

Microfracture Surface Characterizations: Implications for In Situ Remedial Methods in Fractured Rock

Bedrock Bioremediation Center Final Report

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Notice

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Table of Contents

Notice	ii
Foreword.....	iii
Executive Summary.....	E1
List of Tables.....	vii
List of Figures.....	viii
Abbreviations	x
Chapter 1 Introduction.....	1
1.1 Microfractures and Their Role in Reaction and Transport	1
1.2 Microfracture Definition.....	3
1.3 Microbes in Bedrock	4
1.4 Microbe-Mineral Interactions	5
1.5 Chlorinated Solvent Abiotic and Biotic Transformations	6
1.6 Determination of Likely Terminal Electron Accepting Processes	10
1.7 Related Field Work on Bedrock TCE Contaminated Sites.....	10
1.8 Spectroscopic Characterization of Surfaces.....	11
1.9 Objectives of This Study	11
Chapter 2 Materials and Methods.....	12
2.1 Core/Microfracture Locations.....	12
2.2 Core/Microfracture Sample Collection	15
2.3 Microfracture Sample Preparation	16
2.4 Overall Analytical Sequence.....	16
2.5 SEM-Morphology and Biopatch Distribution.....	17
2.6 SEM-EDAX Spatial Maps	18
2.7 Microfracture Surface Precipitate Fixation & Embedding for TEM	18
2.8 TEM-Microbial Ultrastructure	18
2.9 Petrographic Thin Sections.....	18
2.10 SEM-EDAX Spatial Mapping Microfracture Surface Precipitates and Host Rock	19
2.11 XRD Analysis of Microfracture Surfaces and Host Rock.....	19
2.12 XPS Speciation of Microfracture Surfaces	20
2.13 SIMS Fingerprints of Microfracture Surfaces	21
2.14 MIP of Host Rock.....	21
2.15 Packer Water Collection.....	22
2.16 Geochemical Modeling	22
2.17 Microbe Extraction	22
2.18 Microbial Characterization Using Molecular Biological Techniques.....	23
2.19 Primers and Polymerase Chain Reaction Assay.....	24
2.20 Denaturing Gradient Gel Electrophoresis.....	24
2.21 DNA Sequencing & Analysis	24
2.22 Data Quality	25

Table of Contents, continued

Chapter 3 Results & Discussion.....	28
3.1 Microfracture Locations	28
3.2 Microfracture Surface Precipitate Morphology	28
3.3 Microfracture Element Spatial Maps.....	30
3.4 Microfracture Biopatch Distribution and Morphology.....	32
3.5 Microfracture Microbial Populations Situated Within Surface Precipitates	35
3.6 Petrographic Characterization of Host Rock and Microfracture Surfaces	39
3.7 Mineralogy of Microfracture Surfaces and Host Rock Based on XRD	45
3.8 Element Speciation of Microfracture Surfaces Based on XPS.....	49
3.9 Packer Water Characterization and Geochemical Modeling	59
3.10 Mass Fragment Fingerprints of Microfracture Surfaces	63
3.11 Porosity and Pore Size Distribution of Host Rock	65
3.12 Microbes Identified on Microfractures.....	65
3.13 Relationship between Packer Water Samples and Microfracture Geochemical Environment.....	67
3.14 Likely Terminal Electron Accepting Processes in the Open Fracture System.....	68
3.15 Likely Terminal Electron Accepting Processes in the Microfracture Network.....	69
3.16 Microfracture Surface Speciation and Adherent Microbial Population Metabolism and Diversity	70
3.17 Role of Microfracture Surfaces in TCE Transformation and Microbial Ecology	72
Chapter 4 Conclusions.....	73
Acknowledgements	76
References	77

