

Recent progress in monoaromatic pollutants removal from groundwater through bioremediation

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ABSTRACT

Monoaromatic pollutants such as benzene, toluene, ethylbenzene and mixture of xylenes are now considered as widespread contaminants of groundwater. In situ bioremediation under natural attenuation or enhanced remediation has been successfully used for removal of aromatic pollutants, including monoaromatic compounds, from groundwater. Results published indicate that in some sites, intrinsic bioremediation can reduce the monoaromatic compounds content of contaminated water to reach standard levels of potable water. However, engineering bioremediation is faster and more efficient. Also, studies have shown that enhanced anaerobic bioremediation can be applied for many BTEX contaminated groundwaters, as it is simple, applicable and economical. This paper reviews microbiology and metabolism of monoaromatic biodegradation and in situ bioremediation for BTEX removal from groundwater under aerobic and anaerobic conditions. It also discusses the factors affecting and limiting bioremediation processes and interactions between monoaromatic pollutants and other compounds during the remediation processes.

Keywords: Monoaromatic pollutants; Bioremediation; *In Situ*; Groundwater; Biodegradation; Enhanced remediation

1. INTRODUCTION

Benzene, toluene, ethylbenzene and xylenes isomers (BTEX) are important monoaromatic hydrocarbons that have been found in sites polluted by oil production facilities and industries. These organic compounds are toxic and contaminate groundwater sources (An, 2004). Groundwater gets polluted by monoaromatic compounds. These hydrocarbons have higher water solubility than other organic compounds that are present in gasoline such as aliphatic [1]. Generally, solubility of benzene, toluene, ethyl benzene, xylenes and gasoline in water are respectively 18, 25, 3, 20, 50-100 ppm when gasoline is introduced into water. Percent volume of benzene, toluene, ethylbenzene and xylenes in gasoline, are 1, 1.5, <1-1.5 and 8-10, respectively [2]. Groundwater contaminated by toxic pollutant is a very serious problem because many communities in the world depend upon groundwater as sole or major source of drinking water. Maximum levels for monoaromatic compounds in potable water are 0.05, 1, 0.7 and 10 ppm for benzene, toluene, ethylbenzene and isomers of xylenes, respectively [3]. The detection and determination of light aromatic compounds in limits up to part per billion (ppb) for a water sample can be carried out by various methods including gas chromatography (GC)/flame ionization detector (FID), GC/photo ionization detector (PID),

GC/mass spectrometer (MS) or GC/solid phase micro extraction (SPME) through head space or purge and trap depending on sample preparation methods [4]. Other methods such as chemical extraction (benzylsuccinate, trimethylbenzene, catechol 2, 3 dioxygenase), physical methods (depletion of dissolved oxygen, nitrate and sulfate or production of dissolved ferrous iron, sulfide and carbon dioxide), biological (bioassay tools) or numerical, physical and kinetic models can be used for on-line monitoring of monoaromatics degradation during the course of in situ bioremediation.

There are different methods for monoaromatic compounds removal from groundwater, such as physical techniques (electro remediation, air sparging, carbon adsorption, ion exchange, adsorption by zeolites), chemical methods (chemical oxidation, photo catalysis remediation) and biological processes (bioremediation, biodegradation in reactors, phytoremediation, wetland) methods [5]. These approaches can be applied alone or in combination, the use of several of them is generally encountered for polishing purposes. Some of these complementary methods include sand filtration and the permeable reactive barrier technology. All above mentioned methods can be divided into in situ and ex situ (pump and treat) remediation technologies [6]. In situ remediation is treatment of the contaminated material in place. Among all remediation technologies for treating xenobiotics or monoaromatic compounds from contaminated groundwater, bioremediation appears to be an efficient and economical process and environmentally sound approach. Ex situ bioremediation is generally costly and difficult due to extraction of contaminated water from subsurface, treatment and recharging the underground. This has led to an interest in using in situ bioremediation for groundwater contaminated by oil products.

