Numerical Modeling Analysis of Hydrodynamic and Microbial Controls on DNAPL Pool Dissolution and Detoxification: Dehalorespirers in Co-Culture

Eric S. Wesseldyke^{a,1}, Jennifer G. Becker^{a}, Eric A. Seagren^a, Alex S. Mayer^a, and Changyong Zhang^{b,2}*

^a Dept. of Civil and Environmental Engineering, Michigan Technological University, 1400

Townsend Drive, Houghton, MI 49931, USA

^b Chemical and Materials Science Division, Fundamental and Computational Sciences

Directorate, Pacific Northwest National Laboratory, 3335 Q Ave., MSIN K8-96, Richland, WA 99354, USA

¹ Present address: Leggette, Brashers, and Graham, Inc., 511 Valley Ave., P.O. Box 326, Burlington, ND 58722, USA

² Present address: Exxon Mobil Upstream Research Company, Houston, TX, USA.

*Corresponding Author. Mailing address: Dept. of Civil and Environmental Engineering, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931, USA; Telephone: (906)487-2942; e-mail: jgbecker@mtu.edu

ABSTRACT

Dissolution of dense non-aqueous phase liquid (DNAPL) contaminants like tetrachloroethene (PCE) can be "bioenhanced" via biodegradation, which increases the concentration gradient at the DNAPL-water interface. Model simulations were used to evaluate the impact of ecological interactions between different dehalorespiring strains and hydrodynamics on the bioenhancement effect and the extent of PCE dechlorination. Simulations were performed using a two-dimensional coupled flow-transport model, with a DNAPL pool source and two microbial species, *Dehalococcoides mccartyi* 195 and *Desulfuromonas michiganensis*, which compete for electron acceptors (e.g., PCE), but not for their electron donors. Under biostimulation, low v_x conditions, *Dsm. michiganensis* alone significantly enhanced dissolution by rapidly utilizing aqueous-phase PCE. In co-culture under these conditions, *Dhc. mccartyi* 195 increased this bioenhancement modestly and greatly increased the extent of PCE transformation. Although *Dsm. michiganensis* was the dominant population under low velocity conditions, *Dhc. mccartyi* 195 dominated under high velocity conditions due to bioclogging effects.

KEYWORDS

NAPL; dissolution; dehalorespiration; competition; advection; dispersion

1. Introduction

Contamination of groundwater with chlorinated ethenes such as tetrachloroethene (PCE) and trichloroethene (TCE) is common [1]. Although it is difficult to quantify the number of sites at which these contaminants occur in the form of dense non-aqueous phase liquids (DNAPLs), the problem is probably widespread. For example, based on a review of site investigation data from 712 sites in the Superfund program, the EPA [2] DNAPL contamination is likely present at approximately 60% of all National Priority List sites in the Superfund Program [2]. The DNAPL contaminant mass entrapped in saturated porous media can, due to the low aqueous solubility of PCE and TCE, serve as a source of contamination for decades or even hundreds of years under natural conditions [3, 4]. Thus, the presence of a DNAPL source zone can greatly extend the time frames needed to reduce dissolved contaminant concentrations to regulatory levels.

Several approaches for remediating DNAPL source zones and associated dissolved plumes have been evaluated. Engineered bioremediation approaches targeting aqueous-phase chlorinated ethenes generally are designed to overcome rate limitations on dehalorespiration, a form of anaerobic respiration in which the chlorinated ethene serves as the terminal electron acceptor and undergoes reductive dechlorination. This approach usually involves biostimulation through the addition of an appropriate electron donor, such as H₂, and/or bioaugmentation with cultures containing dehalorespiring populations. Two categories of dehalorespiring populations may be naturally present or added via bioaugmentation (see Supplementary Material). The first category encompasses several dehalorespiring bacteria, such as *Desulfuromonas michiganensis*, that dehalorespire PCE and TCE and produce *cis*-dichloroethene (DCE) as the dominant dechlorination product [5-8]. Complete detoxification to ethene can be achieved only if sufficient numbers of dehalorespirers in the second category—members of the phylum *Chloroflexi*,

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4 especially *Dehalococcoides mccartyi* strains—are present, because these organisms appear to be
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6 unique in their ability to dehalorespire DCE and vinyl chloride (VC) [9-13].
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9 When biodegradation reduces contaminant concentrations at the DNAPL-water interface,
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11 the driving force for contaminant dissolution from NAPL source zones is increased, relative to
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13 abiotic dissolution processes. Modeling studies [14-21] have demonstrated the potential for
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15 biodegradation-enhanced NAPL dissolution to decrease the longevity of NAPL source zones.
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17 This potential has been verified in laboratory studies [22-33]. Evidence of bioenhanced
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19 dissolution also has been obtained in field studies [34-36]
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23 While the results of these studies are promising, the extent to which bioenhanced
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25 dissolution can be applied to remediate DNAPL contaminant source zones in the field is still
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27 poorly understood [37]. Little is known about the factors controlling biological enhancement of
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29 dissolution *in situ* because many of the laboratory studies conducted to date have focused on
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31 demonstrating the occurrence of bioenhanced dissolution without attempting to optimize this
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33 phenomenon, e.g., [22-26]. Because of the many processes that can concurrently impact the
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35 bioenhancement effect, modeling studies provide a useful tool for elucidating the controlling
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37 factors [18]. Previous modeling studies indicate that the bioenhancement effect is greatest when
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39 the biodegradation rates are fast compared to the abiotic NAPL dissolution rate [20, 21]. Further,
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41 the degree of bioenhancement depends on several physico-chemical factors, e.g., advection rate,
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43 transverse dispersivity, length scale of the reactive zone, NAPL configuration, and rates of co-
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45 substrate supply, and biological factors, e.g., biokinetics and competition for electron donors [18-
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47 21]. Although informative, most of the modeling studies that have been conducted to date are
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49 limited by their failure to incorporate additional key biochemical phenomena that may impact
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51 bioenhanced dissolution.
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4 In particular, it has been shown that different species of dehalorespiring bacteria may be
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6 present within the microbial community inhabiting a DNAPL source zone, resulting in complex
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8 competitive or complementary ecological interactions between the microbial populations [38].
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11 Becker and Seagren [14] used a one-dimensional (1-D) model to show that these ecological
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13 interactions play a critical role in determining which dehalorespiring population becomes most
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15 abundant at the DNAPL-water interface. Their work also showed that hydrodynamic factors,
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17 especially the relative rates of advection and chlorinated ethene biodegradation, also affect the
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19 outcome of these ecological interactions. Importantly, because of significant differences in the
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21 dehalorespiration kinetics exhibited by different dehalorespiring strains, the extent to which
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23 bioenhanced dissolution was observed depended largely on the dehalorespiring population that
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25 was dominant at the DNAPL-water interface. Chen et al. [16] used the column data of Amos et
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27 al. [29] to develop and test a one-dimensional model incorporating two dechlorinating
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29 populations, one of which reductively dechlorinates PCE to DCE (*Geobacter lovleyi* strain SZ)
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31 and a second population of *Dehalococcoides mccartyi* strains that dechlorinates DCE to ethene.
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33 The model, which accounts for substrate limitations and competitive inhibition, indicated that the
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35 bioenhancement of dissolution was controlled by the rates of PCE and DCE degradation and
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37 *Dehalococcoides* growth, when electron donor availability was not limiting. However, the extent
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39 of dechlorination was influenced by DCE inhibition of VC dechlorination and the column
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41 residence time.
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51 Importantly, the modeling studies conducted to date with multiple dehalorespiring
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53 populations have focused on 1-D systems, which may not capture critical hydrodynamic
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55 phenomena. For example, one-dimensional models cannot be used to model NAPL pool source
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57 zones, which are expected to persist substantially longer than ganglia source zones [39], because
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1-D models are incapable of capturing dispersion perpendicular to the edge of the NAPL-pool source zone. This is key because vertical transverse dispersion is likely to be the process controlling dissolution from the pool surface [40, 41]. Accordingly, the rate of advection along the interface between the aqueous phase and the NAPL also strongly influences the abiotic dissolution rate along the NAPL boundary. Lastly, the potential impacts of biomass growth on spatial variations in hydraulic conductivity, which has been shown to influence bioenhanced DNAPL dissolution [19], cannot be properly simulated in 1-D systems [42].

