

Model Simulations in Support of Field Scale Design and Operation of Bioremediation Based on Cometabolic Degradation

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Abstract

This paper addresses questions fundamental to the design and operation of aquifer bioremediation based on cometabolic degradation. A model of a full-scale, in situ system for bioremediation of chlorinated ethenes relying on cometabolic degradation was developed and applied to a hypothetical aquifer being considered for a large-scale field demonstration of in situ bioremediation with recirculation. The model was used to identify feasible substrate (electron donor and electron acceptor) delivery schedules. Trichloroethylene (TCE) was the target contaminant. Methane and phenol were considered as electron donors. The delivery of the electron donors and the electron acceptor, oxygen, was varied to evaluate the rate and extent of bioremediation under different substrate delivery schedules. Maximum removal of TCE was predicted when substrates are delivered at ratios near the stoichiometric requirement of electron donor and acceptor for net microbial growth.

Additionally, the decrease in TCE removal that results from using substrate delivery schedules other than those achieving the maximum removal of TCE was quantified. This decrease was greater for the methane-oxygen system because the two gaseous substrates compete for transfer into the recirculated ground water. If one substrate is introduced in excess of the amount required for net microbial growth, it accumulates, thus limiting the ability to introduce the second substrate. This imbalance both limits the introduction of the second substrate and accelerates the accumulation of the substrate added in excess. The phenol-oxygen system is less sensitive to deviation away from the best observed substrate delivery schedule because phenol is a relatively soluble liquid and its introduction does not compete with the mass transfer of oxygen.

Introduction

Remediation of contaminated aquifers continues to be a major focus of regulatory activity and an area of scientific and technical challenge for environmental professionals. Improper storage and waste management practices have left a legacy of contaminated soil and aquifers, threatening drinking water supplies in many areas (Fetter, 1993). Chlorinated ethenes, particularly trichloroethylene (TCE), are among the most frequently detected ground-water contaminants (Westrick et al., 1984; Barbash and Roberts, 1986). In addition to being a suspected carcinogen (Fan, 1988), TCE has been shown to undergo reductive dechlorination to vinyl chloride (VC), a known human carcinogen, under anaerobic conditions commonly observed in aquifers (Vogel and McCarty, 1985). Ground-water remediation for chlorinated ethene contamination is usually attempted with pump-and-treat systems that extract contaminated ground water for treatment aboveground. Treatment historically has comprised some combination of air stripping, adsorption onto activated carbon, thermal destruction, or biological degradation. In situ bioremediation is a possible alternative or enhancement to pump-and-treat remediation (National Research Council, 1994).

Aerobic microorganisms transform chlorinated ethenes through cometabolic degradation, i.e., fortuitous degradation of a compound by nonspecific enzymes (oxygenases) that the microorganisms produce to metabolize their primary electron donor. Laboratory investigations (Wilson and Wilson, 1985;

Fogel et al., 1986; Nelson et al., 1986, 1988; Little et al., 1988; Fox et al., 1990; Henry and Grbić-Galić, 1990; Folsom et al., 1990; Hopkins et al., 1993; Wackett et al., 1989; Fan and Scow, 1993) have identified a number of aerobic microorganisms capable of transforming chlorinated ethenes via pathways that do not produce VC. Cometabolic transformation of chlorinated ethenes is an aerobic process, requiring the addition of both oxygen as the electron acceptor and an appropriate electron donor. Methane has been shown to be an effective electron donor for the cometabolic transformation of chlorinated ethenes in both the laboratory (Wilson and Wilson, 1985; Fogel et al., 1986; Little et al., 1988; Fox et al., 1990; Henry and Grbić-Galić, 1990) and the field (Roberts et al., 1990; Semprini et al., 1990). Additional effective electron donors include phenol, toluene, propane, and others (Nelson et al., 1986; 1988; Wackett et al., 1989; Folsom et al., 1990; Hopkins et al., 1993; Fan and Scow, 1993; McCarty and Hopkins, 1995). Methane, phenol, and toluene have been demonstrated to effectively promote cometabolic transformation of chlorinated ethenes in pilot-scale field experiments, with phenol and toluene exhibiting more efficient TCE degradation than methane (Semprini et al., 1990; Hopkins et al., 1993; Hopkins and McCarty, 1995).

In situ bioremediation of a contaminant plume requires that the key components for cometabolic transformation—microorganisms, oxygen, methane or phenol, and the target contaminant—be present at the same place and time. To guarantee full mixing of these ingredients, the pumping scenario assumed in these simulations is a two-well, fully recirculating system (Figure 1). The electron acceptor and donor are introduced in alternating pulses to minimize the presence of both at the injection point and thus minimize excess microbial growth and potential clogging at the injection well (Roberts et al., 1990). Pulsed introduction of electron acceptor and donor (collectively referred to as substrates) results in regions of the aquifer experiencing transient

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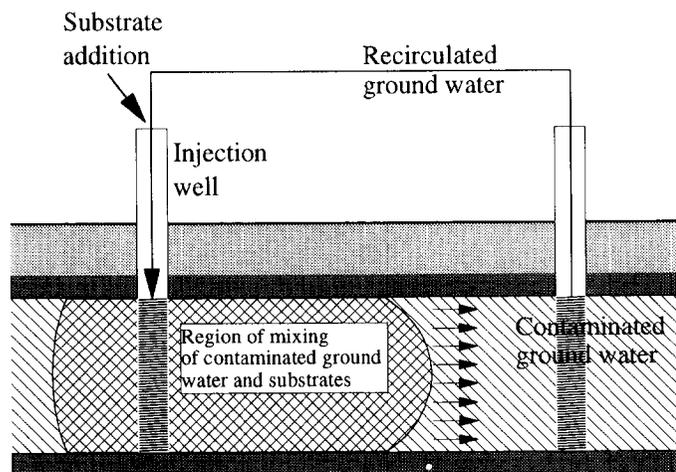


Fig. 1. Substrate injection with recirculation for mixing.

periods in which both, none, or only one substrate is present. Table 1 summarizes the processes assumed active in the simulation model under the four possible conditions of donor and acceptor presence or absence (Semprini and McCarty, 1991; 1992). The assumption of no microbial death in the absence of oxygen follows from laboratory and field evidence that inactive but viable populations are retained for significant time in the absence of oxygen (Alvarez-Cohen and McCarty, 1991; Roslev and King, 1994; Semprini and McCarty, 1991). Note also that contaminant transformation occurs only in the presence of the electron acceptor, oxygen.