List of Tables

Table 1.1	Microfracture Description Protocol.....	3
Table 1.2	Conditions for Abiotic and Biotic Transformation of Chlorinated Solvents (adapted from McCarty, 1997b)	8
Table 1.3	Environmental Conditions for Biological Reductive Dechlorination Reactions (adapted from McCarty, 1997b)	8
Table 1.4	Properties of Some Direct Chlorinated Solvent Dechlorinators (adapted from Gossett and Zinder, 1997).....	9
Table 2.1	Microfracture Location, Cluster Assignment, and Description.....	14
Table 2.2	Analytical Methods Applied to the Host Rock and Microfracture Samples.....	15
Table 3.1	Summary Description of Surface Precipitate Morphologies	29
Table 3.2	Summary Description of Element Association in Spatial Maps.....	31
Table 3.3	Mineral Phases Identified by Petrography in Host Rock.....	42
Table 3.4	Mineral Phases Identified by Petrography in Microfracture Surface Precipitates	44
Table 3.5	Summary of Crystalline Minerals Identified in Host Rock Samples	47
Table 3.6	Microfracture Surface Precipitate Candidate Minerals Based on XRD	48
Table 3.7	Microfracture MF02 - Candidate Minerals by XPS	50
Table 3.8	Microfracture MF03 - Candidate Minerals by XPS	51
Table 3.9	Microfracture MF04 - Candidate Minerals by XPS	52
Table 3.10	Microfracture MF05 - Candidate Minerals by XPS	53
Table 3.11	Microfracture MF06 - Candidate Minerals by XPS	54
Table 3.12	Microfracture MF07 - Candidate Minerals by XPS	55
Table 3.13	Microfracture MF08 - Candidate Minerals by XPS	56
Table 3.14	Microfracture MF09 - Candidate Minerals by XPS	57
Table 3.15	Microfracture MF10 - Candidate Minerals by XPS	58
Table 3.16	Packer Water Characterizations.....	60
Table 3.17	Candidate Controlling Solid Minerals in Packer Waters Identified by Geochemical Modeling.....	62
Table 3.18	Presence or Absence of Prokaryotic Groups on Borehole BBC5 Microfracture Surfaces as Determined by Amplification with Specific Primer Sets.....	66
Table 3.19	Prokaryotic Groups on Borehole BBC5 Microfracture Surfaces Relative to Fe, S, and C	71

List of Figures

Figure 1.1	Conceptual model of adherent biopatch (in cross section) on a microfracture surface	2
Figure 1.2	Suite of analytical methods for characterization of bulk and surface chemistry.....	11
Figure 2.1	Cross section through BBC site	12
Figure 2.2	Plan view of borehole locations at Site 32	13
Figure 2.3	Cross section through boreholes BBC5 and BBC6 showing microfracture locations and hydrologic connections between the two boreholes.....	14
Figure 2.4	Schematic depiction of microfracture-type sampling.....	16
Figure 2.5	Analytical scheme.....	17
Figure 3.1	SEM micrographs of typical microfracture morphology.....	29
Figure 3.2	Typical EDS elemental spatial map in X-Y plane for microfracture MF03.....	30
Figure 3.3	Biopatch SEM micrographs	32
Figure 3.4	Biopatch SEM micrographs	33
Figure 3.5	Biopatch SEM micrographs	34
Figure 3.6	TEM micrographs of stalked morphologies present in calcite precipitates on microfracture MF11	35
Figure 3.7	TEM micrographs of spirillum morphologies in calcite precipitates in microfracture MF11.....	36
Figure 3.8	TEM micrographs of filamentous morphologies in calcite precipitates in microfracture MF11.....	37
Figure 3.9	TEM micrographs of inclusion bodies within cell structures in calcite precipitates in microfracture MF11	38
Figure 3.10	Petrographic thin section of Kittery metasandstone	39
Figure 3.11	Photomicrographs of the same region of a petrographic thin section in (a) plane, (b) cross-polarized, and (c) reflected light.....	39
Figure 3.12	Diabase dike textures and microfracture fillings	40
Figure 3.13	Photomicrographs of microfracture textures and morphology.....	41
Figure 3.14	Petrographic micrographs of host rock and microfracture surfaces	43
Figure 3.15	Element spatial map of microfracture MF07 thin section showing host rock and microfracture face in cross section	44
Figure 3.16	Typical raw diffractogram	45
Figure 3.17	Typical diffractogram after background removal and smoothing	45
Figure 3.18	Typical peak ID as determined by search match routine	46
Figure 3.19	Diffractogram of host rock from microfracture MF07.....	46
Figure 3.20	Diffractogram of microfracture surface precipitate from microfracture MF07	47
Figure 3.21	Typical XPS low resolution survey scan of microfracture MF02.....	49
Figure 3.22	Typical component curve fit exercise for the C1s photoelectron from a high resolution scan for microfracture MF02.....	49

