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Combined nano-biotechnology for in-situ remediation of mixed contamination of groundwater by hexavalent chromium and chlorinated solvents

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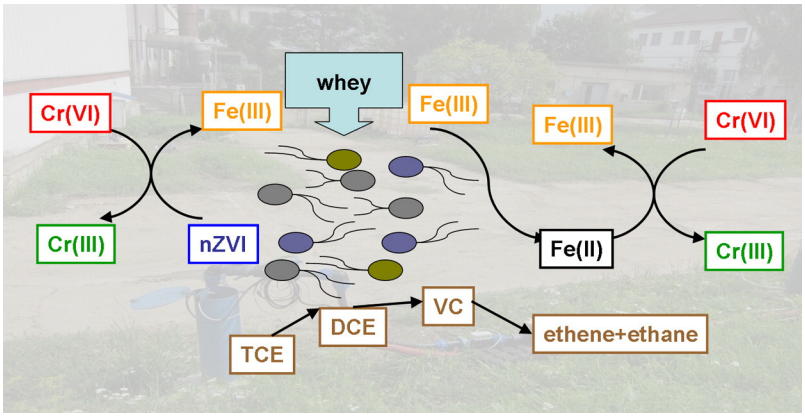
HIGHLIGHTS

- A co-mingled plume was successfully remediated by *in-situ* nZVI and whey application.
- Cr(VI) was removed completely after nZVI and remained stable after whey injection.
- Subsequent whey application also resulted in a high removal of chlorinated ethenes.
- Application of whey assisted microbial partial regeneration of the spent nZVI.
- Detected chlororespiration activity documents utility of the combined technology.

GRAPHICAL ABSTRACT



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ABSTRACT

The present report describes a 13 month pilot remediation study that consists of a combination of Cr(VI) (4.4 to 57 mg/l) geofixation and dechlorination of chlorinated ethenes (400 to 6526 μg/l), achieved by the sequential use of nanoscale zerovalent iron (nZVI) particles and *in situ* biotic reduction supported by whey injection. The remediation process was monitored using numerous techniques, including physical–chemical analyses and molecular biology approaches which enabled both the characterization of the mechanisms involved in pollutant transformation and the description of the overall background processes of the treatment. The results revealed that nZVI was efficient toward Cr(VI) by itself and completely removed it from the groundwater (LOQ 0.05 mg/l) and the subsequent application of whey resulted in a high removal of chlorinated ethenes (97 to 99%). The persistence of the reducing conditions, even after the depletion of the organic substrates, indicated a complementarity between nZVI and the whey phases in the combined technology as the subsequent application of whey phase partially assisted the microbial regeneration of the spent nZVI by promoting its reduction into Fe(II), which further supported remediation conditions at the site. Illumina sequencing and the detection of functional

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Co-mingled plume
PLFA

vcrA and *bvcA* genes documented a development in the reducing microbes (iron-reducing, sulfate-reducing and chlororespiring bacteria) that benefited under the conditions of the site and that was probably responsible for the high dechlorination and/or Cr(VI) reduction. The results of this study demonstrate the feasibility and high efficiency of the combined nano-biotechnological approach of nZVI and whey application *in-situ* for the removal of Cr(VI) and chlorinated ethenes from the groundwater of the contaminated site.

1. Introduction

Chromium is one of the most abundant and toxic metals that cause the pollution of groundwater and soil due to its frequent industrial application. Chromium generally exists in water with two stable oxidation states, the trivalent Cr(III) and hexavalent Cr(VI) forms. The hexavalent form Cr(VI) is well known to be mutagenic, carcinogenic and toxic (Nriagu and Nieboer, 1988). In contrast to the less reactive and toxic Cr(III), which is almost insoluble and can be readily precipitated out of solution, the adverse environmental effects of Cr(VI) are closely linked to its solubility and mobility, that leads to health problems such as liver damage, pulmonary congestion, vomiting and severe diarrhea. Therefore, the geo-fixation of Cr(VI) by its reduction into Cr(III) and subsequent formation of insoluble Cr(III) compounds represent the majority of *in-situ* remediation methods employed to-date, as represented by numerous publications that describe various biological or chemical approaches to achieve this transformation (Jardine et al., 1999; Barrera-Diaz et al., 2012; Dhal et al., 2013; Němeček et al., 2014; Xu and Zhao, 2007; Flury et al., 2009). In this regard, the application of reduced forms of iron materials, mainly zerovalent iron and its nanoscale forms (nZVI), represents one of the promising chemical remediation methods (see Gheju, 2011 and references therein).

Another option is to utilize microbial processes to reduce and transform Cr(VI) to Cr(III). A number of aerobic as well as anaerobic microorganisms are capable of reducing Cr(VI) (Barrera-Diaz et al., 2012) by employing different mechanisms. For instance mechanisms related to chromium resistance are utilized by the aerobic bacteria in order to detoxify hexavalent chromium (Dhal et al., 2013; Cheung and Gu, 2007) whereas the anaerobic bacteria utilize Cr(VI) as an electron acceptor in the electron transport chain related to their respiratory reactions (Barrera-Diaz et al., 2012) or reduce chromates in the periplasmatic space by hydrogenase or cytochrome c3 (Dhal et al., 2013).