This paper describes simulations performed in support of field scale design and operation of in situ bioremediation systems for remediation of aquifers contaminated by chlorinated ethenes, primarily trichloroethylene (TCE). The simulations were designed to address two objectives. First, field and laboratory experiments have not yet been conducted over the full range of feasible substrate delivery combinations. Assuming that observed microbial growth and contaminant degradation kinetics are representative of conditions at a new site, the simulations described here evaluate a broad range of substrate delivery schedules to identify promising operating conditions which have not yet have been tried. The second objective was to apply previously observed values of model parameters to stimulate bioremediation at the scale of plumes in the real world.

In addition to suggesting new criteria for programs to deliver the electron donor and substrate, this study compares system performance for two different electron donors, methane and phenol. This comparison evaluates not only the effects of different microbial growth and degradation kinetics but also the effects of delivery characteristics by comparing system behavior for a very soluble, easily introduced substrate, phenol, to that for a less soluble substrate, methane. Laboratory and pilot field scale experience with both of these substrates has shown that phenol

promotes more rapid TCE transformation than methane (Hopkins et al., 1993; McCarty and Hopkins, 1995). However, because phenol is itself a listed contaminant, its introduction as a substrate to promote in situ bioremediation will require adequate evidence of its behavior and performance. Simulations such as those presented herein aid in identifying substrate delivery schedules that minimize the phenol necessary for TCE destruction while preventing phenol from escaping from the treatment zone.

Methods

Modeling in situ bioremediation requires accurate simulation of (1) the flow regime created by the pumping, (2) the contaminant, electron acceptor, and electron donor fate and transport, and (3) the microbial growth. In this paper, we apply two models to simulate the steady-state flow and the nonsteady-state solute transport and the biodegradation separately. The two-dimensional, semianalytical flow model, RESSQ (Javandel et al., 1984) was used to determine the flow field established by the pumping wells. RESSQ simulates one- and two-dimensional steady-state flow in a confined homogeneous aquifer with multiple injection and extraction wells. The aquifer characteristics assumed for the simulations are representative of sites that were being considered for full-scale application of in situ bioremediation at the time of this work. The aquifer is assumed to be a relatively homogeneous, fine sand aquifer with a hydraulic conductivity of 10^{-4} m/s and porosity of 0.30. Of the sites under consideration, a full-scale system is currently being installed at Edwards Air Force Base (Goltz et al., 1995).

The well configuration assumed for these analyses is a screened interval of 2 m with a 10 m separation between the injection and extraction well. The injection and extraction rate was 10 gpm for all of the simulations. The gas phase substrates (methane and oxygen) are introduced into the subsurface using a down-well venturi device (Bae et al., 1995), whereas the phenol is introduced as a solution injected directly into the recirculating ground water. The output from RESSQ is a two-dimensional representation of the streamlines of equivalent flux established by the pumping. These streamlines define stream tubes along each of which a one-dimensional simulation of the bioremediation is implemented. Figure 2 is a plan view of the 2D cross section of the stream tubes describing the flow system. Each stream tube accounts for five percent of the total flow through the system. Residence times in the stream tubes range from approximately six days for the innermost stream tube to 150 days for the outermost.

Trichloroethylene, at an initial concentration of 1 mg/l in the aquifer, is the contaminant of interest for all of the simulations presented. A concentration of 1 mg/l of TCE has been successfully biodegraded in field studies (Hopkins et al., 1993). Microorganisms have been exposed to, and successfully degraded, much higher concentrations of TCE in laboratory batch reactors, ~9 mg/l (Oldenhuis et al., 1991), 36 mg/l (Alvarez-Cohen and

Table 1. Summary of Processes Active in the Presence and Absence of Electron Donor and Electron Acceptor

Condition	Donor present	Donor absent
Acceptor present	<ul style="list-style-type: none"> - microbial growth - microbial death - contaminant degradation 	<ul style="list-style-type: none"> - microbial death - microbial inactivation - contaminant degradation
Acceptor absent	<ul style="list-style-type: none"> - no microbial growth or death - no contaminant degradation 	<ul style="list-style-type: none"> - no microbial growth or death - no contaminant degradation

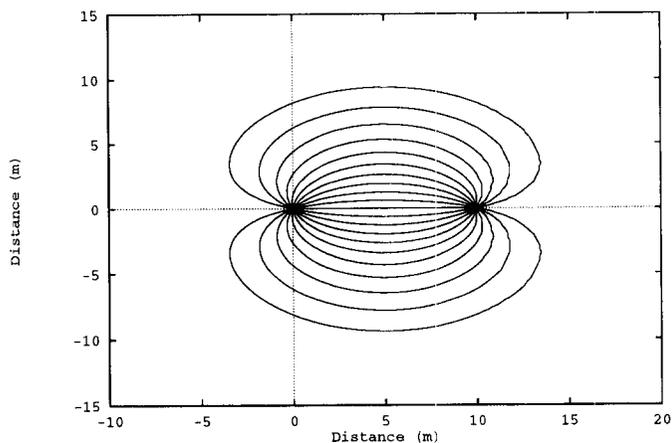


Fig. 2. Plan view of the capture zone developed with extraction rate equal to the injection rate.

McCarty, 1991), and 40 mg/l before a significant rate decrease was observed (Bielefeldt et al., 1995). Toxicity of the degradation by-products, specifically TCE epoxide which reacts with cell protein (Fox et al., 1990), appears to be much greater than the toxicity of TCE itself. Therefore, the upper limit on TCE concentrations for which in situ bioremediation is feasible will depend on the rate of TCE degradation compared to the growth rate of the local microbial population. Preliminary assessment of the local conditions using microcosms with native aquifer material, ground water, and the range of concentrations present at the site should be conducted. Additionally, the presence of other contaminants may prevent effective in situ bioremediation, the presence of 1,1-dichloroethylene greatly decreases the transformation of chlorinated ethenes by methanotrophic microorganisms (Hopkins and McCarty, 1995; Dolan and McCarty, 1995).

