



Effect of Common Site Conditions on Chlorinated Solvent Biodegradation by a Bioaugmentation Consortium

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Abstract

Background/Objectives. The success of bioaugmentation for treatment of chlorinated compounds depends on many factors including site conditions, concentration of contaminants, presence of co-contaminants, etc. To evaluate the impact of these factors on dechlorination by the SDC-9 bioaugmentation consortium, a series of experiments were conducted to evaluate the effect of high and low temperature, exposure to high or low pH, elevated concentration of NaCl, the presence of sulfate and the presence of co-contaminants acetone and methylene chloride. We also evaluated the effects of elevated concentrations of PCE, TCE, cis-DCE, VC and 1,2-DCA on the ability of this *Dehalococcoides* (DHC)-containing consortium to dechlorinate high concentrations of these contaminants. Likewise, because application of bioaugmentation cultures sometimes involves addition of highly concentrated electron donor substrates such as sodium lactate and reducing agents such as L-cysteine or sulfite, we evaluated the impact of these substrates on dechlorination.

Approach/Activities. The impact of environmental conditions, contaminant concentrations, and bioaugmentation additives on dechlorination was investigated by performing a series of laboratory bottle assays. Assays were typically performed in 160-mL serum vials containing anaerobic RAMM medium and the SDC-9 culture.

Results/Lessons Learned. It was found that concentration of sodium lactate greater than 8 g/L inhibited cis-DCE and VC dechlorination by SDC-9. These results suggest that care should be taken when preparing electron donor solutions for in situ injection, and that dechlorinating cultures should not be pre-mixed with these solutions prior to injection.

Addition of the reducing agent L-cysteine at concentration as high as 0.69 g/L (which was 29 times higher than normally recommended for media preparation) did not negatively affect dechlorination of PCE to ethene. Application of the reducing agent sodium sulfite at 0.02% inhibited PCE dechlorination, and it appeared to also inhibit the activity of fermentors and methanogenic bacteria in the culture. Dechlorination of PCE occurred only after sodium sulfite was reduced to sodium sulfide. Elevated concentrations of sulfate up to 4000 mg/L reduced the rate of transformation of PCE to ethene but did not completely inhibit this process, but the rate of PCE dechlorination at 4000 mg/L sulfate was approximately 2.5 times slower than without sulfate. Analysis of degradation of PCE at different temperatures showed that the SDC-9 consortium was able to dechlorinate chlorinated ethenes at temperatures as low as 5°C and as high as 40°C.

Increasing pH of groundwater by adding base or buffers sometimes results in increasing the pH of groundwater to pH 9 or 10, and fermentative activity can reduce pH to as low as pH 4. Therefore, we evaluated the effect of temporary pH increases and decreases on chlorinated solvent biodegradation by SDC-9. It was found that incubating the DHC-containing culture for one day at pH 9.9 or pH 4.0 resulted in complete cessation of bacterial dechlorination activity. Consequently, care should be taken when buffering or adding base to aquifers to increase pH, and when applying high concentrations of electron donors that can reduce pH in poorly buffered aquifers.

