Bioremediation of Tetrachloroethene Contaminated Groundwater by Dechlorinating Microbial Communities

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Abstract

Improper disposal of chlorinated compounds widely used as industrial solvents, intermediates in chemical industries, pesticides and pharmaceuticals has led to severe subsurface contamination. Common chlorinated compounds include tetrachloroethene or perchloroethylene (PCE), trichloroethene (TCE), dichloroethenes (DCE) and vinyl chloride (VC). Enhanced reductive dechlorination (ERD) represents a promising approach for the complete degradation of these compounds. Successful microbial-mediated remediation has to date been associated with major dechlorinating species such as *Dehalococcoides (Dhc), Desulfitobacterium, Desulfuromonas, Dehaloginomonas, Geobacteriaceae* and *Sulfurospirillum.* This research explored the degradation potential of microbial communities other than these well studied groups within groundwater collected from a PCE-contaminated site in Australia. Laboratory based enrichment cultures using groundwater samples with high PCE levels (146 µg L^{-1}) showed the dominance of *Proteobacteria*, *Spirochaetes, Firmicutes, Bacteroidetes, Methanomicrobiaceae, Methanosaetaceae* and *Methanosarcinaceae*groups. The indigenous groundwater community was found capable of the complete dechlorination of PCE to the environmentally safe end product ethene over 24 weeks, with the sequential degradation of PCE via intermediate products. The molecular culture-independent microbial profiling techniques like polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) along with novel statistical Pareto-Lorenz and moving windows analyses were used to assess changes in the indigenous microbial community during PCE removal. A comparison of the effects of using either biostimulation only (BS) with biostimulation plus bioaugmentation (BS-BA) for PCE remediation in a laboratory based system showed that both remediation regimes were successful, with complete PCE degradation occurring over 17 and 21 weeks for BS only and BS-BA, respectively compared to controls which had only 30% PCE degradation. Furthermore, quantitative real time PCR and live-dead cell count (LDCC) analyses

showed a 2-3 fold increase in microbial cell abundance with approximately 70–80% viability in both treatments indicating active growth of PCE dechlorinators. We further employed BS, BS-BA and monitored natural attenuation (MNA) strategies for commercial bioremediation at TCE contaminated site in Victoria, Australia. Over the period of nine months of BS, MNA and BS-BA treatments TCE concentration was reduced from 40, 79 and 150 µg $L^{-1}$to below maximum concentration level of 5µg L^{-1},respectively. Although, this work highlighted ERD as an effective way of PCE remediation, this technology has a few disadvantages. Hence, an alternative microbial electric system (MES) was established where bioenergy was generated through the catalytic actions of microorganisms during PCE dechlorination. Multiple lab-scale MESs fed with acetate and carbon electrode/PCE as electron donors and acceptors, respectively under BS only and BS-BA regimes further highlighted the bio-electrochemical potential of indigenous non-*Dhc* community against previously well studied *Dhc* and *Geobacteriaceae* species. The indigenous non-*Dhc* community was found to contribute significantly to electron transfer with ~61% of the current generated. Microbial colonization and biostimulation resulted in 100% dechlorination in both treatments with complete dechlorination occurring 4 weeks earlier in BS-BA samples and up to 11.5 µA of current being generated than BS only MES. Overall, this study contributes to better understanding of the dechlorinating potential of indigenous non-*Dhc* microorganisms; their structure, dynamics and functional organization in response to PCE dechlorination that will assist to advance the bioremediation field in a rational manner. In addition, evidence of advances in the current bioremediation practices in terms of methodology (LDCC) and techniques (MES) are presented.

Declaration

'I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the ward of any other degree or diploma at Flinders University of South Australia or any other educational institution, except where due acknowledgments is made in the thesis. Any contribution made to the research by others, with whom I have worked at Flinders University or elsewhere, is explicitly acknowledged in this thesis. I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation and linguistic expression is acknowledged.