The current study was undertaken to build on the advances made in earlier studies and overcome several shortcomings in our understanding of bioenhanced dissolution of DNAPLs. Specifically, the overall goal of this research was to develop a two-dimensional (2-D) model that incorporates the ecological interactions among dehalorespiring populations and incorporates coupled systems of non-linear equations for describing dehalorespiration kinetics. The model is used to assess the key hydrodynamic, kinetic, and ecological phenomena that affect DNAPL dissolution rates under intrinsic and enhanced conditions. These phenomena are explored for three model microbial systems: a PCE-to-DCE dehalorespirer alone, a *Dhc. mccartyi* strain alone, and a co-culture of the PCE-to-DCE dehalorespirer and the *Dhc. mccartyi* strain.

2. Modeling Methods

2.1. Domain

Model evaluation of the effects of microbial competition on DNAPL pool dissolution is performed under liquid-saturated conditions in a 5 cm × 5 cm 2-D microfluidic system, which is initially homogeneous and isotropic. The domain is modeled with a third-type boundary condition at the inlet, a free outlet boundary condition, and a no-flow/no-contaminant flux

boundary along the top of the domain. The boundary condition at the PCE DNAPL pool/aqueous phase interface along the bottom of the domain is modeled using either a local equilibrium (first-type) boundary condition, or a non-equilibrium (mass-transfer limited) boundary condition. All simulations reported in this manuscript were performed using the local equilibrium boundary condition, based on preliminary abiotic PCE dissolution studies and model simulations that suggest this assumption is appropriate for the study conditions, as well as previous work with NAPL pools [43, 44]. These boundary conditions produce a flow field that is initially 1-D in the direction parallel to the DNAPL pool/aqueous phase interface, which is referred to as the x-direction in this work. Although we applied the model to describe a microfluidic system in the current study, the model is not constrained to the micromodel scale and can be scaled up to describe domains with much larger dimensions.

2.2. Ecological Interaction Scenarios

The dehalorespiring populations (*Dsm. michiganensis* and *Dhc. mccartyi* 195) modeled in this study were chosen to be consistent with our previous modeling and experimental work with these organisms, including the careful estimation of biokinetic parameters, which were used as model inputs in the current study. *Dhc. mccartyi* 195 does not respire VC and instead transforms it cometabolically. In bioremediation practice, it is important that a *Dhc. mccartyi* strain that respire VC is present downgradient of the DNAPL contaminant source zone. However, in the current study, the VC transformation kinetics of the *Dhc. mccartyi* 195 strains are not important with respect to dissolution bioenhancement because VC is not a significant component of the DNAPL contaminant source. The substrate utilization kinetics of PCE, not VC, drive the bioenhancement of DNAPL dissolution.

Two different ecological interactions can potentially occur between the populations modeled in this study. In one model scenario, *Dhc. mccartyi* 195 competes with *Dsm. michiganensis* for the electron acceptors PCE and TCE (Figure 1a). The secondary scenario is a complementary interaction in which *Dsm. michiganensis* dechlorinates PCE to DCE, which is utilized as an electron acceptor by *Dhc. mccartyi* 195 (Figure 1b). The populations do not compete for the electron donor in either scenario because *Dhc. mccartyi* 195 is restricted to the use of H₂, and *Dsm. michiganensis* is unable to utilize H₂ as an electron donor, but can grow on acetate and other organic compounds. Both H₂ and acetate are produced during fermentation of lactate and other organic substrates that are commonly supplied to a contaminant plume during engineered bioremediation, e.g., [45].

2.3. Numerical Model

Previous modeling studies, e.g., [46, 47], indicate good agreement between pore-scale and 2-D continuum models, if the dispersivity values are fitted properly. Thus, a continuum-scale modeling approach was selected to provide the greatest flexibility in modeling systems with different scales. Reactive solute transport through the domain is modeled using the transient form of the 2-D advection-dispersion-reaction (ADR) equation, shown here for degradation of an electron acceptor substrate:

$$\frac{\partial A_i}{\partial t} = \frac{\partial}{\partial x} \left(D_x \frac{\partial A_i}{\partial x} - v_x A_i \right) + \frac{\partial}{\partial y} \left(D_y \frac{\partial A_i}{\partial y} - v_y A_i \right) \pm \left(\frac{\partial A_i}{\partial t} \right)_{Rxn} \quad (1)$$

where A_i is the concentration of the chlorinated ethene of interest [$M_{solute} L^{-3}$], with i = PCE, TCE, DCE, or VC; t is time; D_x and D_y are the hydrodynamic dispersion coefficients in the x - and y -directions, respectively [$L^2 T^{-1}$]; v_x and v_y are the pore-water velocities in the x - and y -directions, respectively [$L T^{-1}$]; and $(\partial A_i / \partial t)_{Rxn}$ is a source/sink term describing the degradation of the

electron acceptor and, in the case of TCE, DCE, and VC, production of the compound via reductive dechlorination [$M_{\text{solute}}L^{-3}T^{-1}$].

In the full model solution, Eq. (1) is coupled with mass-balance equations for the electron donor substrates and biomass. The governing equation for the electron donor is comparable to Eq. (1):

$$\frac{\partial S_i}{\partial t} = \frac{\partial}{\partial x} \left(D_x \frac{\partial S_i}{\partial x} - v_x S_i \right) + \frac{\partial}{\partial y} \left(D_y \frac{\partial S_i}{\partial y} - v_y S_i \right) + \left(\frac{\partial S_i}{\partial t} \right)_{Rxn} \quad (2)$$

where S_i is the electron donor concentration [$M_{\text{solute}}L^{-3}$]; i is H_2 or acetate; and $(\partial S_i / \partial t)_{Rxn}$ is a sink term describing the degradation of the electron donor [$M_{\text{solute}}L^{-3}T^{-1}$].

The electron acceptor and donor source/sink terms are described using a dual Monod expression as follows:

$$R_{utl,h,i} = \frac{dA_i}{dt} = -q_{\max,i} X_h \left(\frac{S_i - S_d}{K_{S,i} + S_i - S_d} \right) \left(\frac{A_i}{K_{A,i} + A_i} \right) \quad (3)$$

$$R_{utl,h,i} = \frac{dS_i}{dt} = -q_{\max,i} f X_h \left(\frac{S_i - S_d}{K_{S,i} + S_i - S_d} \right) \left(\frac{A_i}{K_{A,i} + A_i} \right) \quad (4)$$

where $R_{utl,h,i}$ is the rate of utilization of compound i by population h ; $q_{\max,i}$ is the maximum specific substrate utilization rate [$M_{\text{solute}}M_{\text{biomass}}^{-1}T^{-1}$]; f is the stoichiometric coefficient relating use of the electron donor to the electron acceptor [$M_{\text{donor}}M_{\text{acceptor}}^{-1}$]; X_h is the biomass concentration [$M_{\text{biomass}}L^{-3}$]; $K_{S,i}$ and $K_{A,i}$ are the half-rate concentrations of the electron donor and the electron acceptor, respectively [$M_{\text{solute}}L^{-3}$]; and S_d is electron donor threshold concentration [$M_{\text{solute}}L^{-3}$]. Following the approach of Chu et al. [19], toxicity and inhibition effects are not included in this study.