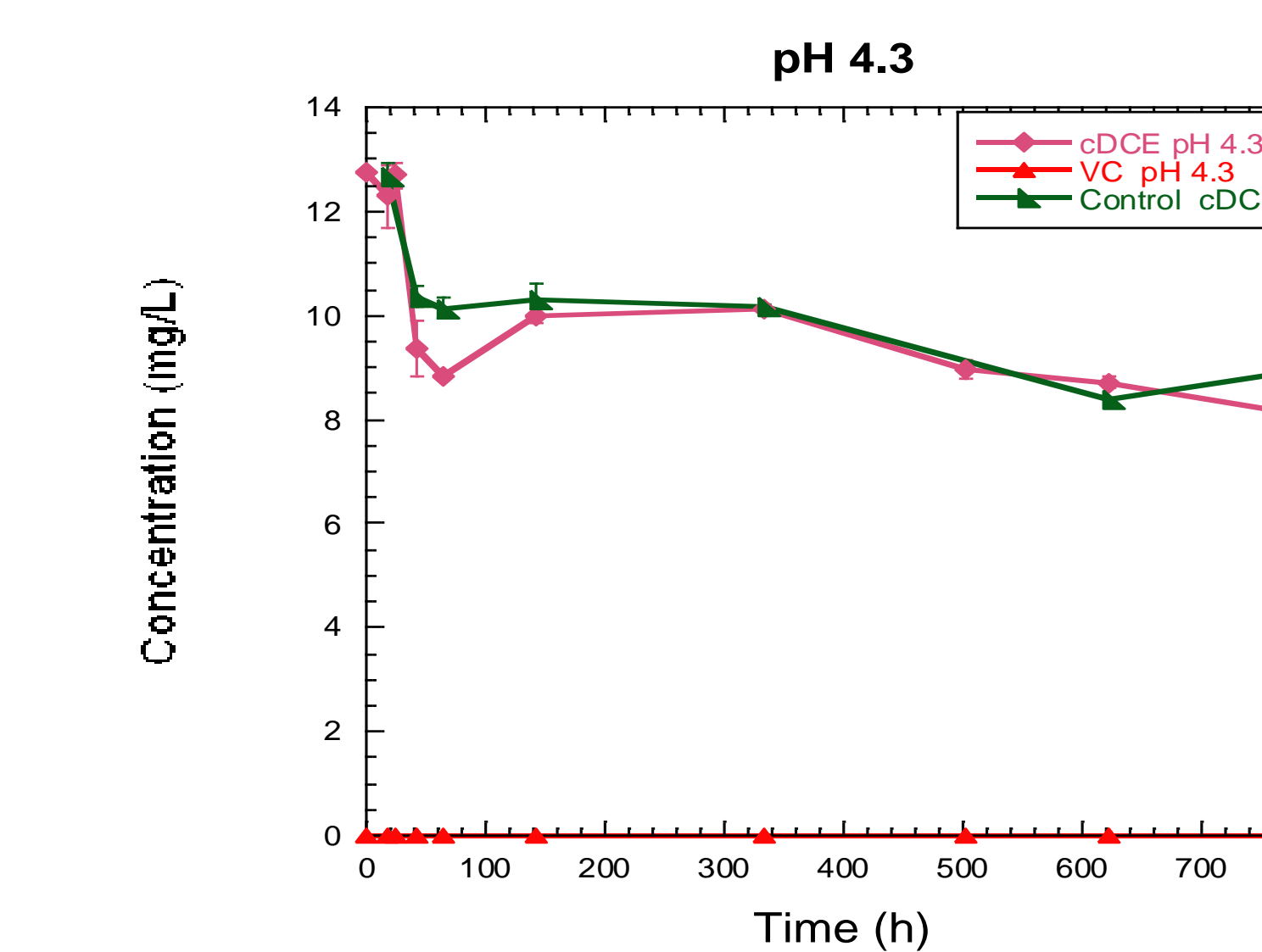
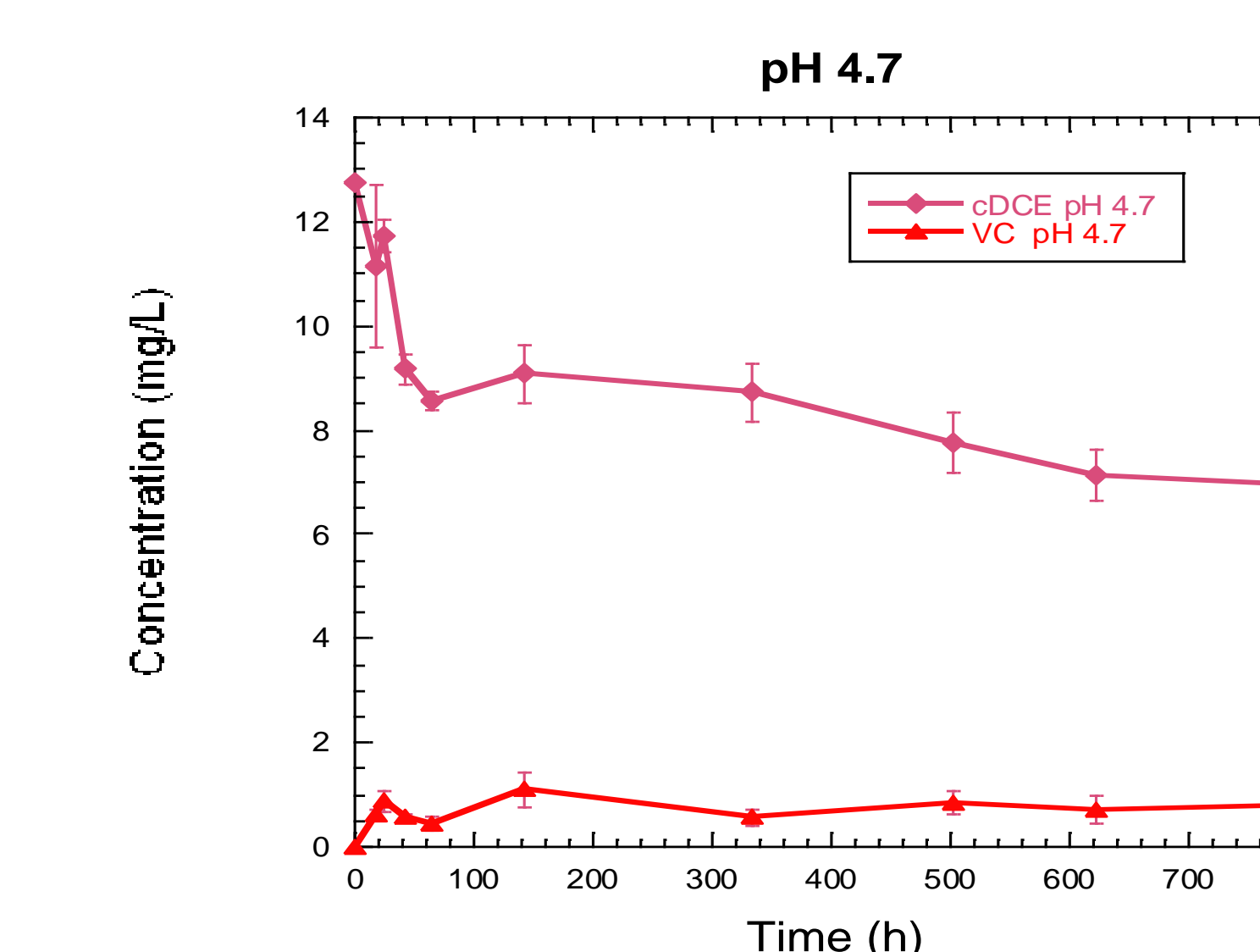
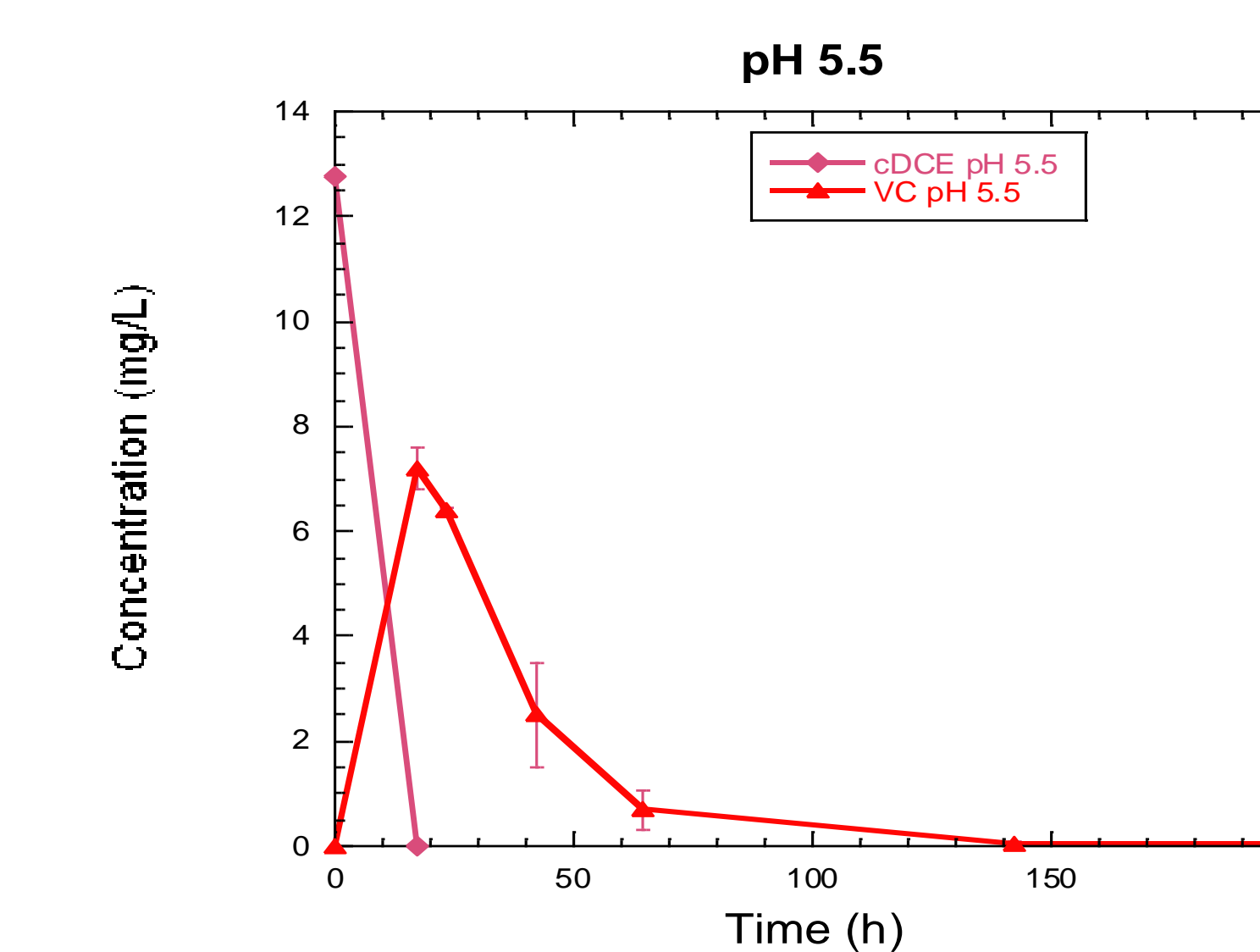
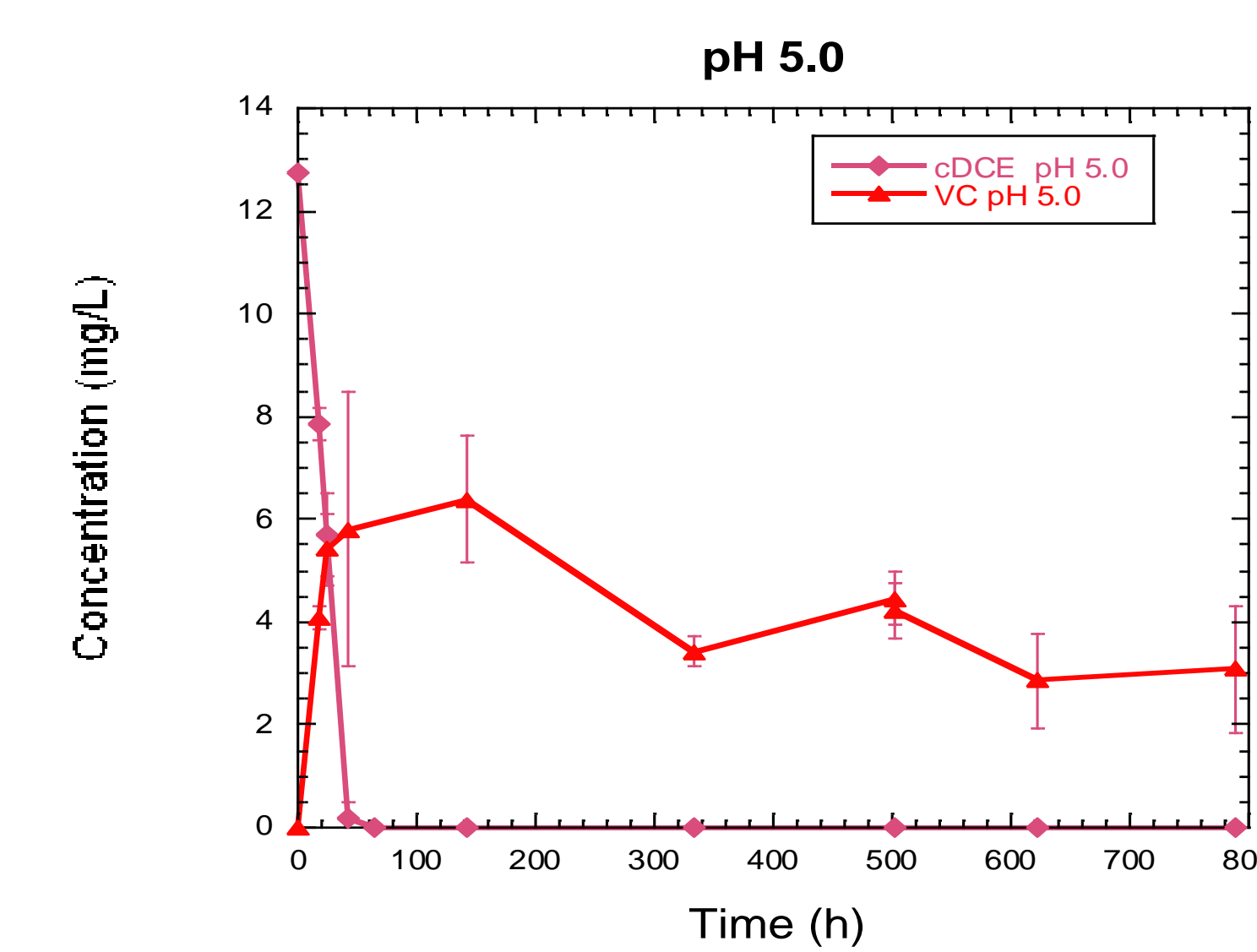
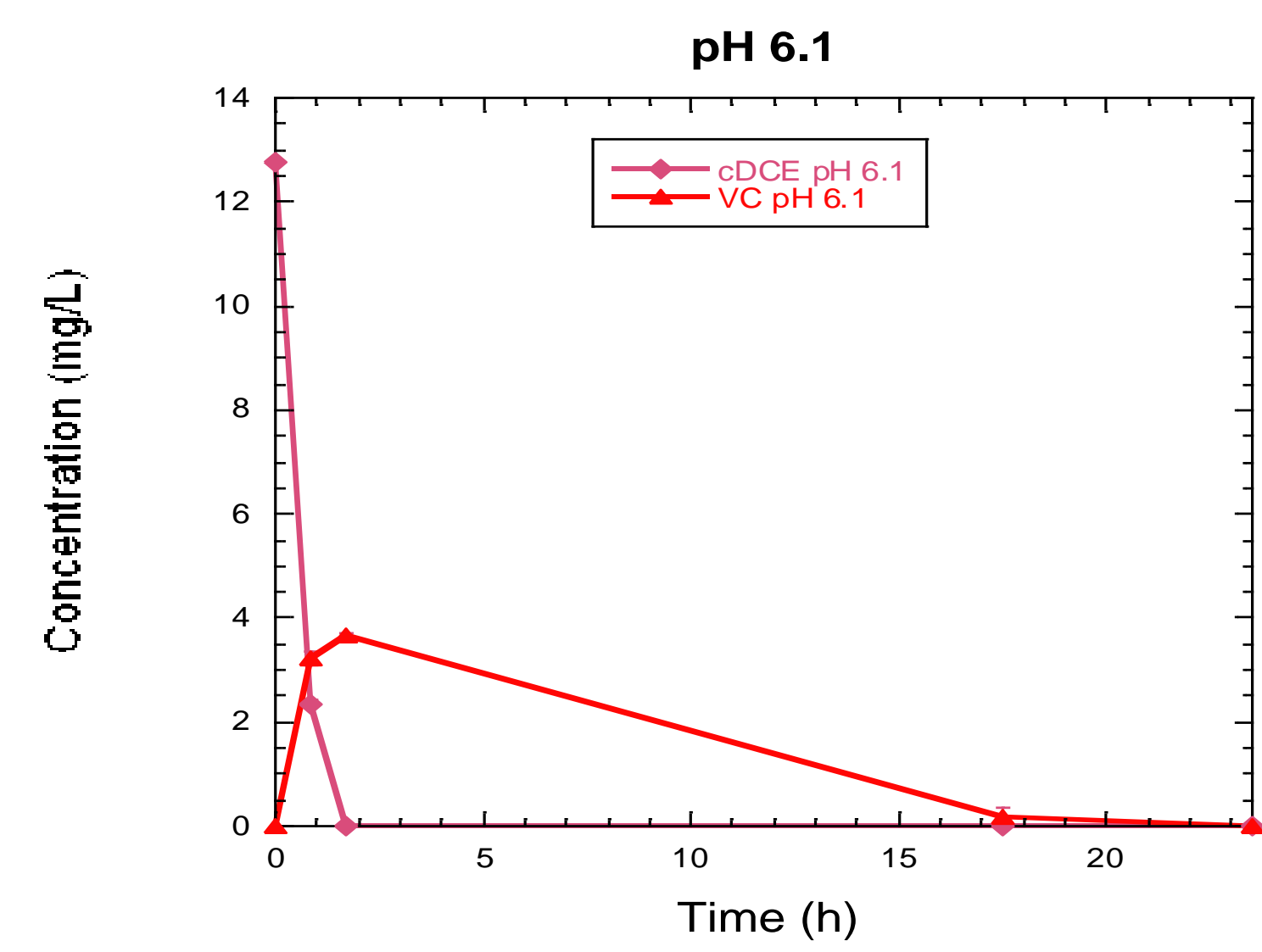
The presence of NaCl up to 6-8 g/L did not inhibit dechlorination activity, and only at 10 g/L of NaCl (6 g/L of chloride) was inhibition, primarily at the level of VC reduction, observed. The presence of acetone (to 40 mg/L), which can be produced in situ via fermentation of electron donor, and ethanol (to 10%) did not inhibit TCE dechlorination. Dechlorination was not observed in the presence of 15% ethanol. The presence of 500 mg/L methylene chloride completely inhibited complete PCE degradation. The exposure the culture to methane at pressure as high as 20 psi did not effect negatively on dechlorination activity of DHC bacteria.