List of Figures, continued

Figure 3.23	SIMS negative mass fragment (0-50 atomic mass units) fingerprint	63
Figure 3.24	SIMS positive mass fragment (0-50 atomic mass units) fingerprint	63
Figure 3.25	SIMS positive mass fragment (50-100 atomic mass units) fingerprint	64
Figure 3.26	MIP cumulative porosity and pore size distribution for borehole BBC5 host rock specimen	65
Figure 3.27	Polymerase chain reaction-denaturing gradient gel electrophoresis bacterial community profiles of borehole BBC5 microfractures MF01 - MF07	66
Figure 3.28	Cluster analysis of the denaturing gradient gel electrophoresis banding patterns of borehole BBC5 microfracture surfaces based on the position of bands using unweighted paired group method with arithmetic averages	67

Abbreviations

AFCEE	Air Force Center for Environmental Excellence
BBC	Bedrock Bioremediation Center
DCA	dichloroethane
DCE	dichloroethene
DGGE	denaturing gradient gel electrophoresis
DPR	drilling parameter recorder
EDAX	energy dispersive analysis of x-rays
ESCA	electron spectroscopy for chemical analysis
FESEM	Field Emission Scanning Electron Microscopy
GC	guanine-cytosine
GC/MS	gas chromatography/mass spectrometry
ICDD	International Center for Diffraction Data
IHSS	International Humic Substances Society
MIP	mercury intrusion porosimetry
NIST	National Institute for Standards and Technology
NOM	natural organic matter
NPDOC	non-purgeable dissolved organic carbon
PCE	perchloroethene
PCR	polymerase chain reaction
SEM	scanning electron microscopy
SIMS	secondary ion mass spectrometry
TCE	trichloroethene
TEM	transmission electron microscopy
TI	Technical Impracticability
TOF	time-of-flight
UST	underground storage tank
VC	vinyl chloride
XPS	x-ray photoelectron spectroscopy
XRD	x-ray diffraction
XRPD	x-ray powder diffraction

Executive Summary

Purpose: The Bedrock Bioremediation Center (BBC) at the University of New Hampshire is a center specializing in multi-disciplinary research on bioremediation of organically-contaminated bedrock aquifers. The focus of its present work is a field research-based program conducted at Site 32 at the Pease International Tradeport (formerly Pease Air Force Base) in Portsmouth, NH. The U.S. EPA supports the overall mission of the BBC to (i) examine whether microbial communities in organically-contaminated bedrock aquifers are capable of biodegrading the contaminants, (ii) more efficiently and economically characterize the direction of groundwater flow and fracture patterns (size, direction, secondary mineralization) in contaminated bedrock aquifers, (iii) improve and develop new field technologies to control hydraulic and flow conditions in the contaminant zone, (iv) develop laboratory and field methods to estimate and accelerate *in situ* rates of bioremediation of organic contaminants in bedrock aquifers, and (v) to develop and apply innovative microbial, molecular biology and other advanced techniques to enhance *in situ* bioremediation and assess the efficacy of remediation strategies. One of the major outreach efforts of the BBC is to transfer information gained during its research to federal, state, and local regulatory agencies and environmental consultants.