The governing equations for net biomass growth of *Dhc. mccartyi* 195 and *Dsm. michiganensis*, respectively, are,

$$\frac{dX_{Dhc}}{dt} = Y_{Dhc} (R_{utl,Dhc,PCE} + R_{utl,Dhc,TCE} + R_{utl,Dhc,DCE}) - b_{Dhc} X_{Dhc} \quad (5)$$

$$\frac{dX_{Dsm}}{dt} = Y_{Dsm} (R_{utl,Dsm,PCE} + R_{utl,Dsm,TCE}) - b_{Dsm} X_{Dsm} \quad (6)$$

where Y_h = the true yield coefficient [$M_{biomass} M_{solute}^{-1}$], $R_{utl,h,i}$ is obtained from Eq. (3) for each organism/electron acceptor combination, and b_h is the biomass decay coefficient [T^{-1}].

The hydrodynamic dispersion coefficients in Eqs. (1) and (2) consist of both molecular diffusion and hydrodynamic dispersion, and are defined as follows [48]:

$$D_x = \alpha_T \bar{v} + (\alpha_L - \alpha_T) \frac{\bar{v}_x^2}{\bar{v}} + \tau d_i \quad (7a)$$

$$D_y = \alpha_T \bar{v} + (\alpha_L - \alpha_T) \frac{\bar{v}_y^2}{\bar{v}} + \tau d_i \quad (7b)$$

where: α_l and α_t are the longitudinal and transverse dispersivities, respectively [L]; $\bar{v}$ is the average velocity ($\bar{v} = \sqrt{v_x^2 + v_y^2}$); τ is the tortuosity factor [dimensionless]; and d_i is the molecular diffusion coefficient [$L^2 T^{-1}$].

Eq. (1) coupled with Eq. (3) can be transformed to a non-dimensional form by defining $t^* = t v_x / (L_x)$, $x^* = x / L_x$, $y^* = y / L_y$, $X^* = X / X_0$, $S^* = S_i / S_0$, $A^* = A_i / A_{eq,i}$, $K^*_{S,i} = K_{S,i} / S_0$, $K^*_{A,i} = K_{A,i} / A_{eq,i}$, a longitudinal Peclet number, Pe_l , a transverse Peclet number, Pe_t , and Damköhler No. 2, Da_2 , where L_x and L_y are the NAPL pool lengths in the x- and y-directions, respectively [L], S_0 is the influent electron donor concentration [$M_{solute} L^{-3}$], and $A_{eq,i}$ is the aqueous concentration of component i at thermodynamic equilibrium with the NAPL [$M_{solute} L^{-3}$]:

$$\frac{\partial A^*}{\partial t^*} = \left(\frac{1}{Pe_l} \frac{\partial^2 A^*}{\partial x^{*2}} - \frac{\partial A^*}{\partial x^*} \right) + \left(\frac{1}{Pe_t} \frac{\partial^2 A^*}{\partial y^{*2}} - \frac{\partial A^*}{\partial y^*} \right) - Da_2 X^* \left(\frac{S_i^* - S_d^*}{K_{S_i}^* + S_i^* - S_d^*} \right) \left(\frac{A^*}{K_{A_i}^* + A^*} \right) \quad (8)$$

The definitions and significance of Pe_l , Pe_t , and Da_2 are given in Table 1. Eqs. (2), (5), and (6) are non-dimensionalized in the same manner.

To reduce the computational effort, Strang operator splitting (OS) is applied to separate the biological reaction system of non-linear ordinary differential equations (ODEs) (the last term on the right side of Eqs. 1 and 2) from the linear partial differential equation (PDE) describing advection and dispersion (AD) (the first two terms on the right side of Eqs. (1) and (2)). Strang OS has been shown to be second-order accurate on a global mass balance [49]. The 2-D AD PDE for each solute is solved for each time step using a 2-D finite volume method (FVM) with rectangular control volumes (CVs) and applying the Crank-Nicholson (CN) method for the time derivative [50]. The FVM is locally conservative, making it an appropriate choice when modeling flow with fast reactions, and the CN method is second-order accurate in time. The system of biochemical reaction ODEs is solved using the fifth-order correct Cash-Karp Runge-Kutta 5(4) method [51].

To obtain values of v_x and v_y , flow through the model domain is described using the 2-D, steady-state, isotropic form of the groundwater flow equation and Darcy's Law,

$$0 = -\frac{\partial}{\partial x} \left(K \frac{\partial h}{\partial x} \right) - \frac{\partial}{\partial y} \left(K \frac{\partial h}{\partial y} \right) \quad (9)$$

where K is the hydraulic conductivity [LT^{-1}], and h is the hydraulic head [L]. The 2-D flow profile is assumed to reach steady state quickly compared to transport phenomena, allowing the assumption of steady-state flow. Eq. (9) is also solved using the FVM. A harmonic average was applied to calculate the hydraulic conductivity used for the Darcy flux calculations at each face of the CVs. At the end of each time step, the hydraulic conductivity is recalculated at each

control volume in the domain after the transport and biomass equations have been solved. If the change in conductivity at any location exceeded 0.5% of the value calculated in the previous solution of the flow field equation, then the flow field is recalculated between time steps.

Following the approach of Chu et al. [19], the pore space is modeled as occupied by moving water or attached biomass. The total porosity (n) of the medium is thus defined as,

$$n = n_w + n_b \quad (10)$$

where n_b is the pore space occupied by biomass, and n_w is the pore space occupied by mobile water. A maximum biomass density is specified so that n_b does not exceed n . The phenomenon of biomass formation in a porous medium is complex [52], and there is currently no unifying theory describing its formation and effect on the porous medium. For this work, biomass-induced changes to K are calculated assuming the biomass is in the form of a smooth biofilm, using the relationship between K and n_b defined by Chu et al. [19]:

$$K = \begin{cases} K_0 \left(1 - \frac{n_b}{n}\right)^2 & \text{if } n_b \leq n \left(1 - \sqrt{\frac{K_{\min}}{K_0}}\right) \\ K_{\min} & \text{if } n_b > n \left(1 - \sqrt{\frac{K_{\min}}{K_0}}\right) \end{cases} \quad (11)$$

where K_0 is the initial isotropic hydraulic conductivity, and $K_{\min}$ is the minimum hydraulic conductivity set equal to $(5 \times 10^{-3})(K_0)$. This modeling approach follows the findings of Seagren et al. [26] who observed sparse biofilm colonization of the porous media in their study of bioenhanced NAPL pool dissolution, consistent with the low substrate fluxes from the NAPL pool source [53].

Several model benchmarking steps were conducted. A 1-D form of the model with a constant, first-order reaction rate and no dissolution was tested against the appropriate analytical

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4 solution [54]. The maximum relative error within the domain was less than 0.0001 using an
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6 operator-splitting time step of 28 seconds. The 2-D form of the model with dissolution, but
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8 without biochemical reactions, was compared to a steady-state 2-D analytical solution for abiotic
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10 pool dissolution that neglects longitudinal dispersion [21]. Using a varied grid in which the size
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12 of the CVs increased moving vertically away from the DNAPL pool interface and horizontally
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14 along the interface in the direction of flow, the model matched the analytical solution well and
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16 generally had a relative error of less than 0.01. The most significant discrepancies were at the
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18 upstream end of the plume, where dispersion in the upstream direction was allowed in the
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20 numerical model, but not represented in the analytical solution. The numerical model is
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22 described in more detail in Wesseldyke [55].
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31 **2.4. Model Application**

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33 The model was used to quantify the effects of population interactions on DNAPL
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35 dissolution bioenhancement under the conditions summarized in Table 2. Preliminary sensitivity
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37 analyses with the abiotic model components indicated that model parameters that most
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39 influenced the plume development in the domain were the mass transfer coefficient, flow rate,
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41 and transverse dispersivity. Of these parameters, the flow rate is most easily modified in the
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43 field; therefore, the model simulations focused on varying v_x , which is proportional to the flow
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45 rate. Specifically, simulations were run with initial velocities of $v_x = 0.0234$ m/hr and $v_x = 0.234$
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47 m/hr, which are referred to hereafter as the "low" and "high" v_x cases, respectively.
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53 The scenarios with biological activity differed with respect to the populations of
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55 dehalorespirers that were present. In all cases, each population was assumed to initially be
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57 present at 0.15 mg volatile suspended solids (VSS)/L (where VSS is used to represent biomass),
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consistent with previous studies [14, 38, 56]. Sensitivity analysis of a model of the interactions between these two populations in a continuous-flow, stirred-tank reactor containing aqueous-phase PCE showed that the initial biomass concentration had little impact on the model predictions [38]. The maximum biomass density allowed was set at 8,000 mgVSS/L [19].