Finally, experiments were performed to test the ability of the SDC-9 culture to degrade high concentrations of chlorinated solvents. The consortium was able to degrade PCE at its maximum soluble concentration (150 mg/L), and to degrade TCE, cis-DCE, 1,2-DCA and VC at concentrations as high as 230-250 mg/L. The results of this work will be useful for remediation practitioners as they prepare for the application of bioaugmentation cultures to remediate chlorinated solvent contamination.

LOW pH

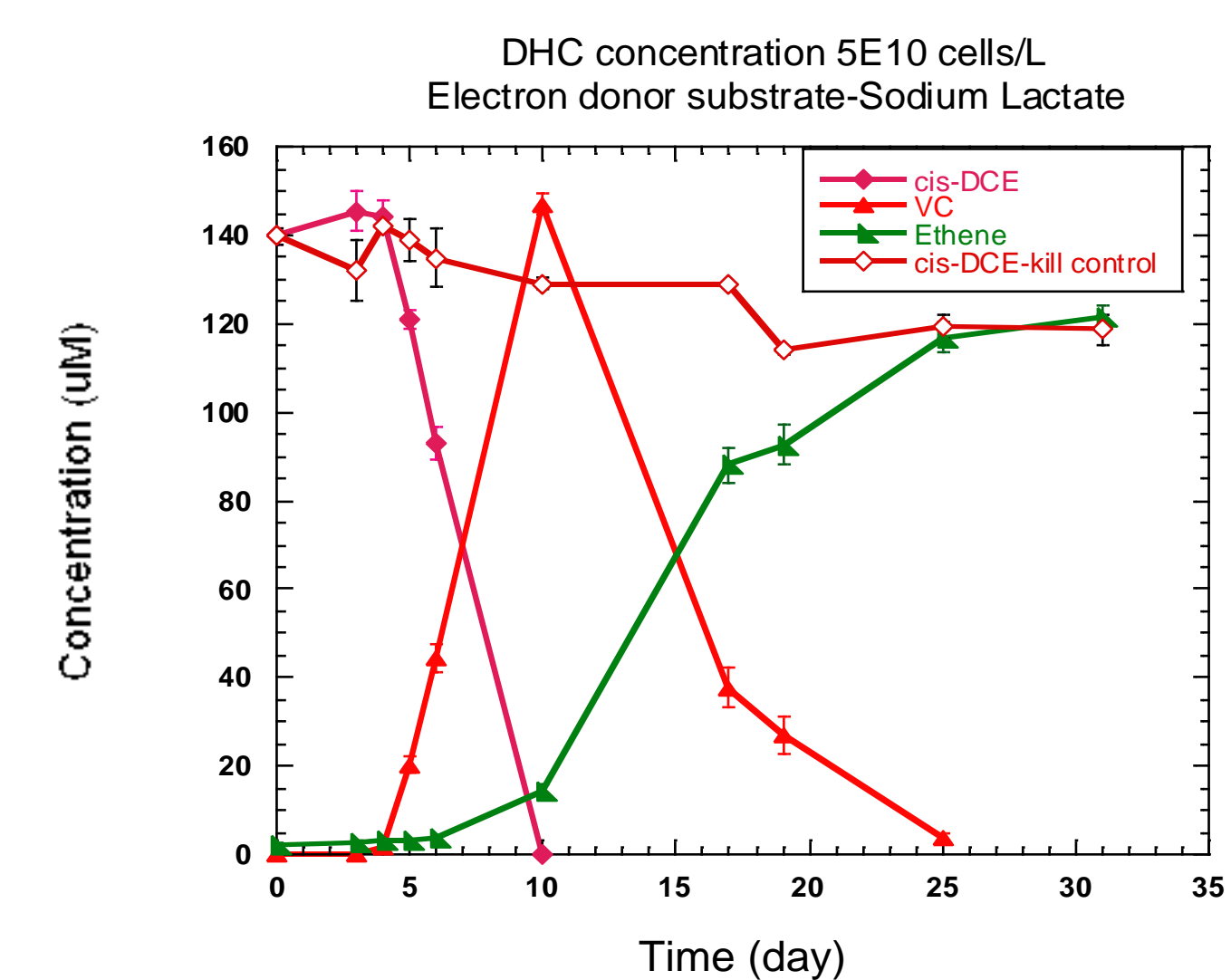
Degradation of cis-DCE by SDC-9 (15°C)