I give consent to this copy of my thesis when deposited in the Flinders University Library, being made available for loan and photocopying, subject to the provisions of the copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder(s) of those works.

List of publications contained in this thesis and the copyright holders(s) for each work are as follows:

Patil, S.S., Adetutu E.M., Aburto–Medina, A., MenzI.R., Ball A.S. 2014. Biostimulation of indigenous communities for the successful dechlorination of tetrachloroethene (perchloroethylene) - contaminated groundwater. *Biotechnology Letters* 36, 75-83 doi:10.1007/s10529-013-1369-1

Patil, S.S., Adetutu E.M., Sheppard P.J., Morrison P., MenzI.R., Ball, A.S. 2014. Site-specific pre-evaluation of bioremediation technologies for chloroethene degradation. *International Journal of Environmental Science and Technology* 11 (7), 1869 – 1880.

Patil, S.S., Adetutu E.M., Rochow, J., Mitchell, J.G., Ball, A.S. 2013. Sustainable remediation: electrochemically assisted microbial dechlorination of tetrachloroethene contaminated groundwater. *Microbial Biotechnology* 7 (1), 54-63 doi: 10.1111/1751-7915.12089

Gundry, T.D., **Patil, S.S.**, Ball, A.S. 2014. Application of molecular biological tools for the assessment of the *in situ* bioremediation potential of TCE. Submitted to *Groundwater Monitoring and Remediation* (Under review)

During the candidature, I attended two conferences, one international and other national which presented an opportunity to present my work. The conferences included:

- Proffered paper presentation on ‘Sustainable remediation: bioenergy generation during the bioremediation of tetrachloroethene contaminated groundwater’ at 54th ‘Annual Scientific Meeting and Exhibition of the Australian Society for Microbiology’ held between 7-10 July 2013, Adelaide, South Australia.

- Poster presentation on ‘Indigenous microbial community dynamics during reductive dechlorination of groundwater at a chloroethene contaminated site in Victoria, Australia’ at 14th ‘International Symposium on Microbial Ecology’ held between 19–24 August, 2012, Copenhagen, Denmark (Appendix 1, page 116).

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Finally, I would like to thank my parents, my beloved father and mother for being a constant source of inspiration, courage and the greatest teacher in my life: this is for both of you!

Structure of the Thesis

This thesis is subdivided into 8 main Chapters. An introduction to the project and a review of the current literature is provided in Chapter 1. Chapter 2 contains details of the general experimental materials and methods used in this study throughout the research chapters (3 to 6). Details of the methodologies used in specific research chapters are given in the relevant result chapters.

Chapter 3 represents the first results chapter and describes how the biostimulation strategy was implemented to enhance indigenous communities for successful dechlorination of tetrachloroethene contaminated groundwater.

Based on the Chapter 3 results, Chapter 4 further describes work on the site-specific pre-evaluation of biostimulation and biostimulation plus bioaugmentation based bioremediation strategies for chloroethene degradation. This chapter also demonstrates the applicability of quantitative microbiological tools like real – time PCR and Live/dead Cell Count analyses for preliminary site assessments.

Chapter 5 extends the laboratory based work described in Chapter 3 and 4 to *in situ* commercial application.

Chapter 6 explores the avenue of Microbial Electric System (MES) as a ground-breaking alternative to current remediation technology. This chapter highlights the potential of indigenous non-*Dehalococcoides* bacterial community in bio-electrochemically reducing tetrachloroethene to enhance MES efficiency for successful bioremediation.

Chapter 7 presents an overall discussion of the research carried out in the thesis and draws final conclusions.

Chapter 8 contains the references cited in Chapter 1, Chapter 2 and Chapter 7. Appendices and Corrigenda have also been included in Chapter 8.

The Results Chapters 3, 4 and 6 represents peer-reviewed articles in international journals and has been reproduced in their published format. Chapter 5 has been submitted to a refereed academic journal and is reproduced in the submitted format.