Background: Site 32 contains a contaminant plume of trichloroethylene (TCE) and its transformation products dichloroethylene (DCE) and vinyl chloride (VC). These are the principal contaminants. The site is situated on a variable thickness upper sand layer overlying a marine clay layer overlying a variable thickness lower sand layer. These unconsolidated layers are situated over the Kittery Formation, a tightly folded, biotite-grade partially metamorphosed sandstone and shale crosscut by numerous porphyritic diabase dikes. The contaminant plume extends downward and laterally northeast ~0.5 km via migration through weathered and competent bedrock. The groundwater in the bedrock is predominately contaminated with *cis*-DCE (280-440 µg/L) with some *trans*-DCE (26-48 µg/L), TCE (24-59 µg/L), and VC (8-22 µg/L). Since 1997 the overburden has been managed using a sheet pile containment system coupled with pump and treat. The bedrock groundwater zone was given a technical impracticability (TI) waiver.

Research Questions: The overarching questions addressed by this portion of the project relate to possible relations between microfracture networks in the bedrock, the surface geochemistry of these microfractures, and the ecology and metabolic activity of attached microbes relative to terminal electron accepting processes and TCE biodegradation. Questions include the following: (1) How does the microfracture surface influence attachment and growth? (2) How does the geochemistry of the microfracture surface influence population ecology and metabolism? (3) What is the relationship between the relatively high specific surface area of the microfracture network and the adjacent relatively open and more voluminous open fracture system? More specifically, how does the microfracture surface influence the dominant terminal electron acceptor processes in the microfracture network? (4) Lastly, what is the precise nature of TCE biodegradative processes within the microfracture network?

As part of the overall research plan to better understand these questions, we studied 11 microfractures extracted from competent bedrock cores from two wells at Site 32 (BBC5 and BBC6) so as to characterize, with a variety of surface spectroscopic and microbial techniques, the relation, if any, between microfracture surface geochemistry and the ecology and metabolic activity of attached microbial populations relative to terminal electron accepting processes or to chlorinated solvent biodegradation.

Results are highlighted relative to host rock and microfracture mineralogy and geochemistry, groundwater geochemistry, microfracture microbiology, and terminal electron accepting processes.

Host Rock and Microfracture Mineralogy and Geochemistry: A variety of spectroscopic techniques are needed to characterize the mineralogy and chemical speciation of the host rock and the minerals coating the microfracture surfaces. Mercury intrusion porosimetry (MIP), petrography, scanning electron microscopy-energy dispersive analysis of x-rays (SEM-EDAX), x-ray powder diffraction (XRD), x-ray photoelectron spectroscopy (XPS), and secondary ion mass spectrometry (SIMS) were all used to characterize the host rock and microfracture surface precipitates. Eleven microfractures (MF 01-11) were extracted from competent rock from cores from two boreholes (BBC5 and BBC6) located at the study site. Microfracture samples were taken at depths > 21.3 m (70 ft) below ground and within the contaminant plume. Using MIP, the partially metamorphosed sandstones and shales were found to be very impermeable. The host rock had three nominal pore throat sizes (131.1, 1.136, and 0.109 µm), a porosity of 0.8%, and a permeability of < 1 µDarcy. The host rock mineralogy was typical of metasandstones and metashales (quartz, feldspar, white mica, chlorite and/or biotite). Carbonate minerals and quartz were the dominant microfracture

surface precipitates. Likely oxidized and reduced iron species were identified on the microfracture surfaces with XPS, including siderite (FeCO_3), pyrrhotite (FeS), wüstite (FeO), goethite ($\alpha\text{-FeOOH}$), hematite (Fe_2O_3), aged hydrous ferric oxide ($\text{Fe}_2\text{O}_3 \cdot 1.57 \text{H}_2\text{O}$), limonite ($\text{Fe}_2\text{O}_3 \cdot n\text{H}_2\text{O}$), and magnetite (Fe_3O_4). Carbon functional groups characteristic of humic substances and aquatic natural organic matter (NOM) were also identified with XPS. SIMS mass fragment fingerprints revealed chlorinated carbon fragments which suggested that TCE or perhaps its transformation products were partitioned to the NOM on the microfracture surfaces. The level of spatial resolution of this technique was on the order of 10s of μm . Heterogeneity in mineral abundance on the microfracture surfaces was seen at that level.