In the model, dissolved H_2 and acetate serve as electron donors and are delivered via the influent flow, which is consistent with ongoing experimental work. Relatively low aqueous concentrations of electron donors ($H_2 = 10 \mu M$ and acetate = $8 \mu M$) were provided in the influent for the "intrinsic bioremediation" condition. Relatively high aqueous influent electron donor concentrations ($H_2 = 600 \mu M$ and acetate = $5,000 \mu M$) were provided for the "biostimulation" condition.

The biokinetic parameters used in the simulations are listed in Table 3. The media and contaminant properties used in the simulations include transverse dispersivity, $\alpha_t = 8.03 \mu m$; longitudinal dispersivity, $\alpha_l = 300 \mu m$; porosity, $n = 0.39$; and PCE aqueous diffusion coefficient, $d_i = 9.0 \times 10^{-6} \text{ cm}^2/\text{hr}$ [14]. Details of the methods used to estimate the dispersivities and n are given in the Supplementary Material.

All scenarios were run for 672 hours (28 days). The time to achieve quasi-steady-state conditions varied for different scenarios and ranged from 0.25 hours for the abiotic scenario at the high v_x (Experiment 1b in Table 2) to approximately 672 hours for the biotic scenario involving *Dhc. mccartyi* 195 and *Dsm. michiganensis* under biostimulation conditions and high v_x (Experiment 7b).

4. Results and Discussion

4.1. Parameter Analysis and Abiotic Dissolution Rate

The longitudinal column Peclet number (Pe_l) and transverse column Peclet number (Pe_t) were calculated according to the equations given in Table 1. The initial Pe_l values (without biomass) for PCE are 1.6×10^2 and 1.7×10^2 , under the low and high v_x conditions, respectively, and Pe_t equals 2.0×10^3 and 5.1×10^3 , under the low and high v_x conditions, respectively. These values indicate that hydrodynamic dispersion, especially transverse dispersion, is rate-limiting compared to advection (Table 1). The only other mass-transfer process in the micromodel system is dissolution. In this study, local equilibrium was assumed at the DNAPL-water interface, implying that the dissolution rate is much faster than advective-dispersive and reactive solute sinks in the domain near the interface. Therefore, hydrodynamic dispersion, in particular transverse dispersion, is initially the rate-limiting mass-transfer process for supplying PCE into the system.

As discussed by Seagren et al. [21, 44] for an idealized NAPL pool, the dispersive mass flux, J [$M_s L^{-2} T^{-1}$], across a unit area of the NAPL-aqueous phase interface, a , ($y=0$) can be described using a relationship analogous to Fick's first law, as long as the advective mass flux is only parallel to the NAPL interface [57]:

$$J = -nD_y \left(\frac{\partial A_i}{\partial y} \right)_{y=0} \quad (12)$$

The dissolution rate, R [MT^{-1}] is then given by:

$$R = Ja \quad (13)$$

The abiotic dissolution mass flux away from the interface can be enhanced by increasing D_y and/or the concentration gradient in Eq. (12). Increasing the groundwater flow rate increases the average pore water velocity and D_y (Eq. 7), and the concentration gradient, $\partial A_i / \partial y$, because of the increased advective solute sink term, i.e., A_i , is lowered by flushing out dissolved solute. For

example, under the conditions used in these simulations, the model predicts an abiotic PCE dissolution rate of 3.6×10^{-4} $\mu\text{mol/hr}$ under the low v_x conditions (Experiment 1a; Figure 2) and 2.3×10^{-3} $\mu\text{mol/hr}$ at the high v_x conditions (Experiment 1b). The predicted high v_x value of R is equal to the value calculated using the analytical abiotic dissolution model of Seagren et al. [21], and the predicted low v_x value of R is within 3% of the calculated analytical model value.

Biodegradation can also increase the concentration gradient in Eq. (12) by serving as a reaction sink that decreases A_i . The bioenhancement of the dissolution flux in Eq. (12) can be quantified as the bioenhancement factor, E , which is defined in Eq. (14) as the ratio of the DNAPL source dissolution flux with biodegradation, J_{biotic} , to the dissolution flux without biodegradation, $J_{abiotic}$ [14, 21]:

$$E = \frac{J_{biotic}}{J_{abiotic}} = \frac{R_{biotic}}{R_{abiotic}} \quad (14)$$

As shown in Eq. (14), E can equivalently be defined as the ratio of the DNAPL source dissolution mass rate with biodegradation (R_{biotic}) to the abiotic dissolution mass rate ($R_{abiotic}$).

4.2. Single bacterial population simulations

Several key observations related to dissolution bioenhancement and PCE detoxification can be made based on the simulations of *Dhc. mccartyi* 195 or *Dsm. michiganensis* in pure culture. When *Dsm. michiganensis* (Experiments 2a and 2b) or *Dhc. mccartyi* 195 (Experiments 3a and 3b) is present alone under intrinsic bioremediation conditions at either the low or high v_x , there is little PCE biotransformation, and accordingly, the biotic dissolution fluxes are similar to the abiotic dissolution fluxes, resulting in $E = 1.0$ (Figure 2). The lack of substantial

bioenhancement effects on dissolution rates suggests that the biodegradation kinetics are the rate-limiting process under the intrinsic bioremediation conditions.

This interpretation was verified by following the approach of Johnson et al. [58] for identifying the overall rate-limiting process. As discussed above, based on the Peclet numbers, transverse dispersion is the rate-limiting mass-transfer process for supplying PCE to the system, assuming a local equilibrium at the DNAPL-water interface. The magnitude of the biodegradation rate under intrinsic conditions can then be compared to the magnitude of transverse dispersion by calculating the value of the dimensionless Damköhler No. 6, Da_6 (Table 1). For these conditions, $A_{eq,i}$ was set equal to the PCE solubility concentration, and the biomass concentration was set equal to the initial biomass concentrations. The resulting values of Da_6 generally range between 1 and 5, at the initial low and high v_x conditions, respectively, with the exception of *Dsm. michiganensis* at the low v_x , for which $Da_6 = 9$. Ramaswami and Luthy [59] suggest that dimensionless parameters need to be significantly smaller or larger than unity (e.g., <0.2 or >5) to provide valid insights regarding the rate-limiting phenomenon. Thus, the results here suggest that the biodegradation rates under these conditions are generally similar in magnitude to transverse dispersion for both populations, and we expect biodegradation to have little or no effect on the dissolution rate.