DHC concentration 5E11 cells/L
Vials were incubated 24 hours at different pH levels before cis-DCE and Sodium Lactate (6 mM) were added to vials.
Error bars represent SD (n=3).

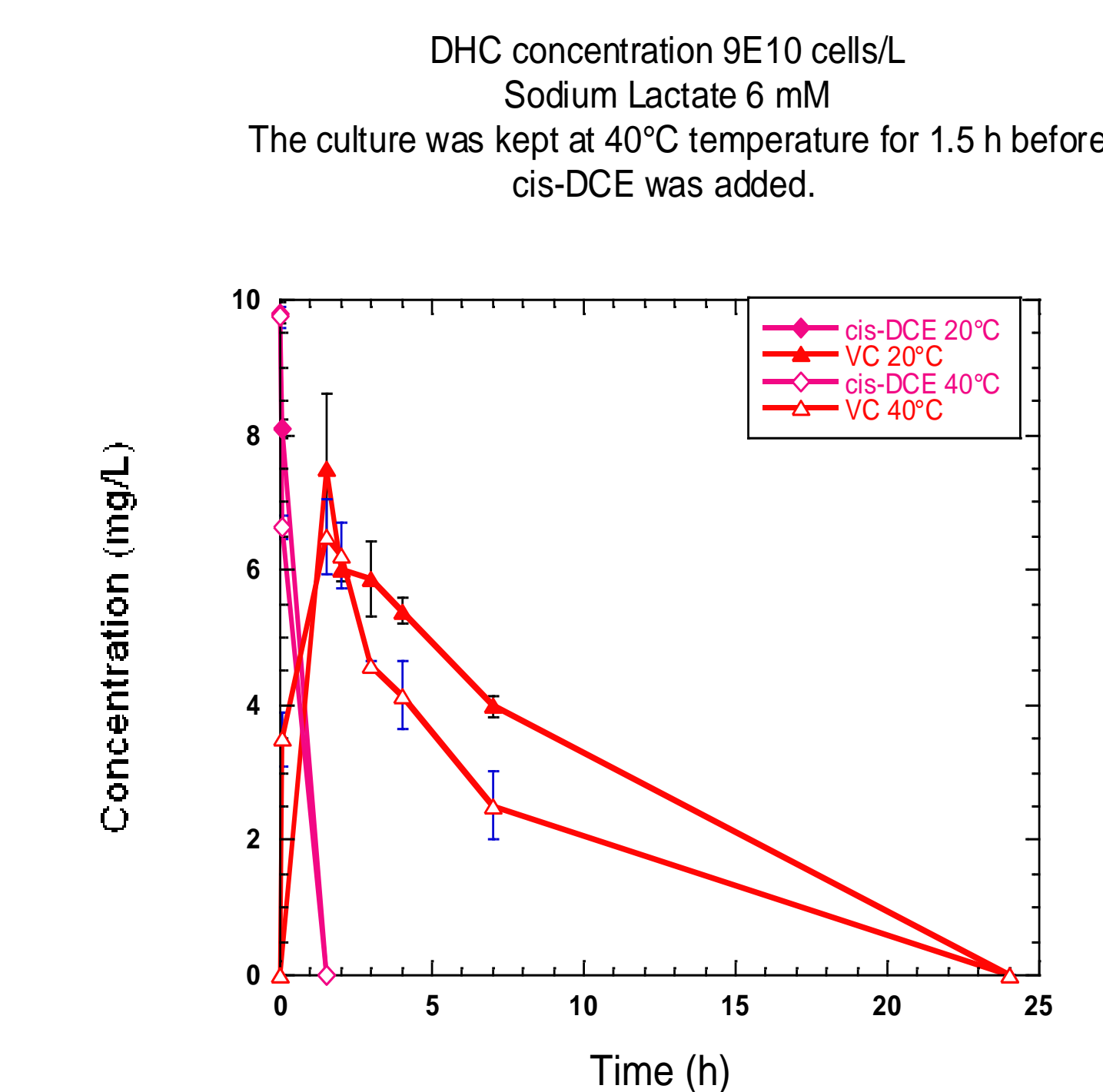


TEMPERATURE

Degradation of cis-DCE by SDC-9 (5.5±0.5 °C)

