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Dynamics of organohalide-respiring bacteria and their genes following *in-situ* chemical oxidation of chlorinated ethenes and biostimulation

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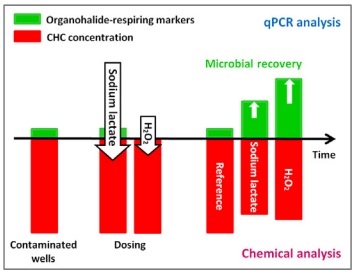
HIGHLIGHTS

- Effect of hydrogen peroxide (Fenton-like reaction) and lactate was evaluated.
- Changes in specific microflora after chemical oxidation/biostimulation documented.
- Stimulation of indigenous organohalide-respiring bacteria with lactate observed.
- Microbial recovery after the Fenton-like reaction was unexpectedly fast.
- Chemical analysis corresponded with the results of molecular genetic analysis.

GRAPHICAL ABSTRACT



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ABSTRACT

Application of Fenton's reagent and enhanced reductive dechlorination are currently the most common remediation strategies resulting in removal of chlorinated ethenes. In this study, the influence of such techniques on organohalide-respiring bacteria was assessed at a site contaminated by chlorinated ethenes using a wide spectrum of molecular genetic markers, including 16S rRNA gene of the organohalide-respiring bacteria *Dehaloccocoides* spp., *Desulfitobacterium* and *Dehalobacter*; reductive dehalogenase genes (*vcrA*, *bvcA*) responsible for dechlorination of vinyl chloride and sulphate-reducing and denitrifying bacteria.

In-situ application of hydrogen peroxide to induce a Fenton-like reaction caused an instantaneous decline in all markers below detection limit. Two weeks after application, the *bvcA* gene and *Desulfitobacterium* relative abundance increased to levels significantly higher than those prior to application. No significant decrease in the concentration of a range of chlorinated ethenes was observed due to the low hydrogen peroxide dose used. A clear increase in marker levels was also observed following *in-situ* application of sodium lactate, which resulted in a seven-fold increase in *Desulfitobacterium* and a three-fold increase in *Dehaloccocoides* spp. after 70 days. An increase in the *vcrA* gene corresponded with increase in *Dehaloccocoides* spp. Analysis of selected markers clearly revealed a positive response of

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organohalide-respiring bacteria to biostimulation and unexpectedly fast recovery after the Fenton-like reaction.

1. Introduction

Chlorinated ethenes are widely occurring contaminants that have been used in a range of industrial applications (Paul and Smolders, 2014). They represent one of the most environmentally persistent pollutants due to their strong carbon-chlorine bonds (Adetutu et al., 2015).

The most commonly used remedial activity for chlorinated ethenes in aquifers is monitored natural biodegradation; however, this has the disadvantage of slow reaction kinetics and possible accumulation of more hazardous by-products, such as *cis*-1,2-dichloroethene (cDCE) and vinyl chloride (VC) (Amaral et al., 2011; Nijenhuis et al., 2007). Nevertheless, the natural biological processes can be stimulated by addition of an organic substrate (carbon and electron source) and nutrients.

Microbial degradation of chlorinated ethenes takes place *via* anaerobic organohalide respiration. Chloroethene-contaminated aquifers may contain a range of coexisting bacteria with differing dechlorination activity, their occurrence and activity depending upon local biogeochemical conditions such as oxidation-reduction potential (ORP), pH and pollutant distribution (Imfeld et al., 2011; Paul and Smolders, 2014). Organohalide respiration is also influenced by competition for hydrogen by different bacteria, i.e. dechlorinating, fermentative, methanogenic and iron and sulphate-reducing bacteria (Aulenta et al., 2007; Chambon et al., 2013). Depending on the environmental conditions, organohalide respiration may lead to cDCE or VC accumulation (Conrad et al., 2010; Mattes et al., 2010; Taş et al., 2010). Accumulation of cDCE or VC occurs because the ORP needed for biodegradation of less-chlorinated ethenes is higher than required (Kansas et al., 1998). As both cDCE and VC are more toxic and carcinogenic than the original contaminants, treatment processes ensuring complete degradation of chlorinated ethenes are required.