The second model employed in this study is the in situ bioremediation model developed by Semprini and McCarty (1991; 1992). This model includes the processes of microbial growth, decay, and electron donor and acceptor utilization; cometabolic degradation of the target contaminant; competitive inhibition between the electron donor and the target contaminant; and the transport processes of advection, dispersion, and rate-limited sorption necessary to simulate contaminant transformation. Model parameters for microbial growth and contaminant transformation and transport are assumed to have the same values as those observed in pilot field-scale experiments (Semprini and McCarty, 1991; 1992; and Semprini et al., 1993). For this work, the model was modified to perform multiple one-dimensional simulations, one along each stream tube. The concentration at the extraction point was calculated as a flux-weighted average of the concentration exiting the 20 stream tubes.

The performance of different substrate delivery schedules was determined through a search method employing multiple simulations in which two variables controlling substrate delivery, X and Y, were varied. Results are plotted as two-dimensional contour plots. The substrate delivery control variables chosen as X and Y differed in the methane-oxygen and phenol-oxygen systems. For a methane-oxygen system, the injected substrate concentrations are fixed by the gas solubility and the gas transfer efficiency. Thus, varying the pulse duration is the only means of varying the mass of methane and oxygen introduced into the system. For the methane-oxygen system, the x-axis, X_{M-O} , was chosen as the methane pulse duration and the y-axis, Y_{M-O} , as

the ratio of the oxygen to methane pulse duration. The phenol-oxygen system has three possible substrate delivery control variables: the phenol injection concentration, the oxygen pulse duration, and the phenol pulse duration. In these analyses, the oxygen-to-phenol pulse duration ratio was initially set at 1.5 (an arbitrary choice for which the sensitivity is addressed later), which leaves X_{P-O} as the phenol pulse duration and Y_{P-O} as the phenol injection concentration.

System performance was evaluated by plotting contour plots of X_{M-O} versus Y_{M-O} and X_{P-O} versus Y_{P-O} for two system performance criteria: the fraction of total TCE mass degraded within 200 days, and the maximum biomass concentration occurring at any time or location during the 200 day simulation (an indication of potential clogging). Experience with this system's geometry and kinetics has shown that after 200 days all of the ground water has circulated at least once and, if a steady-state biomass concentration could develop under the operative substrate delivery schedule, this steady-state biomass concentration had been reached. The initial analysis of each system consisted of 25 model runs simulating 200 days of system performance, five values of X_{M-O} versus five values of Y_{M-O} and similarly for X_{P-O} versus Y_{P-O} . Additional simulations were performed when necessary to clarify interesting or suspect structures present on the contour plots. The contour plots of the system performance criteria are still rather coarse, resulting in some angular contour lines, but behavior of each system is apparent.

Presenting results in this manner clearly illustrates the trends of a particular system performance criterion (the mass fraction of TCE transformed and the maximum biomass achieved are the only two criteria presented) for the range of substrate delivery schedules. Each criterion can be examined independently to identify substrate delivery schedules that are acceptable for all criteria and best for the criteria considered most important in a particular application or at a given time within the operation of a particular in situ bioremediation system.

Results

Methane-Oxygen Systems

The mass of oxygen and methane introduced to promote microbial growth is determined by the solubility of these gases in water as governed by Henry's Law, and the gas transfer efficiency achieved. The dissolved concentrations of these two gases are not independent because saturation of one gas reduces the driving force for transfer of the other gas to the recirculating ground water. In laboratory tests of the down-well venturi device, gas transfer was achieved at 50 percent of the equilibrium concentration when other dissolved gases were present (Bae et al., 1995). The remediation system is assumed to be closed to the atmosphere; thus the additional problem of competition between dissolved gases already present in the recirculating ground water and additional gases being introduced must also be considered in the methane-oxygen system. The effects of equilibrium, mass transfer, and mass balance principles interact in a manner that decreases the ability to transfer the rate-limiting gaseous substrate into the recirculating ground water (Lang, 1995). This negative feedback on the operation of field scale in situ bioremediation, previously unreported, is evaluated in these simulations.

Figure 3 shows the normalized mass of TCE transformed using methane as the electron donor. The y-axis is the ratio of the oxygen-to-methane pulse duration and the x-axis is the methane pulse duration in days. The line labels indicate the fraction

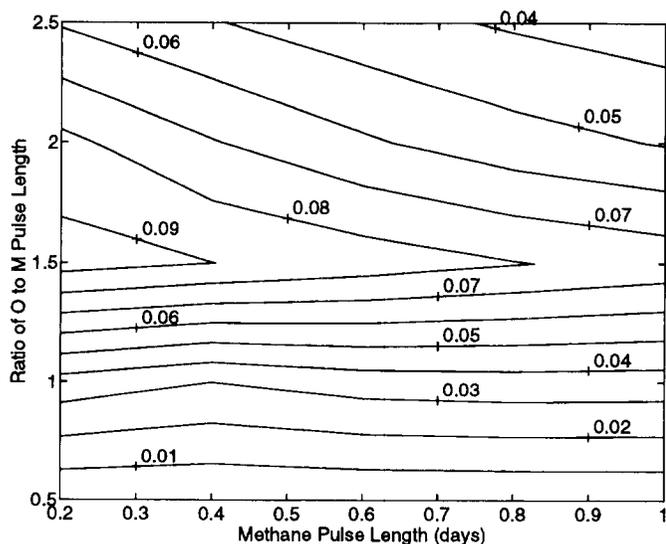


Fig. 3. Fractional mass transformation of TCE within 200 days for a two-well, methane-oxygen system. Gas transfer efficiency = 0.5.

of the total TCE mass (dissolved + sorbed TCE within the capture zone) that is transformed within the 200 days simulated. Figure 3 indicates that the greatest transformation occurs for substrate delivery with a ratio of oxygen-to-methane pulse durations of approximately 1.5 to 1. Accounting for the dissolved concentrations of oxygen and methane achieved in the recirculating ground water, the flow rate, and the pulse durations, this ratio of substrate delivery results in 2.8 grams of O_2 delivered per gram of CH_4 , slightly higher than the stoichiometric requirement for methanotroph growth, 2.4 grams O_2 per gram of CH_4 (Semprini and McCarty, 1991). A 1.5 to 1 ratio of oxygen-to-methane pulse durations establishes a substrate delivery that satisfies the stoichiometric requirement of oxygen and methane for net biomass growth, i.e. the substrate requirements for biomass growth plus the oxygen demand exerted by cell decay. With this substrate delivery schedule, both substrates are almost completely utilized and neither accumulates within the system. This operating schedule has two important characteristics: (1) it minimizes recirculation and interference of one substrate with the injection of the other substrate, and (2) it produces a steady-state biomass concentration over time. For subsequent comparisons, this substrate delivery schedule will be referred to as the steady-state delivery schedule. Figure 3 also shows that more frequent pulsing, i.e., shorter pulse durations, results in better contaminant transformation at any pulse ratio, but (as discussed below) at the cost of a less uniform biomass distribution and consequently higher localized biomass concentrations.