Biodegradation has little impact on dissolution under intrinsic bioremediation conditions because of the low biomass levels near the DNAPL pool source. The levels of *Dhc. mccartyi* 195 and *Dsm. michiganensis* biomass in the domain increased modestly under the intrinsic conditions. The total *Dhc. mccartyi* 195 biomass increased 2.5 and 12.8 times for the low and high v_x cases, respectively. The total *Dsm. michiganensis* biomass increased 4.4 and 24.4 times for the low and high v_x cases, respectively. However, the growth of the biomass occurs at the

margins of the chlorinated ethene plume where the electron donor and acceptor mix (data not shown). This pattern indicates that the biodegradation kinetics of *Dhc. mccartyi* 195 and *Dsm. michiganensis* are electron donor-limited under intrinsic bioremediation conditions because dechlorinating populations grow toward their limiting substrate [19]. Further, electron donors are depleted within the chlorinated ethene plume. Under these low biomass conditions, the electron donor is primarily supplied via advection [19]. The magnitude of the biodegradation rate can be compared to the rate of advection by calculating the value of the dimensionless Damköhler No. 2, Da_2 (Table 1), using $f = 4 \text{ } \mu\text{mole H}_2/\mu\text{mole PCE}$ and $0.5 \text{ } \mu\text{mole acetate}/\mu\text{mole PCE}$, $S_{0,i}$ = feed concentration of H_2 or acetate, and X_0 = the initial biomass concentrations. The resulting values of Da_2 equal 0.9 and 0.3 for *Dhc. mccartyi* 195 and *Dsm. michiganensis*, respectively, at the initial low v_x condition, indicating that the biodegradation rates are similar in magnitude to the rate of supply of electron donor via advection. Da_2 equals 0.09 and 0.03 for *Dhc. mccartyi* 195 and *Dsm. michiganensis*, respectively, at the initial high v_x condition, indicating that biodegradation is the rate-limiting process due to the short contact time. Becker and Seagren [14] obtained similar results in simulations of pure cultures of *Dhc. mccartyi* 195 and *Dsm. michiganensis* in a one-dimensional domain with PCE blobs and intrinsic electron donor levels at low and high v_x .

In comparison, when *Dhc. mccartyi* 195 is grown alone under biostimulation conditions at low v_x (Experiment 5a), there is a relatively rapid increase in total ethenes leaving the system between 100 and 150 hrs (Figure 3a), as the biomass increases (Figure 4a) due to higher electron donor concentrations. The total ethenes efflux rate subsequently plateaus (Figure 3a), although there is a steadily increasing rate of ethene leaving the domain that is not readily visible in Figure 3a. The latter is important because ethene is a non-toxic product of PCE dechlorination and a

desirable bioremediation end-product. In addition, at the end of the 672 hr (28 d) simulation, the total ethenes mass rate of approximately 4.2×10^{-4} $\mu\text{mol/hr}$ represents a small enhancement ($E = 1.2$) of the total dissolution rate observed under abiotic conditions (Figure 2).

Da_6 numbers were calculated for the *Dhc. mccartyi* 195 population alone under biostimulation conditions for the initial v_x , assuming the maximum biomass levels achieved in the domain and setting $A_{eq,i}$ equal to the PCE solubility concentration. Consistent with the bioenhancement results, analysis of the dimensionless parameters indicates that conditions are suitable for bioenhancement because overall the rate is limited by transverse dispersion (i.e., electron acceptor supply), not the biodegradation kinetics, as indicated by $Da_6 = 2.5 \times 10^5 \gg 1$.

The supply of excess electron donor to *Dhc. mccartyi* 195 alone at low v_x also promotes dechlorination of PCE to lesser chlorinated ethenes, including VC and ethene (Figure 2). The positive effects of biostimulation on the extent of PCE dechlorination and dissolution are even more dramatic when *Dsm. michiganensis* populates the domain (Experiment 4a), as can be seen by comparing Figures 3a and 3b. PCE is converted primarily to DCE by *Dsm. michiganensis*. Moreover, bioenhancement of dissolution under biostimulation conditions is almost nine times higher in the presence of *Dsm. michiganensis* ($E = 10.1$) compared with *Dhc. mccartyi* 195 alone ($E = 1.2$), based on the total ethenes efflux rate (Figure 2). Two factors contribute to the greater bioenhancement in the presence of *Dsm. michiganensis*. First, *Dsm. michiganensis* has faster PCE utilization kinetics compared with *Dhc. mccartyi* 195 (Table 3). Rapid depletion of PCE in the aqueous phase creates a strong driving force for dissolution from the DNAPL phase (Eq. 6). Second, unlike the *Dhc. mccartyi* 195 biomass, which grows primarily in an arc extending away from the PCE plume with increasing distance from the inlet (Figure 4a), *Dsm. michiganensis*' growth is concentrated along the DNAPL water interface where it can directly impact PCE

dissolution (Figure 4b). As noted above, previous work showed that dechlorinating populations grow toward the limiting substrate [19]. Thus, the rapid depletion of aqueous-phase PCE (Figure 3b) and growth of *Dsm. michiganensis* along the interface clearly show that *Dsm. michiganensis* is limited by the availability of PCE under the biostimulation conditions. It should be noted that the limiting substrate for given population is determined, in part, by the stoichiometry of the dechlorination reaction. As discussed below in Section 4.3, fewer electron equivalents are required to reduce PCE to DCE (the transformation carried about by *Dsm. michiganensis*) than to reduce PCE to ethene (the transformation carried out by *Dhc. mccartyi* 195). Thus, compared to *Dhc. mccartyi* 195, *Dsm. michiganensis* is less likely to be limited by the electron donor.

In contrast, under the low v_x conditions with intrinsic electron donor levels, *Dsm. michiganensis* grows along the margins of the chlorinated ethene plume, suggesting it is limited by acetate (data not shown), as discussed above. Similarly, the pattern of *Dhc. mccartyi* 195 growing away from the DNAPL-water interface and high electron acceptor concentrations exiting the domain when *Dhc. mccartyi* 195 is the sole population (Figure 3a) suggest that this system was limited by H_2 under both the biostimulation and intrinsic low v_x conditions.

The results for *Dhc. mccartyi* 195 and *Dsm. michiganensis* in pure culture under biostimulation, high v_x conditions are similar to those described above under the biostimulation, low v_x conditions, but with a greater total dissolution rate due to the faster velocity. However, there are some interesting differences between the two organisms. In the case of *Dsm. michiganensis*, good potential for bioenhancement is predicted ($Da_6 = 1.2 \times 10^5 \gg 1$, assuming the maximum biomass level and $A_{eq,i}$ equal to the PCE solubility), acetate is available throughout the domain, and the biomass is growing right at the NAPL interface (Figure 5b), However, bioenhancement is reduced ($E = 2.43$) compared to the low v_x case ($E = 10.1$), because the

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4 biodegradation rate is smaller relative to the flow rate. This is reflected by the decrease in Da_2
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6 for the electron donor from 21.5 at the low v_x , to $Da_2=2.2$ for the high v_x case, assuming the
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8 maximum biomass level and S_0 equal to the input concentration. In comparison, Da_6 for *Dhc.*
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10 *mccartyi* 195 is $6.6 \times 10^4 \gg 1$, assuming the maximum biomass level and $A_{eq,i}$ equal to the PCE
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12 solubility. Under these conditions, $E = 1.2$, which is the same as the enhancement factor under
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14 the low v_x case. This is because as the velocity increases, the impacts of the reduced contact time
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16 and lower chlorinated ethenes concentrations are counteracted by the development of a
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18 chlorinated ethene plume and growth of *Dhc. mccartyi* 195 closer to the pool relative to the low
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20 v_x case (Figure 5a).
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29 **4.3. Competition simulations**

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31 When *Dsm. michiganensis* competes with *Dhc. mccartyi* 195 under intrinsic
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33 bioremediation conditions (Experiments 6a and 6b), there is little biotransformation of the PCE,
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35 and accordingly, minimal bioenhancement at low and high v_x (data not shown). The lack of
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37 significant bioenhancement follows the trends observed for the individual populations under
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39 intrinsic bioremediation conditions. As discussed above, this finding is consistent with the
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41 calculated Da_6 values. These values showed that biodegradation is the overall rate-limiting
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43 process for both populations, or is similar in magnitude to transverse dispersion, the rate-limiting
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45 mass transfer process for supply of PCE.
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51 The co-culture of dehalorespirers exhibits faster biomass growth and biotransformation
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53 rates when higher electron donor concentrations are supplied, as expected. As a result, the
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55 impacts on dissolution and detoxification are much more substantial under biostimulation
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57 conditions. The mass rates of the ethenes exiting the domain for the low v_x condition (Scenario
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7a) are shown in Figure 3c. Several important findings emerge by comparing the results in Figure 3c to those obtained with *Dsm. michiganensis* (Figure 3b) or *Dhc. mccartyi* 195 (Figure 3a) alone. For example, the total ethene mass rate exiting the domain populated by *Dsm. michiganensis* alone is approximately 3.7×10^{-3} $\mu\text{mol/hr}$ (Figure 3b) and increases only to 4.0×10^{-3} $\mu\text{mol/hr}$ ($E = 11$) when the domain is also populated by *Dhc. mccartyi* 195, which alone achieves a total ethene mass rate of approximately 4.3×10^{-4} $\mu\text{mol/hr}$. These results suggest that the roughly ten-fold increase in dissolution resulting from the rapid utilization of aqueous-phase PCE by *Dsm. michiganensis* is modestly enhanced by the activity of *Dhc. mccartyi* 195.