Groundwater Geochemistry: Packer sampling techniques were used to collect groundwater samples from packer intervals associated with some of the collected microfracture samples in boreholes BBC5 and BBC6. The water collected in the packer intervals (termed packer water) was characterized using various field and laboratory techniques to describe pH, alkalinity, dissolved gases (H_2 , O_2), and dissolved geochemical constituents. The analyses were then used to model and interpret (subject to limitations) the geochemistry of the packer waters with the thermodynamic equilibrium model Visual MINTEQ. Given the volume of the microfracture network relative to the open fracture system, the samples were expected to reflect more from the composition of the open fractures. Packer waters were alkaline (131-190 mg/L as CaCO_3 , pH 8.8 to 9.6), mildly reducing (Eh of -208 to 160 mV, DO of 0.4 to 2.5 mg/L), with low NPDOC values (0.8 to 1.7 mg/L), and measurable Fe(II) (0.1 mg/L) and Fe(III) (0.02 to 0.3 mg/L). H_2 was present in a number of the BBC wells at the site (2.2 – 7.3 nM). These levels are capable of supporting reductive dechlorination and are indicative of sulfate reduction as a dominant terminal electron accepting process; however, sulfate was the dominant anion in the packer sample water (110-120 mg/L), and no sulfide was detected. Additionally, no fixed nitrogen was detected. The packer waters were in apparent pseudo-equilibrium with many of the observed major mineral phases (carbonates and iron oxides) in the host rock and on the microfracture surfaces. Estimations of Eh using the Nernst equation and activities of Fe^{2+} and Fe^{3+} suggested that the dominant redox couple was Fe(II)/Fe(III). Estimated values were similar to those measured with a polished platinum inert redox probe and reference Ag/AgCl electrode.

Microfracture Microbiology: The microbiology of the microfracture surfaces was investigated using SEM, transmission electron microscopy (TEM), and a number of molecular biology techniques. SEM of microfracture surfaces revealed occasional biopatches of attached microbes. The biopatches were located in small depressions, cracks, or crevices on the microfracture surfaces. The microbes were predominantly rod-shaped (1.0 μm in diameter by 2.0 μm in length). In some instances, the bacteria had possible extracellular polymeric substances associated with them. In other cases, the microbes appeared encased in a film of organic material or surface precipitate-like material. TEM micrographs of soft calcite surface precipitate samples from one microfracture revealed more diverse prokaryotic morphologies (e.g., spirilla, stalked bacteria, filaments). In some cases, flagella and possible cell division septa may have been present. Many cells contained large, clear organelles and small dark organelles. These may have been storage bodies. Amplification with specific primer sets of microfractures from borehole BBC5 showed the presence of both bacteria and *Archaea* (which includes methanogens) in all of the borehole BBC5 microfracture samples. Positive results were also observed for dehalorespirers (*Dehalococcoides* sp.), sulfate reducing bacteria, and iron reducing bacteria (specifically the *Geobacteraceae*). Denaturing gradient gel electrophoresis community profiles of the polymerase chain reaction-amplified bacterial 16S rDNA showed between 7 and 27 band; indicating significant population diversity of the microfracture surfaces. Dendograms showed that two of seven of the microfractures tested were similar. All other samples showed significantly different banding patterns, indicating the bacterial communities on the fracture surfaces were, in most cases, compositionally unique. Microfracture porewater likely differed from packer water in composition as the microfracture network may have been more reducing than the open fracture system based on the presence of obligate anaerobes found on the microfracture surfaces.

