Response to 'Comment on "Enrichment of High-Rate PCE Dechlorination and Comparative Study of Lactate, Methanol, and Hydrogen as Electron Donors To Sustain Activity"'

SIR: We thank Fennell and Gossett for their analysis of our work and regret that any confusion may have resulted from comparisons between our research and theirs. We appreciate the opportunity to address the issues they have raised in their Comment and to resolve any confusion that may exist. In their Comment to the editor, the correspondents focus on the difference between electron donor to PCE ratios (ED: PCE) used in the two studies. They also comment on the omission from our article regarding the role of preformented yeast extract (FYE) in their long-term studies and introduce an important discussion of relevant engineering design issues for the addition of electron donors to the subsurface. We have addressed these comments in the order in which they are raised.

In both our work (*1*) and the work of Fennell et al. (*2*), experiments were conducted to evaluate the ability of several electron donors to sustain PCE dechlorination in mixed, methanogenic cultures. Despite the similarity in the central objective of these studies, several important differences must be recognized. One difference, as pointed out by the correspondents, was the ED:PCE ratio. Perhaps a more important difference was the electron donors employed, since lactate was the only common substrate between the two studies. Fennell et al. used butyric acid, ethanol, lactic acid, and propionic acid, while we used methanol, lactic acid, and hydrogen. Hydrogen could be considered a common electron donor in both studies, since it is produced through fermentation of organic electron donors. However, the partial pressures of hydrogen in our systems in which hydrogen was fed directly were orders of magnitude greater than would be expected from fermentation processes. Other differences included inoculum, experimental design (batch vs recycle column), duration of experiments, and experimental temperature. Each of these factors may impose some degree of incongruity in the interpretation of findings between the two reports.

Fennell and Gossett raise an interesting point in the evaluation of our systems regarding ED:PCE ratios. We elected not to focus on this issue in our manuscript as electron donor consumption data was available only for the hydrogen-fed recycle columns. For those studies we reported an ED:PCE ratio of 630:1 based on a PCE addition of 0.054 mequiv and the amount of hydrogen that was consumed over a 4-day period (34 mequiv total or 8.5 mequiv per day). At the beginning of the study, dechlorination was not complete (e.g., PCE, TCE, and *cis*-DCE were present without vinyl chloride or ethene production) within the 4-day feeding cycle, and the 630:1 ratio accurately described the operation of the systems. Over time, dechlorination rates increased to the point that dechlorination to vinyl chloride and ethene was complete within a few hours after PCE addition, and the observed daily hydrogen consumption averaged 5 mequiv per day. Thus, by day 474, the ratio of hydrogen equivalents consumed to PCE equivalents fed was approximately 15:1 during active dechlorination. In other words, the observed ratio of hydrogen consumption to PCE dechlorination was not constant throughout the study, due to the continuous increase in dechlorination activity.

We believe that this finding is important, since there has been considerable concern regarding the ability of dechlorinators to compete with other microorganisms (i.e., methanogens and acetogens) at high hydrogen partial pressures. The high partial pressure of hydrogen in our hydrogen fed systems would almost certainly saturate the rates of all competing processes (based on K_s values) and provide the ideal opportunity for competition effects to be observed. Despite the fact that PCE was present for only a few hours every 4 days and that high partial pressures of hydrogen (relative to a fermentation-based system) were available throughout the 474 days of column operation, the extent and rate of dechlorination increased throughout the experimental period. If high hydrogen partial pressures were a strong selective pressure disfavoring dechlorinators, the opposite observation would be expected.

Fennell and Gossett introduce several hypothetical scenarios involving competition for hydrogen between methanogens and dechlorinators under various concentrations of PCE. While it is interesting to investigate all possible scenarios as presented, we feel it is important to focus on "typical" conditions at contaminated sites. As a general rule, source areas (i.e., regions where nonaqueous-phase liquids exist) contain dissolved phase contaminant concentrations at or slightly above 1% of the effective aqueous solubility of the contaminant (*3*). In the case of PCE, this would be approximately 1−2 mg/L (higher concentrations certainly exist at the water−DNAPL interface or may occur in media where dispersion processes are significantly impeded). For this reason, the second scenario presented in their commentary is uncommon. Recognizing that required levels of remediation demand that dechlorination result in μg/L concentrations and that the stoichiometry of dechlorination is quite favorable (i.e., over 20 mg of PCE can be dechlorinated to ethene by 1 mg of hydrogen), the first scenario presented is more likely to be observed in anaerobic remediation systems. This is further supported by the fact that the addition of excess electron donor represents the only safety factor to ensure complete dechlorination. We do agree that ratios as high as 630:1 are probably excessive, but it is difficult at this time to determine *a priori* appropriate levels of electron donor addition required to obtain complete dechlorination.