List of Abbreviations and Terminologies

bgs	Below ground surface
BS	Biostimulation
BA	Bioaugmentation
cDCE	*cis*-Dichloroethene
DCA	Dichloroethane
Dhc	*Dehalococcoides*
DGGE	Denaturing Gradient Gel Electrophoresis
DNAPL	Dense Non-aqueous Phase Liquid
GW	Groundwater
16S rRNA	16S sub-unit of ribosomal DNA gene
MBTs	Molecular Biological Tools
MCL	Maximum Contaminant Level
MES	Microbial Electric Systems
MNA	Monitored Natural Attenuation
MWs	Monitoring Wells
NA	Natural Attenuation
LNAPL	Light Non-aqueous Phase Liquid
PCR	Polymerase Chain Reaction
PCE	Tetrachloroethene (Perchloroethene)
RDase	Reductive Dehalogenase
RNA	Ribonucleic Acid
TCE	Trichloroethene
TCA	Trichloroethane
VC	Vinyl Chloride
VOCs	Volatile Organic Compounds

Terminologies used in this study

Biodegradation: Biologically mediated conversion of one compound to another.

Bioremediation: Use of microorganisms to control, transform and/or destroy contaminants

Biotransformation: Microbiologically catalyzed transformation of a chemical to some other product.

Biostimulation: The addition of nutrients, electron acceptors (or electron donors), and sometimes auxiliary substrates to stimulate growth and activity of specific indigenous microbial populations.

Bioaugmentation: The addition of exogenous, specialized microorganisms with enhanced capabilities to degrade the target pollutant.

Chlorinated Solvent: A hydrocarbon in which chlorine atoms substitute for one or morehydrogen atoms in the compounds structure. Chlorinated solvents commonly are used for grease removal in manufacturing, dry cleaning, and other operations.

Co-metabolism: A reaction in which microbes transform a contaminant even though the contaminant cannot serve as an energy source for the organisms. To degrade the contaminant, the microbes require the presence of other compounds (primary substrates) that can support their growth.

Dechlorination: The removal of chlorine atoms from a compound.

Dehydrohalogenation: Elimination of a hydrogen ion and a halide ion resulting in the formation of an alkene.

Dihaloelimination: Reductive elimination of two halide substituents resulting in formation of an alkene.

***Ex Situ* Bioremediation:** The use of aboveground bioreactors to treat contaminated soil or groundwater that has been extracted from the contaminated site.

***In Situ* Bioremediation**: Bioremediation process that occur below the ground surface, where the contaminated zone becomes the bioreactor.

Electron Acceptor: Compound that gains electrons (and therefore is reduced) in oxidation-reduction reactions that are essential for the growth of microorganisms. Common electron acceptors are oxygen, nitrate, sulfate, iron and carbon dioxide. Highly chlorinated solvents (e.g. TCE) can act as electron acceptors.

Electron Donor: Compound that loses electrons (and therefore is oxidized) in oxidation-reduction reactions that are essential for the growth of microorganisms. In bioremediation organic compounds serve as electron donors. Less chlorinated solvents (e.g., VC) can act as electron donors.

Enhanced Anaerobic Bioremediation: Addition of carbon sources (electron donors) and/or nutrients to the subsurface in order to stimulate bacteria which can destroy chlorinated solvents by using them as an electron acceptor in the process of reductive dechlorination.

Intrinsic Remediation or Natural Attenuation: *In situ* remediation that uses naturally occurring processes to degrade or remove contaminants without using engineering steps to enhance the process.

Reduction: Transfer of electrons to a compound such as oxygen. It occurs when anothercompound is oxidized.

Reductive Dechlorination: The removal of chlorine atoms from an organic compound and their replacement with hydrogen atoms (same as reductive dehalogenation).

Reductive Dehalogenation: A variation on biodegradation in which microbially-catalyzed reactions cause the replacement of a halogen atom (e.g. chlorine) on an organic compound with a hydrogen atom. The reactions result in the net addition of two electrons to the organic compound.