Another significant system response illustrated in Figure 3 is the different rate of decrease in the fraction of TCE transformed when approaching the steady-state substrate delivery schedule from a region of excess methane or excess oxygen. The top half of the contour plot is the operating region where oxygen is introduced in excess. In this upper region, the gradients of the TCE fractional removals are relatively flat and the penalty to TCE destruction for moving away from the steady-state substrate delivery schedule in the direction of increasing oxygen is mild. The bottom half of the contour plot represents substrate delivery schedules with methane in excess. As indicated by much steeper gradients, the fraction of TCE transformed decreases much more rapidly. The more pronounced rate of decrease in fractional TCE

removal in the region of excess methane has two causes: (1) the transformation reaction of TCE with methane monooxygenase (the active enzyme) requires oxygen, so when oxygen is depleted TCE transformation cannot occur, and (2) increased methane leads to increased competitive inhibition (competition between methane and TCE for the active enzyme, MMO).

Figure 4 shows the maximum biomass concentrations predicted to develop under this range of substrate delivery schedules. The maximum biomass concentrations are the maximum concentrations that occur at any location within the capture zone at any time within the 200 days simulated. The maximum biomass contour plot reveals a number of interesting characteristics. The concentration of high biomass around the short pulse intervals with oxygen-to-methane pulse duration ratio of 1.0 illustrates the effects of the model assumption that the microbial population becomes dormant in the absence of oxygen. The operating conditions with high biomass concentration receive methane in excess; thus any oxygen introduced to this region is rapidly utilized for microbial growth resulting in significant periods with no oxygen present. Methane is always present, thus, according to current model assumptions (summarized in Table 1), preventing periods of net microbial death that would occur when oxygen is present but methane absent (Semprini and McCarty, 1991). Consequently, microbial biomass is predicted to accumulate under substrate delivery schedules with methane in excess.

At the steady-state substrate delivery schedule, the maximum biomass concentration, 200 mg dry wt/l, is below levels that have been observed to cause clogging in soil column experiments, i.e. > 550 mg dry wt/l (Taylor and Jaffe, 1990). The biomass concentrations presented in Figure 4 also show that a decrease in maximum biomass concentration can be achieved with longer pulse durations. This decrease is more pronounced away from the net stoichiometric introduction of substrates. At a pulse ratio of 2 to 1, the maximum biomass concentration predicted for the shortest methane pulse duration, 0.2 days, is about 100 mg/l, whereas the concentration for a methane pulse duration of 1.0 days is less than 75 mg/l.

Figures 3 and 4 indicate that the best operating schedule is the net stoichiometric addition of substrates with a tendency to

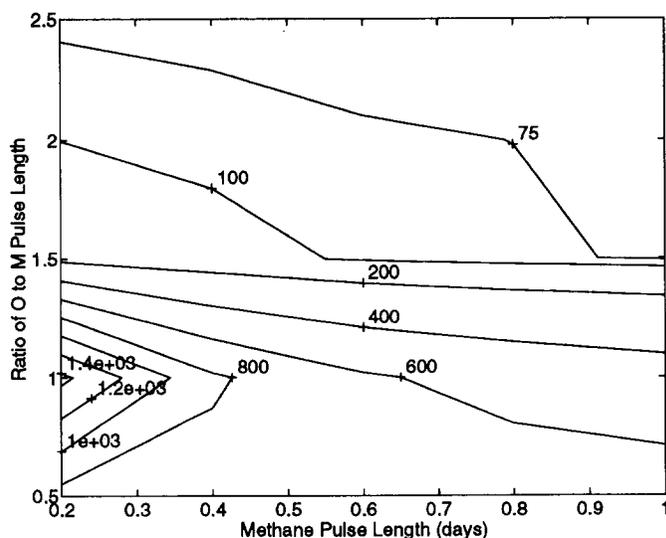


Fig. 4. Maximum biomass concentration (mg/l) occurring within 200 days for a two-well, methane-oxygen system. Gas transfer efficiency = 0.5.

err towards providing excess oxygen. Additional considerations such as maintaining aerobic conditions in the aquifer or maximizing the distribution of active microbial populations can be addressed by introducing excess oxygen or using longer oxygen and methane pulse durations, respectively. These operating conditions correspond to substrate delivery schedules in the upper right quadrant of Figures 3 and 4.

Phenol-Oxygen Systems

In situ bioremediation systems using phenol as the electron donor and oxygen as the electron acceptor have been shown to be much more effective at TCE degradation than methane-oxygen systems (Hopkins et al., 1993; Hopkins and McCarty, 1995). Moreover, because phenol is readily soluble at the concentrations needed to stimulate active microbial populations, the phenol-oxygen systems avoid the competition for introduction of two gas phase substrates that limits the methane-oxygen system. Gas transfer efficiency still influences the system performance because the mass of oxygen transferred determines the mass of phenol that can be utilized to stimulate microbial growth and contaminant transformation.

An important consideration for the phenol-oxygen systems is the extent to which the phenol is degraded. As discussed previously, phenol is itself a contaminant of concern in ground water and its use as an added substrate to promote bioremediation may best be limited to ex situ treatment systems. However, if the benefits observed in faster degradation of contaminants warrant its use in the subsurface, substrate delivery schedules that provide conditions achieving the complete degradation of all added phenol will likely be required.

Microbial degradation is assumed to be the dominant fate of phenol. Under conditions amenable to in situ bioremediation, low fraction of organic carbon, a well-buffered system to maintain near neutral pH, and moderate to high hydraulic conductivity (National Research Council, 1994), phenol remains unionized (pK_a of 9.89). At the Moffett site (organic carbon fraction, $f_{oc} = 0.11$ percent), phenol sorption was not observed as evidenced by the lack of retardation and damping in the pulsed input response at intermediate observation wells (Hopkins et al., 1993).