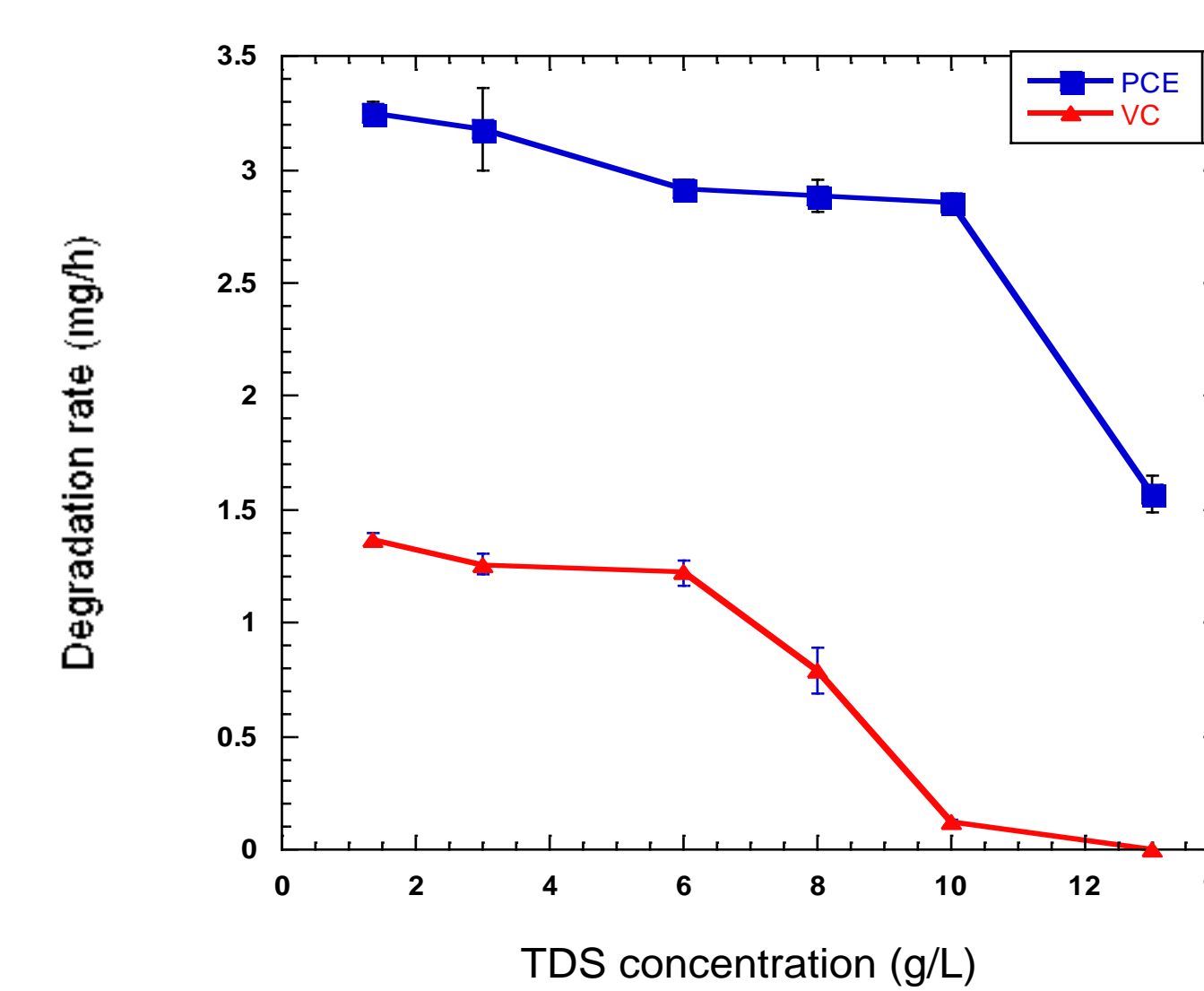


Dechlorination of cis-DCE by SDC-9 (40°C)



TDS

Effect of TDS on Degradation Rates of PCE and VC



HIGH pH

Effect of Elevated Groundwater pH and pH Neutralization Degradation of TCE by SDC-9

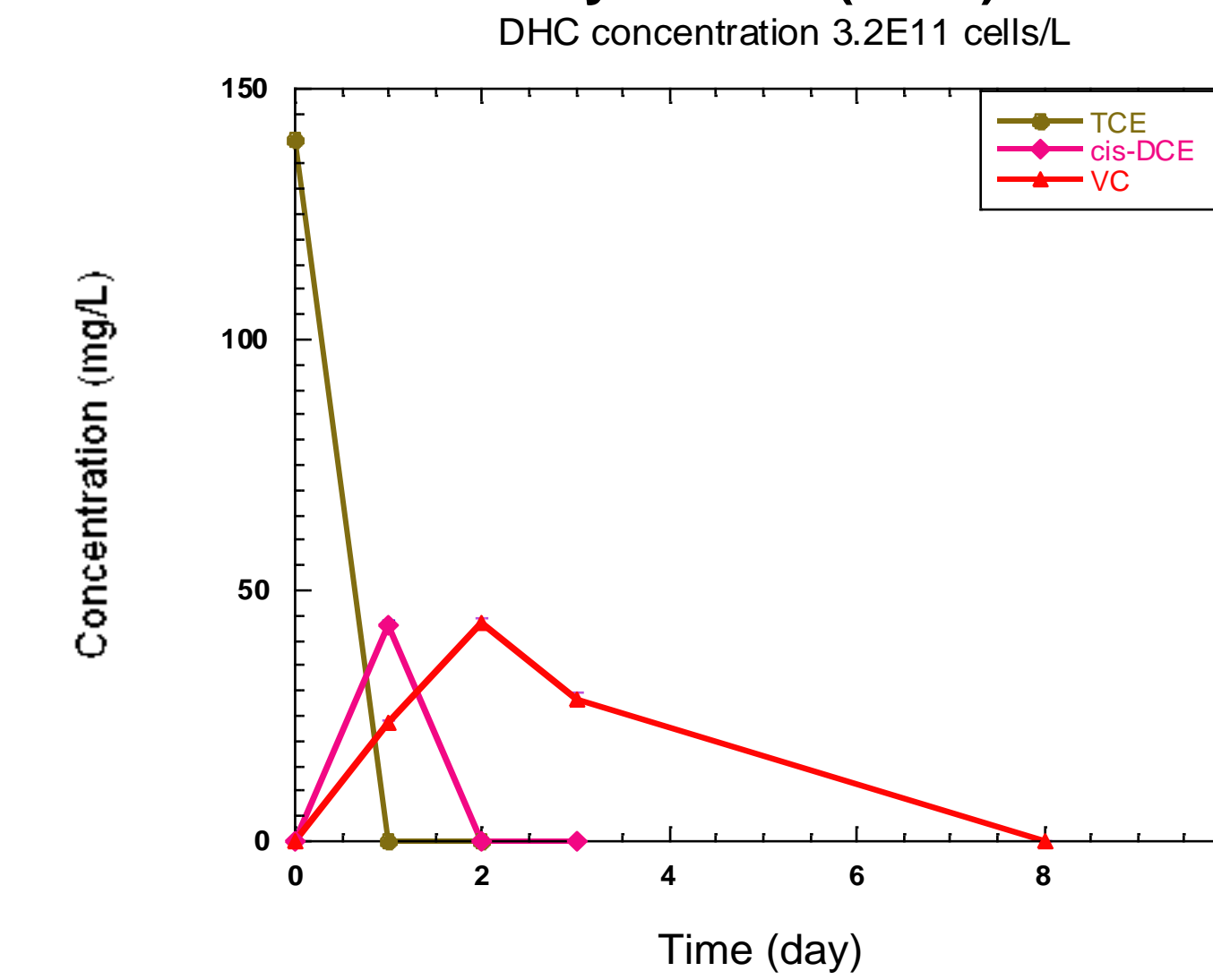
Test Conditions	Storage time (h)	Degradation rate (mg/h x g DW)	
		TCE	VC
1 pH 9.9 Groundwater with SDC-9	22	0.23	0
2 pH 9.9 Groundwater with SDC-9 following Neutralization to pH 7.0	22	0.38	0.07
3 pH 6.1 Groundwater with SDC-9	22	39.8	14