In situ bioremediation is known as long term technology since there is less certainty about the uniformity of treatment because of the variability of aquifer and soil characteristics. However, this process has advantages such as relative simplicity, low cost, and potentially remarkable efficiency in contamination removal [7]. In in situ bioremediation, organic pollutants are completely destroyed, therefore no secondary waste stream is produced. In situ bioremediation is a biological process where microorganisms metabolize organic contaminants to inorganic material, such as carbon dioxide, methane, water and inorganic salts, either in natural or engineered conditions. When naturally occurring metabolic processes are used to remediate pollutants without any additional alteration of site conditions, the process is called as intrinsic or natural attenuation [8]. Present results indicate that biodegradation is the best method for BTEX removal. When working conditions at the site are engineered, i.e. designed to accelerate the bioremediation of contaminants, the process is referred to as engineered or enhanced bioremediation.

Main factors affecting in situ bioremediation of contaminated groundwater have been widely described in the literature. Some of the main points include [9]:

- Source and concentration of pollutant.
- Chemistry and toxicity of contamination.
- Solubility, transport, adsorption, dispersion and volatility of pollutant compounds.
- Detection, determination and monitoring of pollutants.
- Chemistry, physics and microbiology of groundwater.
- Chemistry and mechanics of soil at contaminated site.
- Hydrogeology and hydrology of contaminated site.
- Limitations of environmental standards for water and soil.
- Environment conditions, nutrient sources and presence of electron acceptors.

- Biodegradability of contaminants, and the presence of a competent biodegrading population of microorganisms.

In in situ bioremediation, anaerobic biodegradation plays a more important role than that of aerobic processes. Aerobic bioremediation process requires expensive oxygen delivery systems and process maintenance is often high due to biofouling in subsurface. But anaerobic processes have advantages such as low biomass production and good electron acceptor availability [10]. Anaerobic processes are sometimes the only possible solution to remove pollutants as it is often difficult to inject oxygen into underground waters.

The microbiology and metabolism of BTEX degradation and interaction between BTEX and other compounds (such as ethanol, MTBE) during their biodegradation is an important factor when in situ bioremediation for monoaromatic removal from groundwater is concerned.

2. MICROBIOLOGY AND METABOLISM

Microorganisms such as bacteria, fungi and microalgae play a key role in monoaromatic removal through in situ bioremediation processes. Monoaromatic pollutants act as carbon source for microorganisms. Also, they require macro nutrients (nitrogen and phosphorus), micro nutrients (Ca^{2+} , Mg^{2+} , Na^+ , K^+ , S^{2-} , co-factors such as heavy metals), electron acceptor (oxygen is the electron acceptor for aerobic metabolism and nitrate, sulfate, ferric, manganese and carbon dioxide in anaerobic processes) and optimum environmental conditions for growth (temperature, pH, salinity, presence of inhibitors and of a nitrogen source) [11]. Therefore, the rate of bioremediation of fuel contaminants such as monoaromatic hydrocarbons can be enhanced by increasing the concentration of electron acceptors and nutrients in groundwater.

In aerobic respirometry after degradation of light aromatic hydrocarbons, microorganisms produce carbon dioxide, water, sludge, etc. In anaerobic bioremediation, end products are compounds such as methane, CO_2 , mineral salts. Biomass has also to be taken into account even if, as already stated, its production remains usually quite low. The electron transfers which occur during biochemical reactions release energy which is further utilized for growth and cell maintenance [12].