While a number of bacteria are capable of incomplete degradation of tetrachloroethene (also known as perchloroethylene; PCE) and trichloroethene (TCE) (producing cDCE or VC as by-products), only *Dehalococcoides* spp. (e.g. *Dehalococcoides mccartyi*, phylum *Chloroflexi*) are known to fully degrade PCE to ethene (Chambon et al., 2013; Löffler et al., 2013; West et al., 2013). *Dehalococcoides* spp. are present at many chlorinated ethene contaminated sites (Kranzioch et al., 2013; Rossi et al., 2012) and there is a recognised correlation between *Dehalococcoides* spp. presence and formation of ethene (Rossi et al., 2012). Correspondingly, absence of *Dehalococcoides* spp. is frequently related to accumulation of cDCE and VC (Hendrickson et al., 2002; Kranzioch et al., 2013). Presence or absence of *Dehalococcoides* spp., therefore, may serve as an indicator of biodegradation potential as regards total removal of chlorinated ethenes under anaerobic conditions (Cupples, 2008; Kaster et al., 2014). The dechlorinating activities of *Dehalococcoides* spp. are known to be connected with the presence of reductive dehalogenases *rdhA*, which serves as a catalyst for cleaving the carbon-chlorine bond during organohalide respiration (Badin et al., 2014; West et al., 2013). While complete dechlorination of chlorinated ethenes is known to depend on the presence of one or more *rdhA* enzymes (*pceA, tceA, vcrA* or *bvcA*; Tang et al., 2013), the relationship between enzyme activity and expression is not as yet fully understood.

In addition to biological methods, a number of chemical techniques are also available for *in-situ* remediation, with chemical oxidation, e.g. using Fenton's reagent (a mixture of hydrogen peroxide [H_2O_2] and an iron catalyst [Fe^{2+}]) or a Fenton-like reaction (where there is a sufficient concentration of iron in the environment) being one of the most frequently applied methods for chlorinated ethenes. However, chemical oxidation can significantly restrict the activity of organohalide-respiring bacteria and cause changes in microbial population structure. Chemical oxidation can also oxidise organic matter in preference to chlorinated ethenes, resulting in a lack of carbon for autochthonous microorganisms (Chapelle et al., 2005). While changes to the microbial populations are a side-effect of chemical oxidation, application of a substrate (electron donor), such as lactate, directly improves conditions for biological organohalide respiration. This can be employed either alone or during the remediation of residual contamination, often present following the application of other techniques such as the Fenton reaction. Although biological methods exhibit slower reaction kinetics, they have the advantage of being less expensive than chemical methods and being more environmentally friendly. Despite this, many of the processes involved in biological methods are still not fully understood (Kang, 2014; Maphosa et al., 2010).

Previous studies involving Fenton-like reactions or application of sodium lactate ($NaC_3H_5O_3$) have either focused purely on chemical parameters or on total organohalide-respiring microbial colonisation (Chapelle et al., 2005; Mattes et al., 2010; Sutton et al., 2010). Though a number of studies have also conducted molecular genetics analysis, these have usually been undertaken at a laboratory scale only or following bioaugmentation with organohalide-respiring bacteria (Behrens et al., 2008; Damgaard et al., 2013a; Grostern and Edwards, 2006; Kranzioch et al., 2013; Scheutz et al., 2008).

The aim of this study was to examine the influence of a Fenton-like reaction and biostimulation through sodium lactate application on dynamics of organohalide-respiring bacteria and their genes at actual site contaminated with chlorinated ethenes. To obtain more information for the accurate diagnosis of ongoing organohalide respiration, new approach combining analysis of molecular genetic markers, together with physical and chemical parameters, was used.

2. Materials and methods

2.1. Study site

The Spolchemie Company a.s. (Ústí nad Labem, Czech Republic) has been one of the leading synthetic resins and freons manufacturers in Europe since the middle of the last century. Production of freons along with storage and distribution of raw materials (tetrachlormethane and tetrachlorethene), however, have led to extensive subsurface contamination with organic solvents, including chlorinated ethenes. The geological profile underlying the Spolchemie site consists of Mesozoic Cretaceous siltstones overlaid by a Quaternary terrace comprised mainly of fluvial sediments from the Rivers Bílina and Labe and the Klíšský stream. It is this subsurface Quaternary terrace that has been contaminated.

The Spolchemie site is currently undergoing remediation