Conclusion:

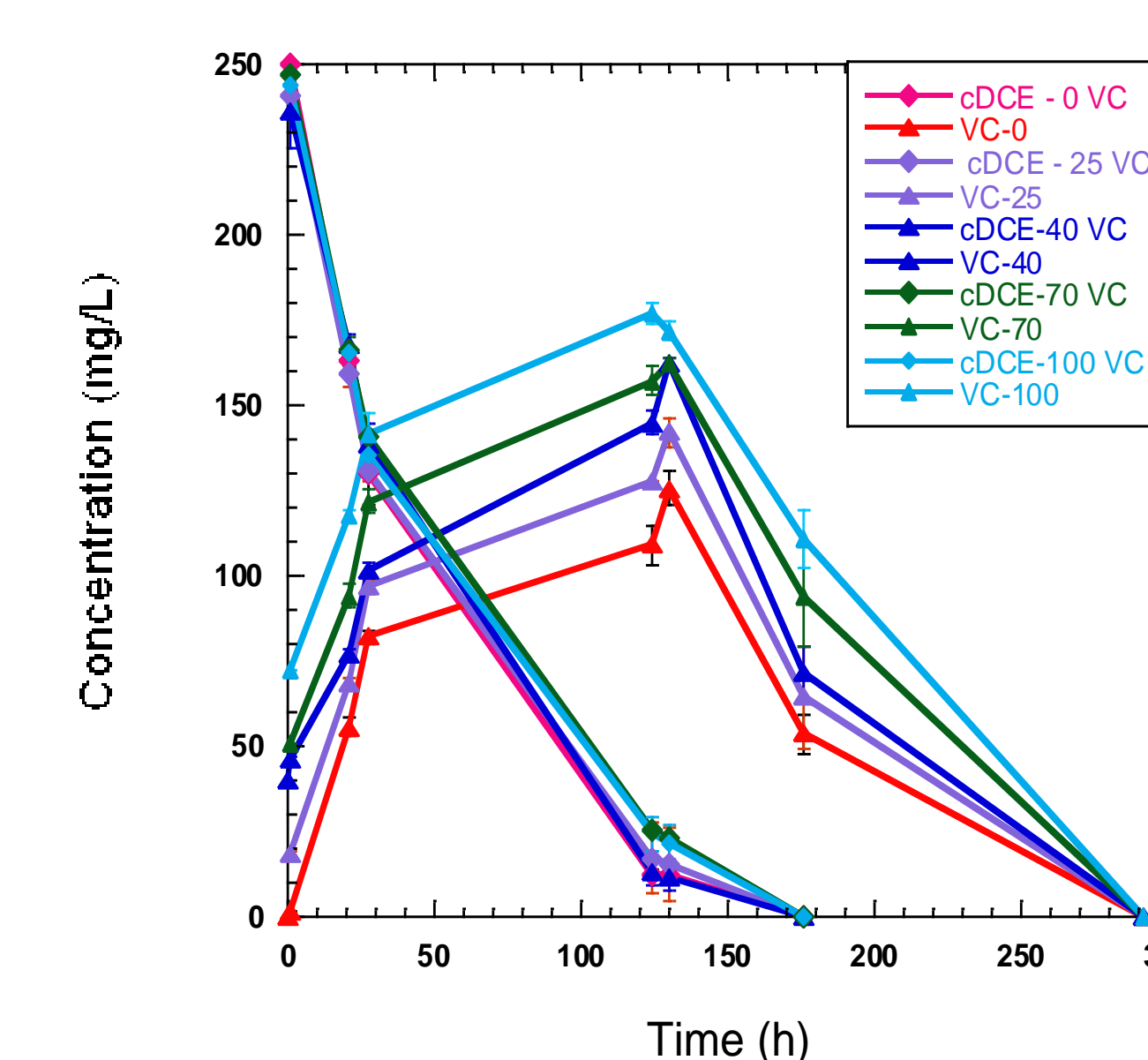
Exposing SDC-9 to pH 9.9 groundwater for one day at 13 °C followed by neutralization of the pH to 7.0 resulted in >99% loss of TCE and VC dechlorination activity