Maximum concentration of electron acceptor compounds that can be added to contaminated groundwater, i.e. oxygen, hydrogen peroxide, nitrate, sulfate and iron are 9-10, 100-200, 80-100, 100-250 and mg/L , respectively [13]. These values are due to practical limitation, aqueous solubility, drinking water standards and microbial activities. Studies on metabolic pathways for BTEX removal in aerobic conditions have indicated that each of these compounds can be degraded through at least one pathway leading to a substituted catechol. For example, benzene is degraded to catechol while toluene and ethylbenzene are degraded via two separate pathways leading to the production of 3-methylcatechol and 3-ethylcatechol, respectively. The xylenes are metabolized to mono-methylated catechols [14]. A mixed culture derived from gasoline-contaminated aquifer has been shown to degrade all BTEX compounds into CO_2 . Also, some enzymes involved in aerobic metabolism, such as catechol 2,3-dioxygenase, are used for monitoring BTEX bioremediation.

Degradation of benzene in anaerobic conditions by mixed populations have been investigated. Details of the biochemical pathways for toluene and ethylbenzene for anaerobic biodegradation are known. Zarlenga and Fiori [15] have shown that for toluene, ethylbenzene and xylene isomers (*ortho* and *meta*), it exists a common intermediate metabolite, which is benzoyl-CoA. This compound appears to be the most common central intermediate for anaerobic breakdown of aromatic compounds. Benzoyl-CoA is further reduced and can be

converted into acetyl-CoA, finally giving carbon dioxide. It must be emphasized that the pathways for *para* xylene metabolization under anaerobic conditions are not completely elucidated. In most cases, electron balances show a complete anaerobic oxidation of these aromatic compounds to CO₂. Also, some intermediates such as benzylsuccinic acid and methylbenzylsuccinic acid isomers have been proposed as distinctive indicators for the monitoring of anaerobic toluene and xylene degradation in fuel contaminated aquifers [16].

Biodegradation kinetics parameters for monoaromatic removal are commonly obtained from cultivation parameters in batch or continuous conditions and fitting the data with the well-known Monod equation. Harrington et al. [17] reported that substrate disappearance in discontinuous operations were 1.32, 1.42 and 0.833, as mmol/L. h for benzene, toluene, and xylene, respectively. Also, maximum growth specific rate value for biomass degradation of monoaromatic compounds has been reported to be in the range of 0.046-0.38 d⁻¹. Many kinetic studies, giving parameters for BTX biodegradation in aerobic batch and column systems have been reported. Experimental data given by Longoria et al. [18] show that the kinetic coefficient values for the individual BTEX compounds are affected by the operating solids retention time (SRT) in the reactor and the combination of growth substrates.

Studies by Lin et al. [19] indicate that the rate of biodegradation of heavy hydrocarbons follows first order kinetics with rate constants up to 0.445 day⁻¹ under aerobic conditions and up to 0.522 day⁻¹ under anaerobiosis. Also, an average reaction rate close to 0.3% day⁻¹ for benzene was estimated from all published data, while the corresponding values for toluene, ethylbenzene, and xylenes were estimated to be 4, 0.1 and 0.4% day⁻¹, respectively.

3. *IN SITU* BIOREMEDIATION

In situ bioremediation has been successful for the treatment of groundwater contaminated with mixtures of chlorinated solvents such as carbon tetrachloride (CT), tetrachloroethylene (TCA), trichloroethylene (TCE), or pentachlorophenol (PCP) [20]. Also, contaminants such as gasoline, fuel, methyl tert-butyl ether (MTBE), alkylbenzene, alkylpyridines, oily wastes, synthetic lubricants, coal tar contaminated site, nitroaromatics and inorganic compounds such as uranium have been successfully removed by in situ bioremediation techniques. These technologies have also been widely used for the treatment of xenobiotic compounds, monoaromatic hydrocarbons or BTEX from groundwater.

Natural bioremediation is the main method for monoaromatic degradation and results indicate that up to 90% of the BTEX removal by this approach can be attributed to the intrinsic biodegradation process [21]. However, natural attenuation is often limited by either the concentration of an appropriate electron acceptor or a nutrient required during the biodegradation. Enhanced degradation accelerates the natural process by providing nutrients, electron acceptors, and competent degrading microorganisms [22].