The biomass concentration plots in Figures 7c and 7d clearly show why *Dsm. michiganensis*, rather than *Dhc. mccartyi* 195, controls the PCE dissolution in the co-culture. *Dsm. michiganensis* is not limited by electron donor availability and thus grows along the DNAPL-water interface where its rapid utilization of aqueous-phase PCE creates a strong driving force for dissolution. While several early studies suggested that the toxicity PCE concentrations might limit the activity of dehalorespiring populations close to DNAPL source zones, Huang and Becker [60] demonstrated that two dehalorespiring populations (including *Dsm. michiganensis*) are able to dehalorespire PCE at aqueous concentrations approaching the solubility limit, provided the initial biomass concentration is sufficiently high. Therefore, growth of dehalorespiring bacteria near the DNAPL-water interface where they can impact dissolution is feasible.

In contrast, the arc of *Dhc. mccartyi* 195 biomass extends further away from the DNAPL source in the transverse direction in the co-culture (Figure 4c), compared with its biomass distribution in pure culture (Figure 4a). The modest impact of *Dhc. mccartyi* 195 on dissolution can be attributed to the narrow region closest to the inlet where H_2 is not yet limiting and thus

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4 *Dhc. mccartyi* 195 can grow adjacent to the DNAPL-water interface. In bioremediation practice,
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6 H₂ (and acetate) are often delivered to the subsurface via the fermentation of simple or complex
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8 organic substrates. This could potentially improve the ability of *Dhc. mccartyi* 195 to obtain H₂
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10 and grow closer to the NAPL-water interface and thus effect greater bioenhancement of PCE
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12 dissolution. However, evaluation of the impact of fermentable substrates on bioenhancement of
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14 DNAPL dissolution was beyond the scope of this study.
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19 The shift in biomass location and simulation results for dechlorination (Figure 6),
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21 indicate that *Dhc. mccartyi* 195 is growing predominantly on DCE produced via dechlorination
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23 of PCE by *Dsm. michiganensis* rather than the PCE dissolving from the DNAPL source, which
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25 occurs in the pure culture experiment. Thus, by out-competing *Dhc. mccartyi* 195 for the PCE
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27 dissolving from the DNAPL source (Figure 1a), *Dsm. michiganensis* causes a switch to
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29 complementary substrate interactions with *Dhc. mccartyi* 195 (Figure 1b).
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34 Becker and Seagren [14] observed similar spatial trends with DNAPL blobs in a 1-D
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36 cells-in-series model. In that model, *Dhc. mccartyi* 195 eventually outcompeted *Dsm.*
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38 *michiganensis* for chlorinated ethenes in the first cell, and its activity was controlled by the H₂
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40 input into the domain. *Dsm. michiganensis* dominated in the second cell, due to the availability
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42 of acetate, which was not utilized by *Dhc. mccartyi* 195. Importantly, in Becker and Seagren's
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44 [14] model, the DNAPL blobs were originally distributed throughout the domain, allowing
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46 growth of both populations throughout the domain. In the current study, the DNAPL source is
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48 present on only one side of the domain. Although both populations are initially distributed
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50 uniformly throughout the domain, the ability of *Dsm. michiganensis* to use acetate as an electron
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52 donor in regions where H₂ levels were depleted, coupled with its faster kinetics, resulted in *Dsm.*
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54 *michiganensis* being the population that grew nearest the DNAPL source and the population
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4 primarily responsible for bioenhancement of DNAPL dissolution. The inclusion of multiple
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6 dimensions in the modeling of the competing populations may also explain why the levels of
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8 bioenhancement predicted in this work are closer than those of Becker and Seagren [14] to the
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10 levels previously observed in laboratory column and tank studies at relatively slow average pore
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12 water velocities with mixed microbial cultures ($E = 2$ to 13), e.g., [24, 25, 31-33].
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16 Although the increase in the dissolution bioenhancement with the co-culture under
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18 biostimulation and low v_x conditions is relatively modest, these results show that having multiple
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20 dehalorespirers that utilize different electron donors can greatly enhance the extent of PCE
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22 transformation compared with a single dehalorespiring population, as previously suggested [14,
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24 38]. For example, PCE and VC are the predominant chlorinated ethenes exiting the domain at
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26 steady-state when it is populated by *Dhc. mccartyi* 195 alone (Figure 3a), while DCE represents
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28 nearly 100% of the chlorinated ethenes exiting the domain populated by *Dsm. michiganensis*
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30 alone (Figure 3b). DCE is also the dominant chlorinated ethene produced by the co-culture under
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32 the biostimulation and low v_x conditions; however, a substantial amount of DCE is transformed
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34 further to VC (Figure 3c). Thus, the extent of PCE transformation is greater compared to what
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36 can be achieved by either population alone. These observations can be explained as follows.
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38 Growing alone, *Dhc. mccartyi* 195 has to invest 3 mol H_2 /mol PCE to synthesize VC. The
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40 investment of H_2 into the dechlorination of the higher chlorinated ethenes diverts H_2 away from
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42 VC and ethene production. The limited availability of H_2 also restricts the total amount of
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44 dechlorination that can be achieved by *Dhc. mccartyi* 195 alone and explains why the PCE
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46 concentration remains relatively high in Experiment 3a. In contrast, when grown in the co-
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48 culture, *Dsm. michiganensis* reductively dechlorinates PCE to DCE using electron equivalents
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50 derived from acetate. Therefore, *Dhc. mccartyi* 195 focuses the investment of electron
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equivalents derived from H₂ in the dechlorination of the lesser chlorinated ethenes, and greater transformation of PCE to VC is observed.

The co-culture simulations also highlight the impact of hydrodynamic conditions on the outcome of the population interactions. Most importantly, v_x has a major impact on the relative abundance of each population (Figure 7) and, consequently, on dissolution bioenhancement and plume detoxification. Under the low v_x (Experiment 7a) conditions, *Dsm. michiganensis* is initially dominant due to its kinetic competitive advantage, which allows it to utilize nearly all of the dissolved PCE and TCE. One way to characterize the competitiveness of bacterial populations is to compare their $q_{\max}/K_S$ ratios [61]. A high $q_{\max}/K_S$ ratio indicates that utilization of the limiting substrate by a population approaches the maximum rate at relatively low substrate concentrations. Populations with relatively low $q_{\max}/K_S$ ratios do not approach optimal kinetics unless high substrate concentrations are present. The $q_{\max}/K_S$ ratios for *Dsm. michiganensis* growing on PCE and TCE are 1.4 and 6.0, respectively, compared to $q_{\max}/K_S$ ratios of 0.32 and 0.27 for *Dhc. mccartyi* 195 growing on PCE and TCE, respectively (Table 3). As a result of the ability of *Dsm. michiganensis* to establish itself near the DNAPL-water interface, the bioenhancement factor, E is approximately 9.7 by 200 hours (similar to that achieved with *Dsm. michiganensis* alone), and the *Dsm. michiganensis* biomass is nearly twice that of the *Dhc. mccartyi* 195 biomass at 672 hours (28 days; Figure 7a). Although the *Dhc. mccartyi* 195 biomass is relatively small, it still has an impact on detoxification and, to a lesser extent, on bioenhancement (as discussed above), because *Dhc. mccartyi* 195 continues to grow primarily on DCE produced by *Dsm. michiganensis* (Figure 6c). Due to the activity of *Dhc. mccartyi* 195, VC levels also slowly decline (Figure 6d) and ethene levels gradually increase up to a maximum of 1.3% of the total ethenes mass flow rate by the end of the simulation.