Besides chromium, chlorinated organic compounds also pose a serious problem for the environment. For example, tetrachloroethene and other related organochlorine aliphatic compounds have been massively used in the past in various industry-related technological processes, resulting in a large number of contaminated sites (Tiehm and Schmidt, 2011; Koenig et al., 2015). Interestingly, nanoscale zerovalent iron (nZVI) can act on chlorinated ethenes through a sequential mechanism that involves reduction and dechlorination of the ethene structure, (Katsenovich and Miralles-Wilheirn, 2009) and the validity of this process has already been tested *in-situ* (Lacinová et al., 2012; Wei et al., 2012). As well as, the subject of bioremediation of the contaminated sites by chlorinated ethenes has been studied for more than two decades and bacterial reductive dechlorination has already been introduced into practice. Chlorinated ethenes can be dechlorinated under favorable redox conditions by abiotic and biotic reductive processes—β-elimination and/or hydrogenolysis (Cwiertny and Schrerer, 2010). During this process of reductive dechlorination, indigenous microflora chlororespirates upon biostimulation with organic substrates and the hydrogen atoms replace the chlorine substituents one-at-a-time, in a sequential manner, resulting in the production of less chlorinated analogues, starting from tetrachlorethene (PCE), via trichloroethene (TCE), dichloroethene (DCE) and vinylchloride (VC) to ethene and ethane. Unfortunately, the slowest step in the sequence of hydrogenolysis is the reduction of VC to ethane, a serious drawback because VC represents a carcinogenic metabolite on the pathway (Futagami et al., 2008). It is noteworthy that the transformation relying on a β-elimination pathway does not cause accumulation of VC (Roberts et al., 1996; Wei et al., 2010; Liu et al., 2005).

In the recent past we reported both the successful *in-situ* application of nZVI suspension in removing Cr(VI) from a contaminated aquifer (Němeček et al., 2014) and a multistep remedial approach with long-term effectivity that included geofixation with nZVI followed by whey injection supported microbial reduction (Němeček et al., 2015). This leads us to the challenging question whether nZVI could be successfully applied in case of co-mingled pollution and whether a promising way could be targeting on pollutants that require reductive conditions.

Regarding the new applications of nanomaterials such as nZVI, attention was primarily focused on the concern of its possible negative effect on natural microbiota and the phenomenon of nZVI interaction with chlororespiring bacteria is excellently reviewed by Bruton et al. (2015). However, it is important to bear in mind that contaminated sites that require a call for remediation are generally represented by sufficiently damaged environments where the most toxic component is often represented by a particular pollutant. For example a rapid decrease of the pollutant concentration together with hydrogen evolution and redox potential shifts caused by nZVI can finally lead to favorable conditions for consequent biological processes (Němeček et al., 2015; Fajardo et al., 2012; Liu and Lowry, 2006).

The present study was performed to assess the feasibility of remediating a site co-contaminated by Cr(VI) and chlorinated ethenes by injecting nZVI suspensions and subsequently cheese whey as an organic substrate. In light of our previous successful studies and the promising results obtained in the presented study, we suggest that this remedial design may be employed effectively to enhance the removal of pollutants from a co-mingled plume. The remediation process was monitored using a combination of techniques, ranging from physical–chemical analyses to molecular biology approaches, in order to elucidate the mechanisms involved in pollutant transformations and to describe the background of the treatment process in general.

2. Materials and methods

2.1. Test site

The pilot test was performed at a site in the Czech Republic. The site is polluted with chlorinated ethenes and Cr(VI) originating from historical degreasing and chromium coating activities. The aquifer is developed in Quaternary sandy gravels with silty admixture and is overlain by clay and clayey loam with a thickness of 5 m. The aquifer has a saturated thickness of approximately 4 m. The hydraulic conductivity of the aquifer is 7.6×10^{-4} m/s based on a pumping test. The average seepage velocity of the groundwater flow is 1.5 m/day. The groundwater discharges into a local river located at a distance of 430 m from the site. The groundwater is of the Ca-HCO_3^- type and is characterized by elevated mineralization (total dissolved solids from 0.9 to 1.2 g/l), neutral pH (6.9–7.0), oxidation–reduction potential ranging from 130 to 490 mV and TOC from <1.0 mg/l to 5.4 mg/l. The initial Cr(VI) concentration in the groundwater ranged from 4.4 to 57 mg/l. The total concentration of chlorinated ethenes ranged from 400 to 6526 μg/l. Trichloroethene (TCE) and cis-1,2-dichloroethene (cis-DCE) were the dominant chlorinated contaminants (TCE and cis-DCE represent 45% to 93% and 5% to 53% of the total chlorinated ethenes on a molar basis, respectively).

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