4^- with free nucleotide bases, found that the permanganate ion reacts strongly and rapidly with pyrimidine bases uracil and thymine, weakly and slowly with the pyrimidine base cytosine, and minimally with purine bases. MnO_4^- forms an unstable cyclic permanganate ester with pyrimidine bases resulting in decomposition.

Laboratory and Field Evidence. Peterson and others (1995) reported that $KMnO_4$ was second to chlorine in toxicity (with respect to N_2 fixation), non-stimulatory at low concentrations (≤ 0.18 mg/L), inhibitory at higher concentrations, and released substantial amounts of dissolved organic carbon due to cell wall damage. $KMnO_4$ was thus described as a chemical oxidant that causes harsh physiological toxicity and releases significant amounts of cellular organic components at concentrations less than those required for water treatment.

A field study was undertaken at a permanganate-pretreated, TCE-contaminated site to describe effects of $KMnO_4$ application on the subsurface microbial community (Klens and others, 2001). Effects on microbial viability as determined by plate counts on complex medium, revealed a community shift from a consortium composed predominantly of aerobic heterotrophs, anaerobic heterotrophs, nitrate reducing bacteria, sulfate reducing bacteria, and methanogens to primarily aerobic heterotrophs.

IMPLICATIONS TO SUBSURFACE RESTORATION

Application of chemical oxidation has been demonstrated to transform subsurface organic contaminants and/or generate products more biodegradable than the parent contaminants. However, implications for contaminant-degrading microbial communities contacted by chemical oxidants may include community changes and/or death. Reacclimation of degrading microbial consortia to a chemical oxidant treated area, a potentially lengthy process, will be necessary to reestablish previous rates of contaminant biotransformation.

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