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4 In comparison, under the high v_x conditions (Experiment 7b), the general patterns are
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6 similar to the low v_x case (Figure 3d), but there is a switch in the dominant population. *Dsm.*
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8 *michiganensis* is again initially dominant; however, *Dhc. mccartyi* 195 becomes the more
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10 abundant population over time. In fact, the *Dhc. mccartyi* 195 biomass is nearly twice the mass
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12 of the *Dsm. michiganensis* biomass by the end of the simulation (Figure 7b). Several physical,
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14 chemical, and biological factors contribute to this population shift.
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19 One, the increased velocity results in the plume of chlorinated ethenes being tighter to the
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21 PCE source compared to the low v_x case, due to the increased dominance of advection over
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23 dispersion. This results in the microbial biomass, in particular *Dhc. mccartyi* 195, growing closer
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25 to the PCE source in the high v_x case (Figure 5c) compared to the low v_x case (Figure 4c).
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29 Two, the increased mass rate of supply of the electron donors H_2 and acetate due to the
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31 faster influent flow, and the increased mass rate of supply of PCE due to dissolution (and of TCE
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33 and DCE produced by *Dsm. michiganensis*) leads to greater biomass growth. In particular, *Dhc.*
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35 *mccartyi* 195 grows well under these conditions due to its relatively large Y (Table 3). In
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37 addition, after initially growing primarily on the DCE produced by *Dsm. michiganensis*, as its
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39 biomass increases, the *Dhc. mccartyi* 195 population begins to successfully compete for and
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41 grow on a larger fraction of PCE and TCE. This growth of *Dhc. mccartyi* 195 occurs in a narrow
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43 region closest to the inlet where H_2 is not yet limiting and at the expense of *Dsm. michiganensis*.
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48 Three, the accumulation of *Dhc. mccartyi* 195 biomass at the inlet where it starts to form
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50 a parabolic boundary, and of *Dsm. michiganensis* biomass along the DNAPL/water interface,
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52 impacted the hydrodynamics in the domain (Figure 5c). Specifically, the biomass accumulation
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54 in those regions impacted the hydraulic head (Figure 8e) and relative porosity (Figure 8f) and
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56 resulted in a reduction in the hydraulic conductivity and average pore water velocity (Figure 8d).
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4 However, although biomass reached its maximum of 8,000 mg VSS/L near the inlet and along
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6 the DNAPL/water interface, due to the assumptions inherent in the model for the relationship
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8 between hydraulic conductivity and biomass concentration (Equation 11), the velocity in either
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10 direction was not reduced to zero. In addition to the zones of reduced pore water velocity, pore
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12 water velocities were increased where the flow is deflected around the biomass accumulation
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14 zone (Figure 8d). The changes in the average pore water velocity after the biomass developed in
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16 turn affected the hydrodynamic dispersion in the y - (transverse) direction, which was the rate
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18 limiting mass-transfer process in the system, as discussed above. In the case of *Dhc. mccartyi*
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20 195, the supply of H_2 occurs mainly via transverse dispersion and diffusion, as does the supply of
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22 DCE produced via dechlorination of PCE and TCE by *Dsm. michiganensis* [19]. Nevertheless,
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24 the accumulation of biomass did not negatively impact the supply of H_2 to *Dhc. mccartyi* 195
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26 very much because the deflected flow is still moving past the biomass. In fact, the increased pore
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28 water velocity due to the deflected flow may have actually increased the supply of electron donor
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30 by dispersion in some regions. As a result, its biomass continued to increase over time (Figure
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32 7b), and the mass rate of removal of H_2 in co-culture is very similar to that when *Dhc.*
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34 *mccartyi* 195 is growing alone (Experiment 5b), with the H_2 depleted within the chlorinated
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36 ethene plume.
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46 On the other hand, the decrease in advection and transverse dispersion due to the biomass
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48 accumulation did reduce the supply of electron donor and acceptor to *Dsm. michiganensis*. For
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50 example, the mass rate of removal of acetate in the domain peaked at around 160 hours at about
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52 the same rate as in the scenario with *Dsm. michiganensis* alone (Experiment 4b), but
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54 subsequently decreased and this trend continued throughout the remainder of the simulation.
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56 *Dsm. michiganensis* thus experienced net decay after about 200 hours and *Dhc. mccartyi* 195
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4 became the dominant population (based on the total mass of biomass throughout the domain)
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6 (Figure 7b). The shift in dominance to *Dhc. mccartyi* 195 results not only in an increase in the
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8 bioenhancement effect, but also a shift in the chlorinated ethenes exiting the domain compared to
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10 the low v_x case. Specifically, VC and ethene make up a greater fraction of the total ethenes mass
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12 flow under the high v_x condition.
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17 The trends are similar in the low v_x scenario, but the effects are less significant because of
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19 the smaller biomass accumulation (Figures 4c and d). The velocities are still significantly
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21 reduced near the inlet and along the DNAPL/water interface (Figure 8a); however, outside of
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23 those high biomass zones, the impact of the biomass on the hydraulic head (Figure 8b) and
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25 porosity (Figure 8c) was relatively small. As a result, the velocity rebounded to the initial value,
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27 or close to it, throughout most of the domain (Figure 8a). Nonetheless, the deflected flow did
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29 reduce the utilization of acetate by *Dsm. michiganensis* over time, resulting in the *Dhc.*
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31 *michiganensis* biomass declining slightly after peaking at about 260 hours (Figure 7a). In
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33 comparison, the mass rate of utilization of H_2 in the domain by *Dhc. mccartyi* 195 was actually
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35 slightly higher in the co-culture case, possibly due to the enhanced dispersion resulting from the
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37 increased velocity of the deflected flow. These results highlight the impact of the complex
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39 interactions between microbial ecology, biokinetics, electron donor supply, and hydrodynamics
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41 on DNAPL plume detoxification and dissolution bioenhancement.
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51 **5. Conclusions**

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53 Although there are many potential interactions between microbial populations in the
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55 field, these modeling results illustrate some of the complexities involved in understanding the
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57 outcome of microbial competition for electron acceptor substrates at sites where chlorinated
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ethene DNAPL source zones are present. Clearly the outcome of competition and the resulting effects on plume detoxification and dissolution bioenhancement are a function of not only the populations' affinities for the substrates, as represented by q_{max}/K , but also the relative magnitude of Y , the rate of supply of electron donor, and hydrodynamic conditions. Consistent with the conclusions of Becker and Seagren [14], these results suggest that biostimulation and bioaugmentation strategies for DNAPL source zones should focus on increasing the abundance of not only *Dehalococcoides* species, but also heterotrophic PCE-respiring populations like *Dsm. michiganensis*. As pointed out by Becker [38], organisms like *Dsm. michiganensis* that specialize in the dechlorination of PCE to DCE (or TCE), are beneficial because of their faster kinetics compared to *Dehalococcoides* strains. An important implication of the ability of heterotrophic PCE-respiring strains to use acetate as an electron donor in regions where H_2 levels are depleted, coupled with their faster dechlorination kinetics, that arises from this work is that organisms like *Dsm. michiganensis*, may be the populations primarily responsible for bioenhancement of DNAPL dissolution. Nevertheless, *Dehalococcoides mccartyi* strains are critical to achieving detoxification of dissolved chlorinated ethene plumes, and may become the dominant population under some conditions.

