

Contaminated environments in the subsurface and bioremediation: organic contaminants

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Abstract

Due to leakages, spills, improper disposal and accidents during transport, organic compounds have become subsurface contaminants that threaten important drinking water resources. One strategy to remediate such polluted subsurface environments is to make use of the degradative capacity of bacteria. It is often sufficient to supply the subsurface with nutrients such as nitrogen and phosphorus, and aerobic treatments are still dominating. However, anaerobic processes have advantages such as low biomass production and good electron acceptor availability, and they are sometimes the only possible solution. This review will focus on three important groups of environmental organic contaminants: hydrocarbons, chlorinated and nitroaromatic compounds. Whereas hydrocarbons are oxidized and completely mineralized under anaerobic conditions in the presence of electron acceptors such as nitrate, iron, sulfate and carbon dioxide, chlorinated and nitroaromatic compounds are reductively transformed. For the aerobic often persistent polychlorinated compounds, reductive dechlorination leads to harmless products or to compounds that are aerobically degradable. The nitroaromatic compounds are first reductively transformed to the corresponding amines and can subsequently be bound to the humic fraction in an aerobic process. Such new findings and developments give hope that in the near future contaminated aquifers can efficiently be remediated, a prerequisite for a sustainable use of the precious subsurface drinking water resources.

Keywords: Organic contaminant; Anaerobic degradation; Reductive transformation; Subsurface microbiology; Bioremediation; Hydrocarbon; Chlorinated compound; Nitroaromatic compound

Contents

1. Introduction	518
2. Hydrocarbons	518
3. Chlorinated compounds	519
4. Nitroaromatic compounds	520
5. Conclusions	520
References	521

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1. Introduction

The natural organic subsurface products coal and crude oil have been and are still used to cover the tremendous energy demand of industrialized countries and to produce almost innumerable synthetic organic chemicals. Due to leakage of underground storage tanks and pipelines, due to spills at production wells, refineries and distribution terminals, and due to improper disposal and accidents during transport, organic compounds have become subsurface contaminants that threaten important drinking water resources. One strategy to remediate such polluted subsurface environments is with the help of the degradative capacity of bacteria.

Two ways to bioremediate groundwater are (i) to pump the groundwater to the surface and to treat it above ground in a bioreactor and (ii) to remediate the aquifer in situ. The biological part of the pump-and-treat method is easy to control and manipulate. However, by pumping groundwater not the whole contamination is recovered and, during treatment, sludge or biomass and waste gas is produced which must be correctly disposed of. Two kinds of in situ bioremediation (ISB) processes are distinguished, they are 'intrinsic ISB' and 'engineered ISB' [1]. It is often difficult and too expensive to manipulate a subsurface environment and often natural microbial degradation is already taking place, although very slowly. Therefore, it is of interest to only monitor the degradation process that is already going on to ensure that the contamination plume is not expanding. This kind of action is called intrinsic ISB. If natural degradation is not occurring or if the degradation is too slow, the subsurface environment has to be manipulated in such a way that biodegradation is stimulated and the reaction rates are increased. This is called engineered ISB. Measures to be taken are supplying the subsurface with nutrients such as nitrogen and phosphorus and with electron acceptors. The most often considered electron acceptor is oxygen. Due to disadvantages such as low solubility of oxygen in water and high production of biomass, anaerobic processes have recently also been applied. In addition, for some contaminants anaerobic degradation is the only possibility to biologically transform the contaminating compound.

In the following, we will focus on three important

groups of environmental organic contaminants: hydrocarbons, chlorinated and nitroaromatic compounds. The anaerobic degradation potential for each group of contaminants is presented and in some cases compared with aerobic degradation. Furthermore, the possible application of an anaerobic process to treat a contamination with one of these compounds is discussed.

2. Hydrocarbons

The monocyclic aromatic compounds benzene, toluene, ethylbenzene and xylene – a group called BTEX – and the polycyclic hydrocarbons (PAHs) belong to the most often encountered subsurface contaminants and they are the most threatening compounds within the hydrocarbons. Aerobic bacteria able to degrade aromatic hydrocarbons are widespread. However, several reasons make the application of an aerobic treatment in the subsurface difficult. The limited availability of oxygen due to its low solubility restricts not only the respiration process, but also the degradation itself. Oxygen is needed by aerobic bacteria to activate and cleave the aromatic ring by the action of oxygenases. In contrast to the oxidative attack of the ring during aerobic degradation, aromatic compounds are reductively activated under anaerobic conditions [2]. First, the aromatic compound is transformed into central intermediates (e.g. benzoyl-CoA), subsequently the ring is reduced and finally cleaved by hydrolysis. Several groups of anaerobic bacteria are able to utilize monocyclic aromatic hydrocarbons as substrates. They include denitrifiers [3–5], Fe(III) [6–8] and sulfate reducers [9–12], and fermentative bacteria [13–15]. Whereas pure cultures of denitrifiers, Fe(III) and sulfate reducers have been isolated and their metabolism has been studied in detail, fermentative bacteria responsible for degradation under methanogenic conditions are not yet known. Recent reviews describe in more detail the organisms and the biochemistry involved in anaerobic degradation of monocyclic aromatic hydrocarbons [2,16–18]. Although degradation of PAHs has been reported under anaerobic conditions [19,20], the bacteria involved are unknown.