For the initial set of simulations, the oxygen pulse duration was set at 1.5 times the phenol pulse duration. Sensitivity to this pulse duration is addressed at the end of this section. For the figures summarizing the behavior of phenol-oxygen systems, the upper half of the plots (increasing phenol injection concentrations) are the regions with electron donor-rich substrate delivery schedules and the lower half corresponds to excess oxygen.

The fraction of the initial TCE mass transformed for the range of phenol injection concentrations and pulsing frequencies is shown in Figure 5. A phenol injection concentration of approximately 23 mg/l phenol achieves the maximum TCE transformation and, again, the shorter pulse durations perform better than the longer ones. A phenol injection concentration of 23 mg/l matches the net stoichiometric requirement for growth of phenol degraders for the mass of oxygen (oxygen concentration ~ 30 mg/l from introduction of pure gas) being introduced. This corresponds to a net stoichiometric requirement of 1.57 grams O_2 to 1 gram of phenol. Operating under these conditions achieves the most efficient transformation of TCE, and nearly complete utilization of both added substrates minimizes disruption of discrete pulsed introduction of the substrates at the injection well. The problem with operating in this region, however,

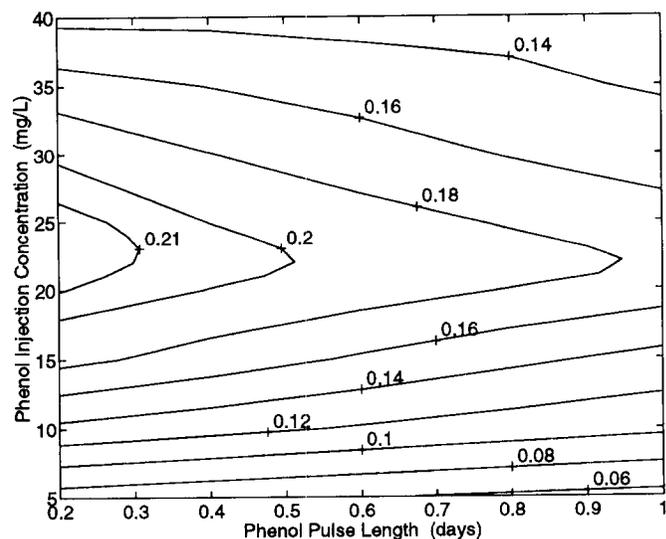


Fig. 5. Fractional mass of TCE transformed within 200 days for a two-well, phenol-oxygen system. Oxygen:phenol pulse length ratio = 1.5; gas transfer efficiency = 0.5.

is that complete utilization of the added phenol is not necessarily achieved. Balancing the requirement of complete phenol utilization and efficient contaminant degradation requires using substrate delivery schedules in the region with oxygen in excess, i.e. oxygen is never depleted in the recirculating ground water.

The region on the contour plots representing substrate delivery schedules introducing excess oxygen is the lower half of Figure 5, phenol injection concentrations less than 20 mg/l. Substrate delivery schedules introducing excess phenol correspond to the upper half of Figure 5, phenol injection concentrations exceeding 25 mg/l. Unlike the methane-oxygen system, the decrease in TCE transformation is slightly greater when moving away from the steady-state substrate delivery schedule in the direction of excess oxygen than in the direction of excess phenol. The same phenomena that apply in the case of excess methane, i.e. dependence of the TCE transformation reaction on the presence of oxygen and competitive inhibition, are observed with phenol as the electron donor (Hopkins et al., 1993). However, because phenol and oxygen do not compete for gas transfer introduction, phenol or oxygen can be added at a constant rate independent of the concentration of the other substrate. Therefore, unlike the methane-oxygen system, the decrease in TCE transformation incurred by moving in the direction of a phenol-rich substrate delivery schedule is predicted to be less than moving in the direction of a methane-rich substrate delivery. However, phenol-rich substrate delivery is not a recommended operating procedure, as it fails to achieve complete utilization of the added phenol and thus would further degrade the ground-water quality. The decrease in TCE transformation when moving away from the steady-state substrate delivery schedule in the phenol-oxygen system in either direction, excess oxygen or excess phenol, can be explained by the biomass concentrations developed, rather than by competition for substrate introduction as in the methane-oxygen system.

Figure 6 shows the maximum biomass concentrations occurring within the 200 day simulations. Similar to the methane-oxygen system, biomass is predicted to accumulate to a greater extent under substrate delivery schedules injecting excess phenol. In contrast to the methane-oxygen system, oxygen continues to be introduced with phenol in excess and the high biomass concen-

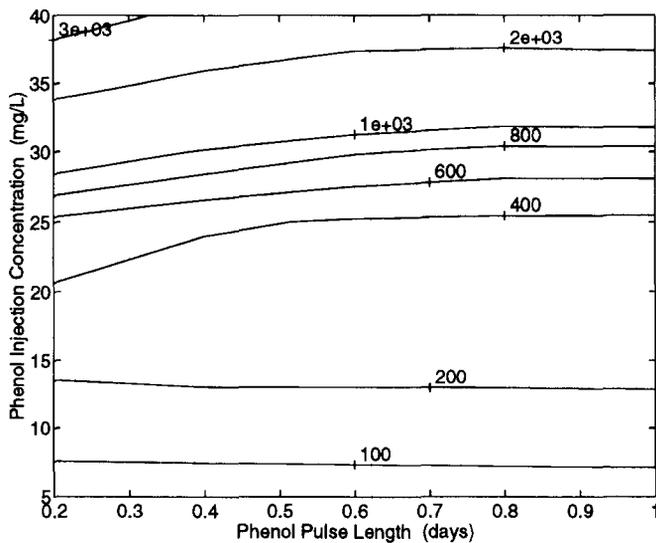


Fig. 6. Maximum biomass concentrations (mg/l) occurring within 200 days for a two-well, phenol-oxygen system. Oxygen:phenol pulse length ratio = 1.5.

trations resulting in this region maintain significant contaminant transformation.