6. Acknowledgements

This research was supported by the National Science Foundation under Grant No. 1034700. A portion of the research was performed using EMSL, a national scientific user facility sponsored by the Department of Energy's Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory.

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Table 1. Definition and significance of the dimensionless numbers

	Symbol	Definition ^a	Significance with respect to rate-limiting process ^b	
			Dimensionless number << 1	Dimensionless number >> 1
Dimensionless numbers comparing mass transfer rates	Pe_l (Longitudinal Peclet No.)	$= \frac{\text{Advection Rate}}{\text{Longitudinal Dispersion Rate}}$ $= \frac{v_l L_x}{D_l}$	advection is slower than longitudinal dispersion	Longitudinal dispersion is slower than advection
	Pe_t (Transverse Peclet No.)	$= \frac{\text{Advection Rate}}{\text{Transverse Dispersion Rate}}$ $= \frac{v_t L_x}{D_t}$	advection is slower than transverse dispersion	Transverse dispersion is slower than advection
Dimensionless numbers comparing biodegradation and mass transfer rates	Da_2 (Damköhler No. 2)	$= \frac{\text{Biodegradation Rate}}{\text{Advection Rate}}$ $= \frac{q_{\max} X_0 L_x}{C_i v_x}$	biodegradation is slower than advection (biokinetics limit)	slow advection of substrate limits biodegradation
	Da_6 (Damköhler No. 6)	$= \frac{\text{Biodegradation Rate}}{\text{Dispersion Rate}}$ $= \frac{q_{\max} X_0 L_x^2}{D_i C_i}$	biodegradation is slower than dispersion (biokinetics limit)	slow dispersion of substrate limits biodegradation

^a $C_i = A_{eq,i}$ or $S_{0,i}$.

^b Following the convention of Ramaswami and Luthy [59], a dimensionless number is considered <1 if it is 0.2 or lower and >1 if it is 5 or higher.

Table 2. Conditions used in simulations.

Experiment	Scenario	Experimental Conditions		
		Initial Pore Water Velocity, v_x , (m/h)	Electron donor(s)	Population(s) Present
1a	Abiotic	0.0234	None	None
b		0.234		
2a	<i>Dsm.</i> Pure Culture, Intrinsic	0.0234	8 μ M Acetate	<i>Dsm. michiganensis</i>
b		0.234		
3a	<i>Dhc.</i> Pure Culture, Intrinsic	0.0234	10 μ M H ₂	<i>Dhc. mccartyi</i> 195
b		0.234		
4a	<i>Dsm.</i> Pure Culture, Biostimulation	0.0234	5 mM Acetate	<i>Dsm. michiganensis</i>
b		0.234		
5a	<i>Dhc.</i> Pure Culture, Biostimulation	0.0234	600 μ M H ₂	<i>Dhc. mccartyi</i> 195
b		0.234		
6a	Scenario 1, Intrinsic	0.0234	8 μ M Acetate + 10 μ M H ₂	<i>Dsm. michiganensis</i> + <i>Dhc. mccartyi</i> 195
b		0.234		
7a	Scenario 1, Biostimulation	0.0234	5 mM Acetate + 600 μ M H ₂	<i>Dsm. michiganensis</i> + <i>Dhc. mccartyi</i> 195
b		0.234		

Table 3. Biokinetic parameter values for microbial populations used in simulations.

Constant	<i>Dhc. mccartyi</i> 195 ^a	<i>Dsm. michiganensis</i> ^b
q_{max} (μmol/mg VSS/h)		
PCE	6.8	12.6
TCE	7.9	17.0
DCE	13.2	NA ^c
VC	0.9	NA
$K_{S,donor}$ (μM)	0.03	5.8
$K_{S,chloroethene}$ (μM)		
PCE	21.5	9.31
TCE	29.0	2.83
DCE	33.6	NA
VC	637	NA
Y (mg VSS/μmol donor)	0.0047	0.0033
k_d (h ⁻¹)	0.004	0.0054
H ₂ threshold (μM)	0.00187	NA

^a [62, 63]

^b [64]

^c Not applicable.

Figure Captions

Figure 1. Potential interactions between the chlorinate ethene-respiring populations (*Dsm. michiganensis* and *Dhc. mccartyi* 195) evaluated in this project: (a) Competition between the two populations for PCE, and (b) Complementary interaction between the two populations in which *Dsm. michiganensis* dechlorinates PCE to DCE, which is respired by *Dhc. mccartyi* 195.

Figure 2. Mass rate of ethene and chlorinated ethenes leaving the simulation domain under abiotic, low v_x conditions and with a pure culture of either *Dhc. mccartyi* 195 or *Dsm. michiganensis* present under intrinsic bioremediation (10 μM H_2) and biostimulation (600 μM H_2) conditions.

Figure 3. Mass rate of ethene and chlorinated ethenes exiting the domain for: (a) Experiment 5a with *Dhc. mccartyi* 195 under biostimulation conditions and low v_x ; (b) Experiment 4a with *Dsm. michiganensis* under biostimulation conditions and low v_x ; (c) Experiment 7a with *Dhc. mccartyi* 195 and *Dsm. michiganensis* under biostimulation conditions and low v_x ; and (d) Experiment 7b with *Dhc. mccartyi* 195 and *Dsm. michiganensis* under biostimulation conditions and high v_x .

Figure 4. Biomass concentrations at 14 d under low v_x , biostimulation conditions for (a) *Dhc. mccartyi* 195 in pure culture (Experiment 5a); (b) *Dsm. michiganensis* in pure culture, (Experiment 4a); (c) *Dhc. mccartyi* 195 in co-culture with *Dsm. michiganensis* (Experiment 7a); and (d) *Dsm. michiganensis* in co-culture with *Dhc. mccartyi* 195 (Experiment 7a). The portion of the domain shown is truncated to the lower 0.7 cm because only this region contained significant biomass.

Figure 5. Biomass concentration at 14 d under high v_x , biostimulation conditions for (a) *Dhc. mccartyi* 195 in pure culture (Experiment 5b); (b) *Dsm. michiganensis* in pure culture (Experiment 4b); (c) *Dhc. mccartyi* 195 in co-culture with *Dsm. michiganensis* (Experiment 7b); and (d) *Dsm. michiganensis* in co-culture with *Dhc. mccartyi* 195 (Experiment 7b). The portion of the domain shown is truncated to the lower 0.7 cm because only this region contained significant biomass.

Figure 6. Dechlorination maps for the low v_x biostimulation conditions with *Dhc. mccartyi* 195 and *Dsm. michiganensis* in co-culture (Experiment 7a): (a) PCE dechlorination rate by *Dhc. mccartyi* 195; (b) TCE dechlorination rate by *Dhc. mccartyi* 195; (c) DCE dechlorination rate by *Dhc. mccartyi* 195; (d) VC dechlorination rate by *Dhc. mccartyi* 195; (e) PCE dechlorination rate by *Dsm. michiganensis*; and (f) TCE dechlorination rate by *Dsm. michiganensis*.

Figure 7. Total *Dhc. mccartyi* 195 and *Dsm. michiganensis* biomass (mg VSS) in the domain for (a) Experiment 7a under low v_x , biostimulation conditions; and (b) Experiment 7b under high v_x , biostimulation conditions.

Figure 8. Hydrodynamic effects due to accumulation of *Dhc. mccartyi* 195 and *Dsm. michiganensis* biomass in a co-culture: (a) and (d) v_x , (b) and (e) hydraulic head, and (c) and (f) relative porosity $(n - n_b/n)$. Results for the low v_x biostimulation conditions are shown in (a)-(c). The high v_x biostimulation condition results are given in (d)-(f).

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