Contamination of groundwater with monoaromatic compounds is often accompanied by other oxygenated molecules such as methyl tert-butyl ether (MTBE), tert-butyl alcohol (TBA), methanol, and ethanol. These compounds have been added to gasoline as octane enhancers and stabilizers at levels close to 10-20% by volume [23]. Generally, alcohols and oxygenated derivatives have a relatively high solubility in water and high mobility in the subsurface.

Methanol and ethanol increase the solubility of oil constituents such as monoaromatic compounds in the water. For example studies indicate that ethanol in oil increases the solubility of BTEX from 30% to 210% by volume [24]. The biodegradation of methanol or

ethanol in groundwater would first deplete the oxygen and then the anaerobic electron acceptors that potentially reduce the rate of monoaromatic pollutant. Also, high concentration of these alcohol spills can inhibit the biodegradation of oil contaminants, especially monoaromatic compounds [25]. Thus, the presence of methanol and ethanol in gasoline is likely to hinder the natural attenuation of BTEX, which would contribute to longer BTEX biodegradation processes and a greater risk of exposure [26]. It must be emphasized that MTBE and TBA are difficult to remove from groundwater because they have high water solubility and low biodegradation rates. Present results demonstrate that MTBE is the most recalcitrant compound, followed by TBA.

3.1. Engineered bioremediation

Oxygen is the main electron acceptor for aerobic bioprocesses. Aerobic in situ bioremediation of monoaromatic pollutants is often limited by the dissolved oxygen tension. As a result, various methods such as air sparging, injection of oxygen-releasing compounds (hydrogen peroxide, magnesium peroxide) and trapped gas phase have been used to increase dissolved oxygen concentrations in ground water [27]. Oxygen can be applied by air sparging below the water table, which has been shown to enhance the rate of biological degradation of monoaromatic or oil pollutants. For oxygen generating compounds, a dilute solution is circulated through the contaminated groundwater zone in order to increase its oxygen content and enhance the rate of aerobic biodegradation [28]. However, some researches have shown that significant difficulties, such as toxicity and microbial inhibition may be encountered when using inorganic nutrients and high concentrations of hydrogen peroxide [29,30].

Monoaromatic pollutants in groundwater can be removed by anaerobic in situ bioremediation. Important electron acceptors that are used to accelerate the rate of anaerobic monoaromatic biodegradation are chemical compounds such as Fe^{3+} , nitrate and sulfate [31,32]. Electron acceptors can be injected alone (which may even selectively speed up the biodegradation of monoaromatic compounds), or in combination with other activating compounds [33,34].

3.2. Natural bioremediation

Intrinsic bioremediation, which is also known as natural attenuation or passive bioremediation, is an environmental site management approach that relies on naturally occurring microbial processes for oil hydrocarbon removal from groundwater, without the engineered delivery of nutrients, electron acceptors or other stimulants [35,36]. Natural bioremediation reduces and decreases organic pollutants from many contaminated sites. It is more cost-effective than engineered conditions but it takes more time for organic biodegradation [37,38]. Mineralization of organic compounds in groundwater under natural bioremediation is, just like with engineered situations, connected to the consumption of oxidants such as oxygen, nitrate and sulfate and the production of reduced species such as Fe^{2+} , Mn^{2+} , H_2S , CH_4 and CO_2 .

4. CONCLUSION

Monoaromatic pollutants in groundwaters are threatening drinking water resources and therefore have, when present, to be removed. The analysis presented here suggests that in some case, naturally-occurring aerobic biodegradation phenomena can take place at a rate

high enough to reach environmental standard limits in a reasonable time. However, the most common situation is that it is necessary to artificially improve the performances of this process. This approach corresponds to the so-called engineered in situ bioremediation, which is most often really able to increase the rate of organic pollutant biodegradation.

It is also possible to make use of anaerobic approaches, since anaerobic microbial pathways able to fully decompose aromatic hydrocarbons do exist. Present data demonstrate that enhanced anaerobic bioremediation is already successfully applied in some areas contaminated with oil products.

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