At the steady-state substrate delivery schedule, the biomass concentrations are roughly twice as high as those predicted for the methane-oxygen system. As discussed previously, the primary difference in kinetics between phenol- and methane-oxidizing bacteria is not the ability of their enzymes to transform TCE, but rather the microbial yield from a given amount of substrate; the yield coefficient for methane oxidizers is 0.5, whereas that for phenol-oxidizing bacteria is 0.8 (Semprini et al., 1993). This higher growth rate for phenol-oxidizers may act as either a benefit to remediation, i.e. greater microbial stimulation for the equivalent mass input of substrate, or an impediment, depending on the aquifer's sensitivity to clogging by bacterial growth. Biomass concentrations of 400 to 600 mg dry wt/l are in the range Taylor and Jaffe (1990) reported to cause clogging (550 mg dry wt/l) but much lower than the level of 3000 mg dry wt/l reported by Vandivivere and Baveye (1992). It is not clear whether clogging would be a problem in this case, because clogging potential depends on a number of factors (soil type, microorganism species, etc.).

Phenol-Oxygen System's Sensitivity to the Oxygen Pulse Duration

As discussed previously, the phenol-oxygen system has three possible control variables for the substrate introduction: the phenol injection concentration, the phenol pulse duration, and the oxygen pulse duration. For the analyses shown above, the ratio of the oxygen-to-phenol pulse durations was set to a constant value of 1.5 to reduce the number of variables to two. The sensitivity of the system to this ratio of the pulse durations is addressed here. Figures 7 and 8 show a set of simulations equivalent to those presented previously, i.e. with the same net stoichiometric ratios, but setting the oxygen-to-phenol pulse duration ratio equal to 1.0.

Figure 7 shows the fraction of TCE mass transformed for the range of substrate delivery schedules considered in the base case simulations. The phenol injection concentration predicted to have the greatest rate of TCE transformation is lower, approximately 15 mg/l, compared to 23 mg phenol/l predicted for an

oxygen-to-phenol pulse duration of 1.5 (Figure 5). The steady-state phenol injection concentration has decreased to match the decrease in the mass of oxygen being introduced into the system as a result of the shorter oxygen pulse duration. This results in a somewhat lower fraction of TCE transformation over the 200 days simulated, 0.18 compared to the previous value of 0.21.

Figure 8 shows the maximum biomass concentrations predicted within the 200 days simulated. The maximum biomass concentration at the optimal substrate delivery schedule is approximately the same as that predicted for the substrate delivery schedule with an oxygen-to-phenol pulse duration ratio of 1.5, i.e. 400 mg dry wt/l (Figure 6). This biomass concentration represents the steady-state biomass concentration that will develop given the microbial growth kinetics of phenol degraders. The substrate delivery schedules that maintain this biomass concentration are those that introduce oxygen and phenol to satisfy the requirements of phenol and oxygen for growth with additional oxygen to balance the oxygen consumed by the decay of cell decay and maintain a steady-state population.

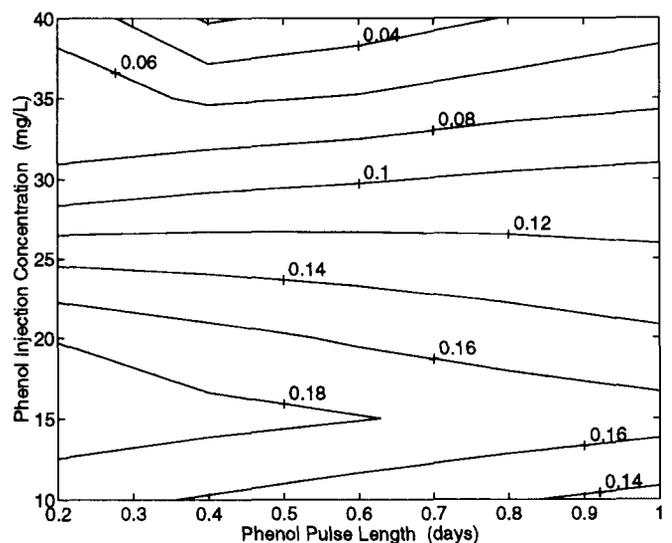


Fig. 7. Fractional mass transformation within 200 days for a two-well, phenol-oxygen system. Oxygen:phenol pulse length ratio = 1.

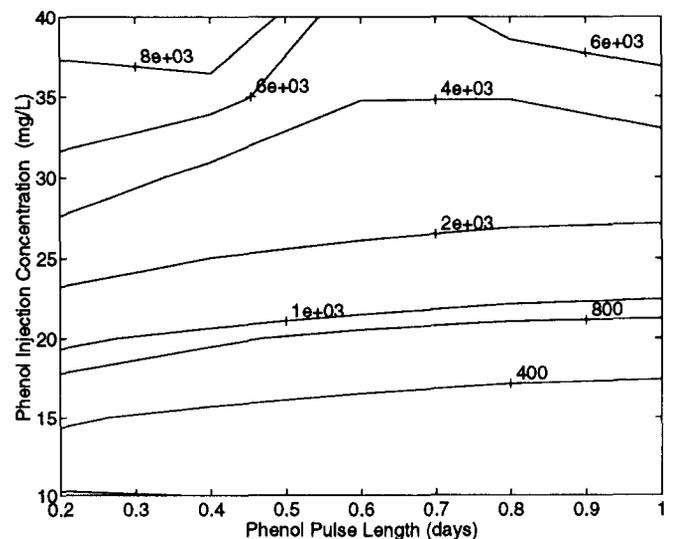


Fig. 8. Maximum biomass concentration (mg/l) occurring within 200 days for a two-well, phenol-oxygen system. Oxygen:phenol pulse length ratio = 1.

Sensitivity to Gas Transfer Efficiency

The method employed to introduce gas phase substrates into the recirculating ground water will determine the efficiency of gas transfer into the system. The simulations presented so far all assume the gas transfer characteristics measured during experimental investigation of a prototype down-well venturi device conducted by Bae et al. (1995). These experimenters found that oxygen, when competing with another dissolved gas, could be dissolved at approximately 50 percent of the oxygen's theoretical saturation concentration after taking into account the partial pressure of a competing dissolved gas. In a full-scale operation, gas transfer efficiencies might be either lower, as a result of competition with dissolved gases other than the introduced substrates, or higher, through the use of more efficient transfer mechanisms such as in-line mixers. Sensitivity analysis describing the effects of increasing gas transfer efficiency from 0.5 to 0.9 is presented here.