Terminal Electron Accepting Processes: The preceding information can be used to infer about possible terminal electron accepting processes occurring in the open fracture system and the microfracture networks. The microfracture network, by virtue of its smaller volume, reduced communication with the open fracture system, and likely mass transfer limitations probably did not significantly contribute to the contaminant or biogeochemical signatures seen in the packer waters collected under fairly transmissive conditions for fractured bedrock at the site. In terms of identification of likely terminal electron accepting processes in the open fracture system, the H_2 values observed for borehole BBC6 suggested sulfate reduction. However, high levels of sulfate and the non detection of sulfide in the packer water samples suggested that sulfate reduction was not dominant, rather, Fe(III) reduction might have been the dominant terminal electron accepting process. Iron was a dominant microfracture surface element. Both Fe(II) and Fe(III) candidate minerals were observed on the microfracture surfaces. The spatial prevalence of Fe as well as its situation in the top few nm of the microfracture surface suggested that Fe(III) was available for iron-reducing bacteria. The spectroscopic characterization of the microfracture surfaces points to Fe(III) reduction as perhaps a dominant process in the microfracture network. There was generally good agreement between SEM-EDAX, XRD, and XPS about identification of C, S, and Fe within the microfracture surface precipitates and on their surfaces. However, the observed population diversity cannot be related to the speciation of any of the three elements on the MF surfaces. The spatial heterogeneity of minerals was quite high on the microfracture surfaces. Mineral grain sizes were on the order of μm . While minerals may have been common to all observed microfracture surfaces, their relative

spacing and proximity to each other and to surface topography were quite varied. It may be that the biopatches that were observed with SEM reflect more localized microbial population response to microfracture surface mineral speciation. The level of resolution of SEM, SEM-EDAX, and XPS, however, was not high enough to discern such spatial relationships though such relations are likely.

Possible TCE Biodegradative Processes: The presence of transformation products of dehalorespiration as well as H₂ concentrations supported the role of *Dehalococcoides* sp. in dehalorespiration in the microfracture networks under conditions where Fe(III) reduction was strongly correlated to the presence of oxidized iron species on the microfracture surfaces. Other means of TCE biodegradation, including abiotic as well as aerobic and anaerobic respiratory and cometabolic processes, cannot be excluded.

Significance: The bulk of the data suggested that the microfracture networks supported diverse microbial communities. The communities differed spatially and were not similar to open fracture system planktonic population compositions. The adherent populations were patchy and associated with microfracture topography. Microbes were also found within the microfracture surface precipitates themselves, suggesting a more complex mineral-microbe spatial relationship. The dominant mineralogy on the microfracture surfaces (Fe(II) and Fe(III) oxides and carbonates) was related to the microbial metabolism of some of the identified isolates, notably iron reducers. However, other types, including obligate anaerobes, suggested that the microfracture network was perhaps more reducing than the open fracture system, perhaps particularly within the microfracture surface precipitate structure. *Dehalococcoides* sp. was a predominant component of the microfracture microbial population and suggested that reductive dechlorination was one principal process whereby TCE was transformed.

A number of follow on activities are suggested. Methods to collect and characterize microfracture porewaters may help to better describe terminal electron accepting processes and may elaborate on real differences with packer sample composition. The relative absence of NOM in the system, as well as the concentration of NOM on microfracture surfaces deserves further examination. Understanding NOM bioavailability on microfracture surfaces may help to explain the phylogenetic and metabolic diversity seen on the microfracture surfaces. Studies looking at partitioning of TCE and transformation products to partitioned NOM under controlled isotherm conditions may help to better describe partitioning with respect to microfracture surface organic carbon fractions, particularly if more sensitive SIMS methods (such as time of flight SIMS) are used. Understanding the spatial proximity of adhering microbes of terminal electron accepting process activity to minerals necessary to that terminal electron accepting process may help to describe the heterogeneous nature of terminal electron accepting processes in the microfracture network and at the microscale within the formation. Determining the extent of the microfracture specific surface area relative to that of the open fracture network would help in determining the role of microfractures in terminal electron accepting processes and biodegradative processes within contaminated bedrock aquifers. The role of mass transfer between the open fracture system and the microfracture network, as well as redox zonations that might develop relative to proximity to the open fractures might be subjected to mass transfer and reaction path modeling exercises. Additional work defining the complex microbial communities, their metabolic interactions, and their possible syntrophy with respect to TCE degradation may help to explain observed accumulations of transformation products. Further, the expression of enzymatic activity relative to terminal electron accepting processes and TCE biodegradation would help determine the metabolic activity on microfracture surfaces and why these might differ from those occurring in the open fracture groundwaters.