Anaerobic treatment of a hydrocarbon-contami-

nated site was considered as a possible means of remediation for a site in Germany as early as in the 1980s [21]. Groundwater was withdrawn from the aquifer by pumping, the water was aerated and oxidized iron and manganese were removed by sedimentation. After filtration, the water was heated to 20°C, supplemented with nitrate, and reinjected into the subsurface. The effectiveness of the treatment was evaluated by following the decrease in concentrations of hydrocarbons and nitrate in the extraction water and by showing an increase of carbon dioxide concentration and oversaturation of dinitrogen. For a diesel fuel-contaminated site where in addition to nitrate phosphate was added as nutrient, the mineralization of hydrocarbons was quantified by alkalinity, inorganic carbon and stable carbon isotope balances [22]. Another possibility is to remediate under Fe(III)-reducing conditions. Insoluble Fe(III) oxides are generally abundant potential electron acceptors in aquifers. Indications have been obtained with geochemical studies that Fe(III) reduction was an important process for the oxidation of hydrocarbons at a crude oil spill in Bemidji, MN, USA [23]. At this site, anaerobic degradation of the crude oil was rapid enough to allow the application of intrinsic ISB. For cases where an enhanced bioavailability of the insoluble Fe(III)-oxides is desirable to increase degradation rates, the addition of suitable ligands has been suggested as a potential strategy for the bioremediation of hydrocarbon-contaminated aquifers [7]. Although hydrocarbon degradation under sulfate-reducing conditions has been documented, injection of sulfate into the subsurface is not the preferred solution due to hydrogen sulfide production.

3. Chlorinated compounds

Within the group of chlorinated compounds, chlorinated ethenes are the most often detected groundwater pollutants. Tetrachloroethene (PCE) is the only chlorinated ethene that resists aerobic biodegradation [24]. Trichloroethene (TCE), all three isomers of dichloroethene, and vinyl chloride are mineralized in aerobic co-metabolic processes by methanotrophic or phenol-oxidizing bacteria [25]. Oxygenases with broad substrate spectra are respon-

sible for the co-metabolic oxidation [26,27]. Vinyl chloride is furthermore utilized by certain bacteria as carbon and electron source for growth [28]. All chlorinated ethenes are reductively dechlorinated under anaerobic conditions with possibly ethene or ethane as harmless end-products [24]. This process has been observed at different locations. There is evidence that vinyl chloride is oxidized under Fe(III)-reducing conditions [29]. PCE is dechlorinated to TCE in a co-metabolic process by methanogens, sulfate reducers, homoacetogens and others [30–33]. Enzymes containing tetrapyrrole cofactors such as corrinoids, porphyrins and factor F₄₃₀ catalyze this dechlorination reaction [32]. Furthermore, PCE and TCE serve in several bacteria as terminal electron acceptors in a respiration process [34–38]. The majority of these isolates dechlorinate PCE and TCE to *cis*-1,2-dichloroethene although they have been isolated from systems where complete dechlorination to ethene occurred. Only in a methanol-fed enrichment culture one single bacterium seems to dechlorinate PCE completely to ethene [39,40]. Hence, not much is known about the organisms catalyzing the dechlorination of dichloroethene and vinyl chloride. Pyruvate, lactate, ethanol, formate, acetate and molecular hydrogen were identified as electron donors for PCE and TCE dechlorination. *Dehalospirillum multivorans* has a rather broad substrate spectrum, '*Dehalobacter restrictus*', in contrast, only utilizes hydrogen. The generation of a proton motive force upon hydrogen oxidation and PCE reduction was demonstrated with '*D. restrictus*' cells by the oxidant pulse method [41]. The PCE reductive dehalogenase from *D. multivorans* was shown to be a corrinoid-containing iron-sulfur protein [42].