The TCE mass transformed in a methane-oxygen system assuming a gas transfer efficiency of 0.9 is shown in Figure 9; compare to Figure 3 where gas transfer efficiency equals 0.5. The substrate delivery that results in the optimum fraction of the initial mass of TCE transformed remains the same, an oxygen-to-methane ratio of about 1.5 with short pulse lengths. For the same range of operating conditions simulated previously, a gas transfer efficiency of 0.9 increases the TCE transformation. The fractional mass of TCE transformed at the optimum is predicted to increase from 0.09 mass TCE transformed/initial mass of TCE with a gas transfer efficiency of 0.5 to greater than 0.14 fraction of initial mass of TCE transformed with a gas transfer efficiency of 0.9. As expected, the increased ability to inject substrate gases increases the biomass growth, and thus the capacity for transformation. The fractional mass of TCE transformed under any substrate delivery schedule increases with an increase in gas transfer efficiency.

Figure 10 shows the maximum biomass concentrations predicted to occur assuming the higher gas transfer efficiency. The trends are similar to those seen with a gas transfer efficiency of 0.5. For most of the substrate delivery schedules simulated, maximum biomass concentrations were predicted to be almost twice as high, 400 mg/l with a gas transfer efficiency of 0.9 compared to

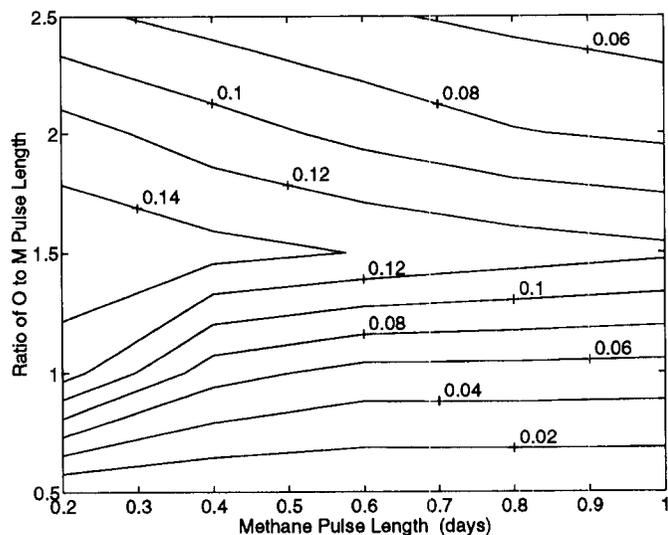


Fig. 9. Fractional mass of TCE transformed within 200 days for a two-well, methane-oxygen system. Gas transfer efficiency = 0.9.

200 mg/l with a gas transfer efficiency of 0.5 for short pulse durations with a ratio of oxygen-to-methane pulse duration of 1.5. The maximum biomass concentrations for substrate delivery schedules with methane in excess are lower than those predicted for a gas transfer efficiency of 0.5. Under substrate delivery schedules introducing methane in excess (lower half of Figure 10), the higher gas transfer efficiency increases the oxygen introduced, which in turn increases the loss of microbial biomass to cell death, and slightly lowers the maximum biomass concentration predicted.

Figure 11 shows the fraction of TCE transformed with the gas transfer efficiency increased from 0.5 to 0.9 in a phenol-oxygen system for the same range of substrate delivery schedules as the previous phenol-oxygen simulations. Comparing Figure 11 to Figure 5 (gas transfer efficiency of 0.5), the optimal phenol injection concentration has increased from approximately 23 mg/l to 42 mg/l. For the methane-oxygen system, no change in the steady-state substrate delivery schedule was observed with an increase in

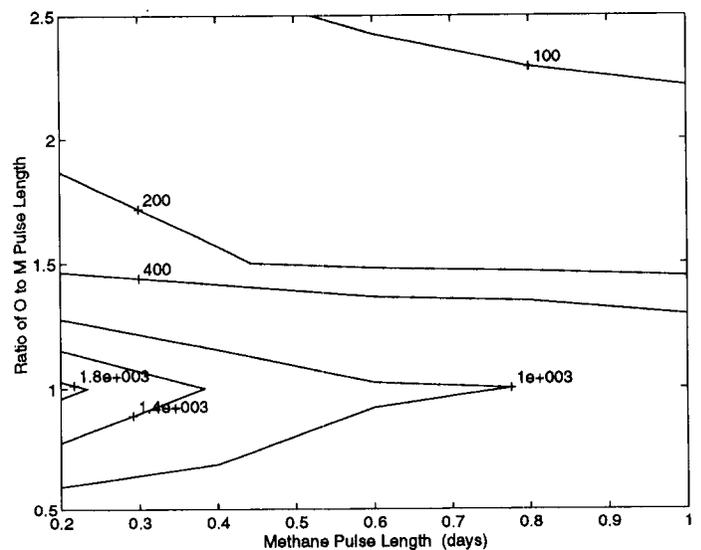


Fig. 10. Maximum biomass concentration (mg/l) occurring within 200 days for a two-well, methane-oxygen system. Gas transfer efficiency = 0.9.

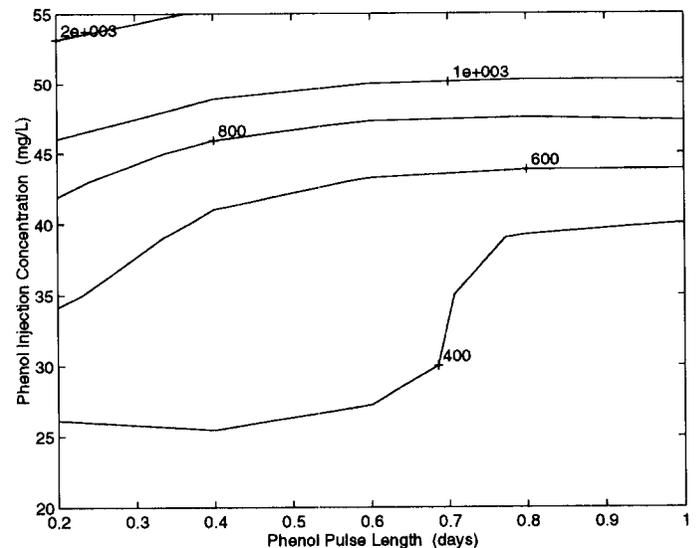


Fig. 11. Fractional mass of TCE transformed within 200 days for a two-well, phenol-oxygen system. Oxygen:phenol pulse length ratio = 1.5; gas transfer efficiency = 0.9.