In the discussion section of our manuscript we draw comparisons between our long-term tests to those of Fennell et al. We did not address the role of FYE in these studies as noted by the correspondents. Our reason for neglecting this point in our manuscript is based on a comment from their discussion. That is, Fennell et al. reported that in long-term study controls, fed only FYE, dechlorination was incomplete with "significant amounts of remaining PCE and TCE". This statement was inconsistent with their conclusion that "...the addition of FYE significantly influenced the outcome of the long-term tests"—a conclusion resulting from short-term experiments evaluating the role of FYE in sustaining dechlorination. We were unclear as to reason for the apparent discrepancy between the long-term and short-term study results and were most interested in the long-term studies as they were more closely related to our own experiments. For that reason, we chose to neglect the matter in our discussion.

The last point raised in the correspondence of Fennell and Gossett focuses on engineering considerations for electron donor delivery. We agree with the correspondents that biofouling is a management concern for any liquid delivery system. From our limited experience there are also regulatory issues that complicate the permitting of liquid delivery systems if contaminated water is reinjected. We agree

TABLE 1. Estimates of True Yields from Various Organic Substrates and Hydrogen

substrate	true yield[a] (mg cells·eq substrate^{-1})	true yield[a] (mg cells·eq hydrogen^{-1})
propionate	0.271	0.633
butyrate	0.266	1.328
ethanol	0.610	1.831
lactate	0.723	2.170
methanol	1.187	3.560
H$_2$ (heterotrophic)	1.011	1.011
H$_2$ (autotrophic)	0.237	0.237

[a] Free energy of formation data was taken from ref 4 or calculated from Table AI.1 in ref 5. pH = 7.0. For calculation of mg cells produced, ammonia was used as the nitrogen source and the chemical formula for biomass was assumed to be C$_5$H$_7$O$_2$N. [b] Moles of hydrogen produced by the fermentation of organic substrates (per mole basis) to acetate were the following: propionate, 3; butyrate, 2; ethanol, 2; lactate, 2; and methanol, 1.

that it may be possible to minimize fouling concerns through electron donor selection—while maintaining adequate electron donor dose—using substrates that result in low yields of nondechlorinating organisms, in particular fermentors and methanogens. Table 1 presents calculated true yields of mixed methanogenic cultures (i.e., methanogens and fermentors) for a range of substrates. Yields were normalized to net hydrogen production during organic substrate fermentation (shown in the right most column) assuming that dechlorination is supported by hydrogen and not the organic substrates themselves. From this analysis, it is clear that electron donor selection may influence the dose allowed to avoid biofouling concerns. For example, the substrates butyrate, ethanol, lactate, and methanol result in more biomass production per equivalent of hydrogen produced than propionate or the direct addition of hydrogen.

It is also important to note that a variety of electron donor delivery systems are being evaluated that do not require liquid injection, in part to avoid certain practical concerns (including biofouling) and regulatory issues that influence reinjection. Alternative methods include hydrogen-based biosparging, *in situ* cathodic hydrogen production, and "slow release hydrogen" materials that can be injected into the formation. Iron slurry walls may serve as electron donor delivery systems in addition to their ability to dechlorinate via abiotic mechanisms. Other systems of which we are currently unaware may also be in development. Any comments regarding the extent to which any of these processes would be effective in mitigating biofouling would be premature. However, it is important to note that alternative electron donor delivery systems may offer methods to provide adequate electron donor delivery and minimize the specific issues raised.

In closing, we would like to thank Drs. Fennell and Gossett again for their thorough and thoughtful comments. We hope that any confusion resulting from our manuscript has been resolved through this process.

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