Aerobic as well as anaerobic processes have been considered for bioremediation of sites contaminated with chlorinated compounds. At the Savannah River Site near Aiken, SC, USA, a TCE plume was treated by stimulating the methanotrophic population [43]. Methane and air were injected through horizontal wells at a depth of 50 m below ground and extracted in the vadose zone via parallel horizontal wells. It was shown that 41% more TCE was removed by biodegradation than by physical stripping alone. To improve growth conditions nitrous oxide and triethyl phosphate were injected in addition to methane and air [44]. In a pilot field-scale *in situ* biore-

mediation, TCE co-oxidation was stimulated by injecting phenol into the aquifer [45]. Reductive dechlorination of chlorinated ethenes has already been found to occur under natural conditions in an aquifer at a TCE spill site in 1984 [46]. After the finding that PCE is completely dechlorinated to ethene in laboratory systems [47,48], it has been confirmed that the transformation to ethene occurs also in aquifers [25,49]. There are studies, however, where incomplete dechlorination to the more toxic products dichloroethene and vinyl chloride was documented and it is not yet possible to guarantee that the carcinogenic vinyl chloride does not accumulate. If reductive dechlorination of chlorinated ethenes is considered as a possible solution in an engineered ISB, denitrifying conditions should be avoided and a suitable electron donor should be injected into the subsurface to enhance dechlorinating activity [24].

4. Nitroaromatic compounds

Nitroaromatic compounds are widespread in the environment and are mainly of anthropogenic origin [50]. One of the most problematic is 2,4,6-trinitrotoluene (TNT), a munition compound that is found wherever munition is produced, loaded, handled or packed. Aerobic bacteria can use nitroaromatic compounds as growth substrates and derive carbon, nitrogen and energy from their degradation [50]. The mineralization of TNT by an aerobic bacterium has also been reported [51]. The nitro group is either eliminated as nitrite by the action of oxygenases, or completely or partially reduced followed by elimination as ammonia [50,52]. Under anaerobic conditions the nitro group is reduced to an amino substituent, e.g. TNT is reduced to 2,4,6-triaminotoluene [50]. In general, these reduction products are even more toxic than the parent nitroaromatic compounds. Anaerobic bacteria such as clostridia [53], sulfate reducers [54,55] and methanogens [56] reduce nitro groups, similar to reductive dechlorination, in a co-metabolic process. However, evidence has been presented for *Desulfovibrio* species that the nitroaromatic compound could serve as nitrogen source [54] and even as terminal electron acceptor [55]. Reductive transformations of nitroaromatic compounds have been also described under Fe(III)-reducing conditions in

sediment columns [57,58]. Fe(III)-reducing bacteria produced Fe(II), which reacted in a chemical reduction with the nitroaromatic compound. Only Fe(II) adsorbed to Fe(III)-containing minerals readily reduced nitroaromatic compounds [59]. A close interdependence between the biological and chemical processes was observed. Almost no nitroaromatic compound was reduced if the sediment column was not amended with acetate as electron donor and much less acetate was consumed in the absence of the nitroaromatic compound [58].

Although many aerobic bacteria have the potential to degrade nitroaromatic compounds, aerobic processes seem less promising to remediate the subsurface. Composting, an aerobic treatment, has been used for field-scale cleanup of soil. The increased volume of waste material, the badly chemically characterized end-products, and the evidence that residual toxicity is still present after composting make this process not very suitable [50]. Bioremediation under anaerobic conditions seems to be much more favorable. Simply creating anaerobic conditions in a soil slurry by adding a starchy potato-processing by-product was sufficient to stimulate an anaerobic bacterial consortium able to completely degrade the nitroaromatic herbicide Dinoseb [60]. This treatment apparently did not lead to the formation of the toxic amines that are often observed as products of anaerobic transformations. The same treatment was also applied to soils contaminated with TNT [61]. Another approach to treat TNT-contaminated material includes a sequence of an anaerobic and aerobic process [62]. First the nitroaromatic compounds are reductively transformed in an anaerobic stage to the corresponding amines, and subsequently the amino compounds are irreversibly bound to the humic fraction under aerobic conditions [62,63].

5. Conclusions

Organic contaminants in the subsurface are threatening important drinking water resources and therefore have to be removed. Subsurface microorganism have a high potential to biodegrade many of the contaminating compounds. In some cases, biodegradation occurs naturally without taking any engineering steps to enhance the process, in others, nutrients

and electron acceptors have to be injected into the subsurface, or specific microbial consortia have to be stimulated by creating selective growth conditions. Anaerobic processes are promising for bioremediation of sites contaminated with hydrocarbons, chlorinated and nitroaromatic compounds. For some compounds such as PCE and TNT, anaerobic biodegradation is even the only solution to transform these compounds into harmless products or products that can be further treated under aerobic conditions.

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