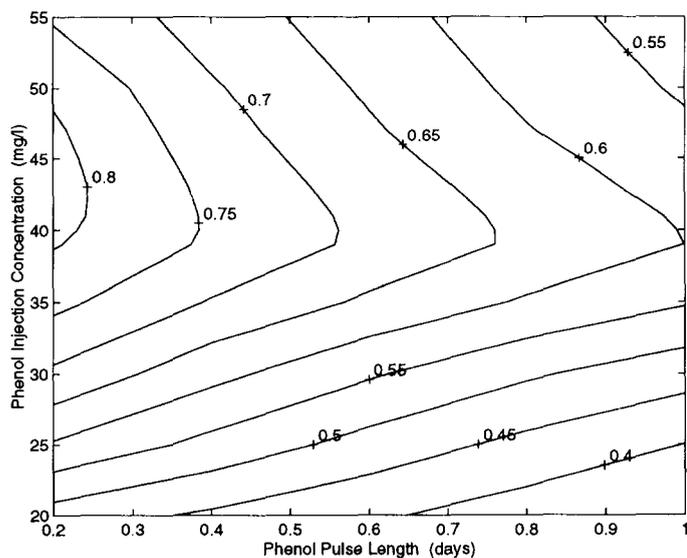


Fig. 12. Maximum biomass concentration (mg/l) occurring within 200 days for a two-well, phenol-oxygen system. Oxygen:phenol pulse length ratio = 1.5; gas transfer efficiency = 0.9.

the gas transfer efficiency, because the gas transfer efficiency influenced the introduction of both substrates. In the phenol-oxygen system, an increase in the gas transfer efficiency increases the amount of oxygen introduced into the system and the optimal phenol injection concentration increases to match this increase in the delivery of oxygen. The predicted fractional transformation of TCE increases from 0.21 to 0.80 with the increase in substrate addition.

Figure 12 shows the maximum biomass concentrations predicted assuming a gas transfer efficiency of 0.9. Comparing Figure 12 to Figure 6, the maximum biomass concentrations for a gas transfer efficiency of 0.5, shows that the maximum biomass changes very little for most of the substrate delivery schedules. The main difference in maximum biomass concentrations with the change in gas transfer efficiency is for those substrate delivery schedules receiving phenol in excess, roughly the upper half of Figures 6 and 12. The maximum biomass concentrations in this region are lower for a gas transfer efficiency of 0.9 because more oxygen is introduced, thus increasing the endogenous respiration and decreasing the biomass concentration.

Summary

For both microbial systems, the greatest rate of TCE transformation was achieved using the substrate delivery schedule that maintains a steady-state biomass concentration. The substrate delivery schedule that will maintain a steady-state biomass concentration is the substrate delivery that supplies the mass ratios of electron acceptor and electron donor required for net microbial growth, i.e. the requirements for microbial growth plus the oxygen demand for endogenous respiration. This steady-state rate of substrate delivery completely utilizes both the electron donor and electron acceptor within the biologically active zone (a limited zone of actively transforming microorganisms typically limited to within 2-3 m of the injection well). The ratio of electron donor to electron acceptor at which this complete substrate utilization is achieved is slightly higher than the stoichiometric mass ratio of the electron acceptor (oxygen) to the electron donor required for microbial growth.

In addition to identifying the substrate delivery schedule achieving the greatest transformation of TCE, these results also

define the decrease in TCE transformation moving away from the steady-state substrate delivery schedule. Constraints not included in the model, such as a requirement to guarantee complete degradation of the electron donor (phenol or methane) or to maintain aerobic conditions within the aquifer, will most likely dictate that a substrate delivery schedule introducing oxygen in excess be adopted. The TCE transformation possible under different substrate delivery schedules can be estimated directly from the plots of TCE mass fraction transformed.

For a methane-oxygen system, the decrease in TCE transformation is much greater moving away from the steady-state schedule in the direction of excess electron donor (methane) than in moving toward excess oxygen. Introducing excess oxygen is predicted to decrease only slightly the extent of TCE transformation. This is due to the fact that the contaminant transformation reaction continues in the absence of the electron donor, though at a decreasing rate, but does not continue in the absence of oxygen. Also contributing to a much greater penalty for moving in the direction of excess donor is that the competition for introduction of gases increases as methane builds up in the recirculating water, severely limiting the introduction of oxygen and continuation of the transformation reaction.

The decrease in TCE transformation operating away from the steady-state substrate delivery schedule for the phenol-oxygen system is predicted to be just the opposite: greater decreases in the rate of contaminant transformation in the direction of excess oxygen than toward excess phenol. Like the methane-oxygen system, simulations introducing excess electron donor in the phenol-oxygen system predict very high biomass concentrations. The phenol-oxygen system avoids the competition for substrate introduction and oxygen can be introduced at a high, constant concentration with excess phenol present and TCE transformation continues. However, operating an in situ bioremediation system under electron donor-rich conditions is not recommended, because complete degradation of all added phenol is unlikely. Substrate delivery schedules introducing oxygen in excess provide conditions that will achieve complete utilization of all added phenol.

The simulations presented here also provide information on the differences in sustainable biomass for different substrate delivery schedules. The longer pulse intervals introduce substrate further into the capture zone than the shorter pulse lengths, resulting in more evenly distributed but generally lower total biomass. This finding suggests that a topic for future exploration might be the adoption of temporally varying substrate delivery schedules to control the size of the biologically active zone.

Conclusions

The simulations presented here examine the range of substrate delivery schedules deemed feasible for promoting cometabolic degradation of TCE using both methanotrophic and phenol-oxidizing bacteria. This modeling effort was designed to identify promising operating conditions for in situ bioremediation which have not yet been applied in field and laboratory experiments. Many assumptions were made for this analysis, the major one being that the microbial growth and contaminant degradation kinetics observed at one site are representative of another site. Obviously, site-specific model parameters are always desirable but extrapolating the findings at one site to design an application at a new site provides important guidance and constraints. The simulations presented are intended to identify trends and implica-

tions of current understanding of the models and model parameters used to evaluate in situ bioremediation relying on cometabolic degradation. Additional experience with full-scale application of these systems is necessary to fully evaluate the feasibility in terms of economics and long-term sustainability of the microbial populations for contaminant degradation.

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