HIGH VOC CONCENTRATION

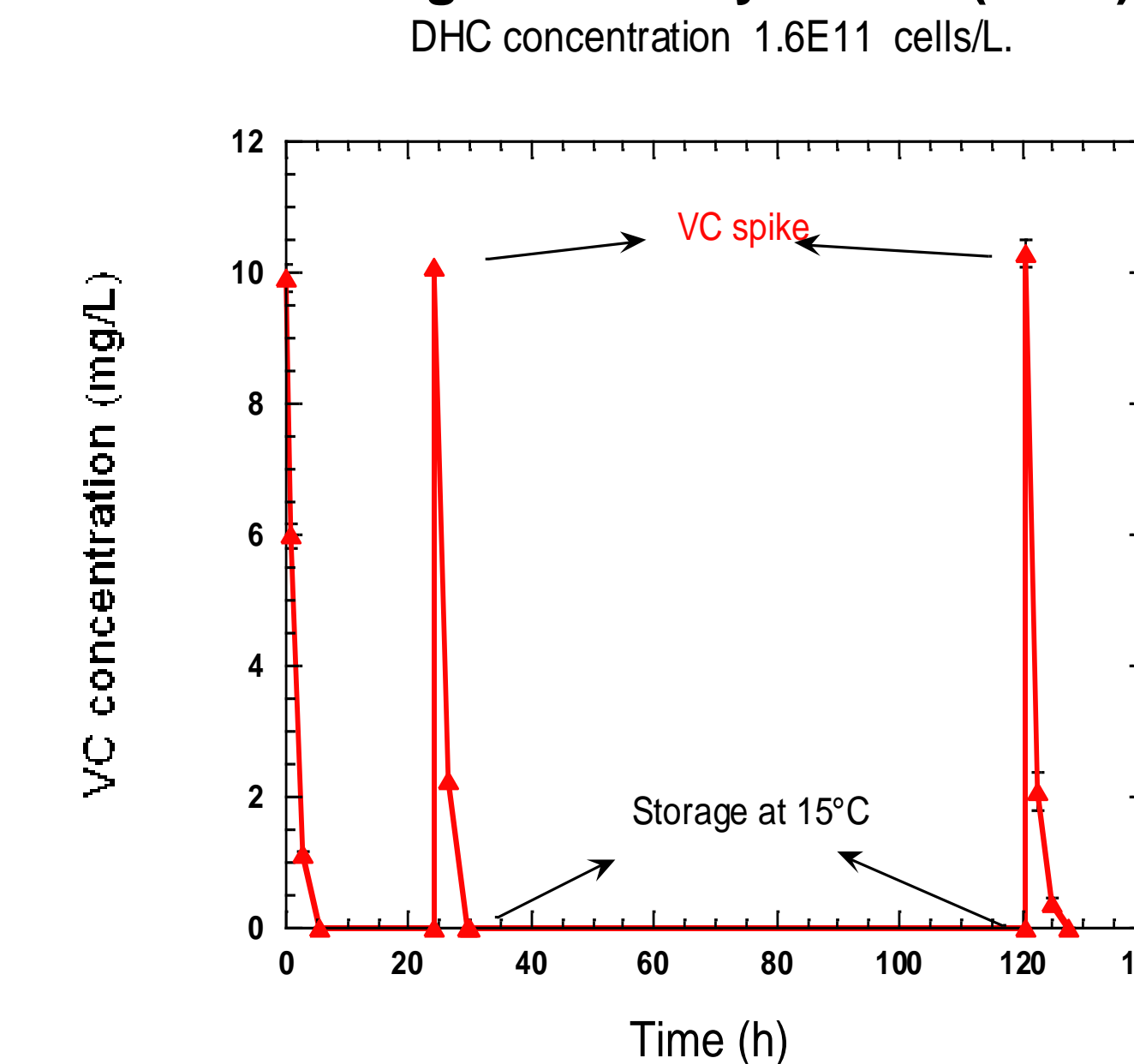
Degradation of Elevated Concentration of TCE by SDC-9 (28°C)



Degradation of Elevated Concentration of cis-DCE at Different Concentrations of VC

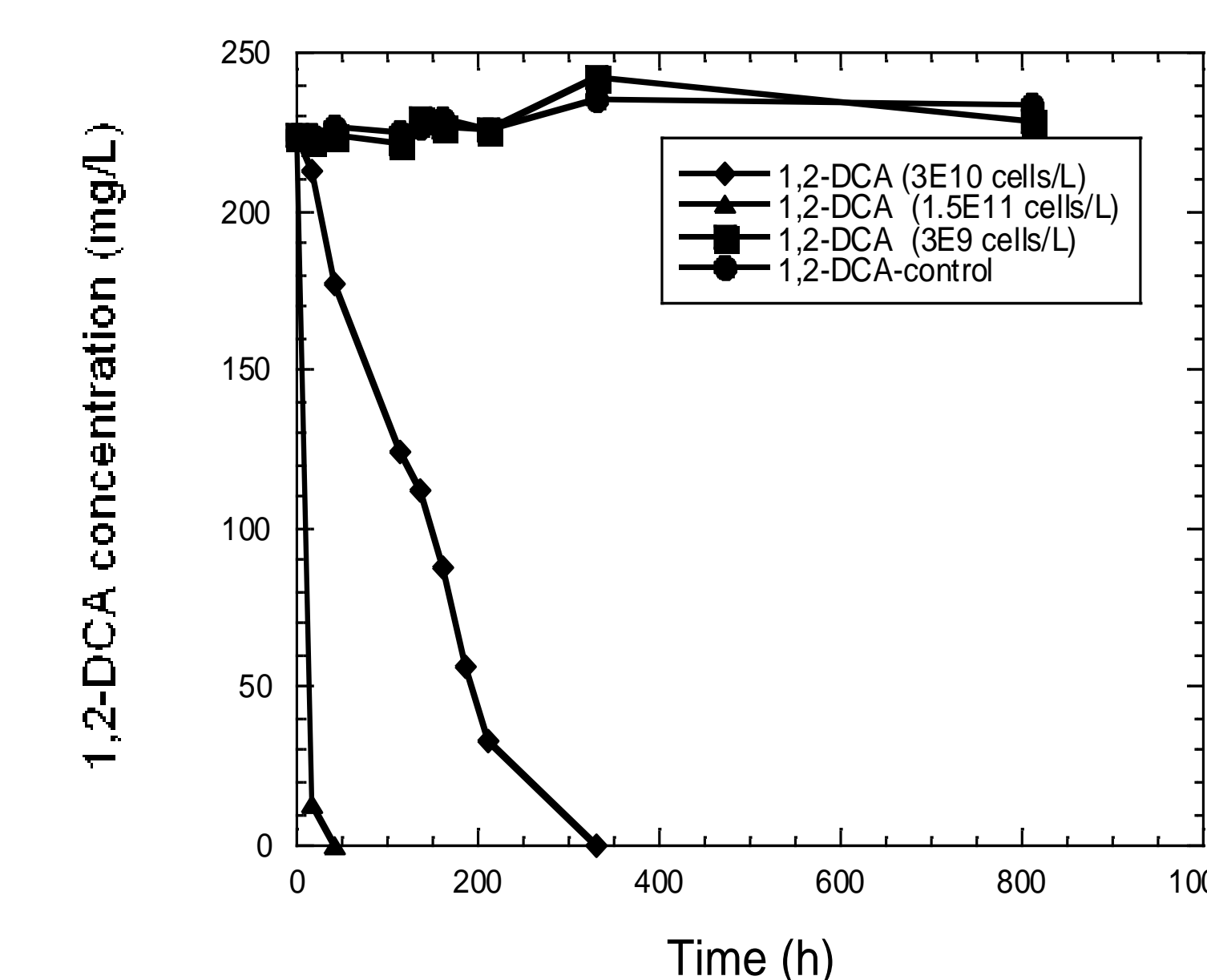


VC degradation by SDC-9 (30°C)



Degradation of elevated concentration of 1,2-DCA by SDC-9

22 °C. Sodium lactate was added at 6 mM. Error bars (SE; n=3) are smaller than symbols.



OTHER FACTORS

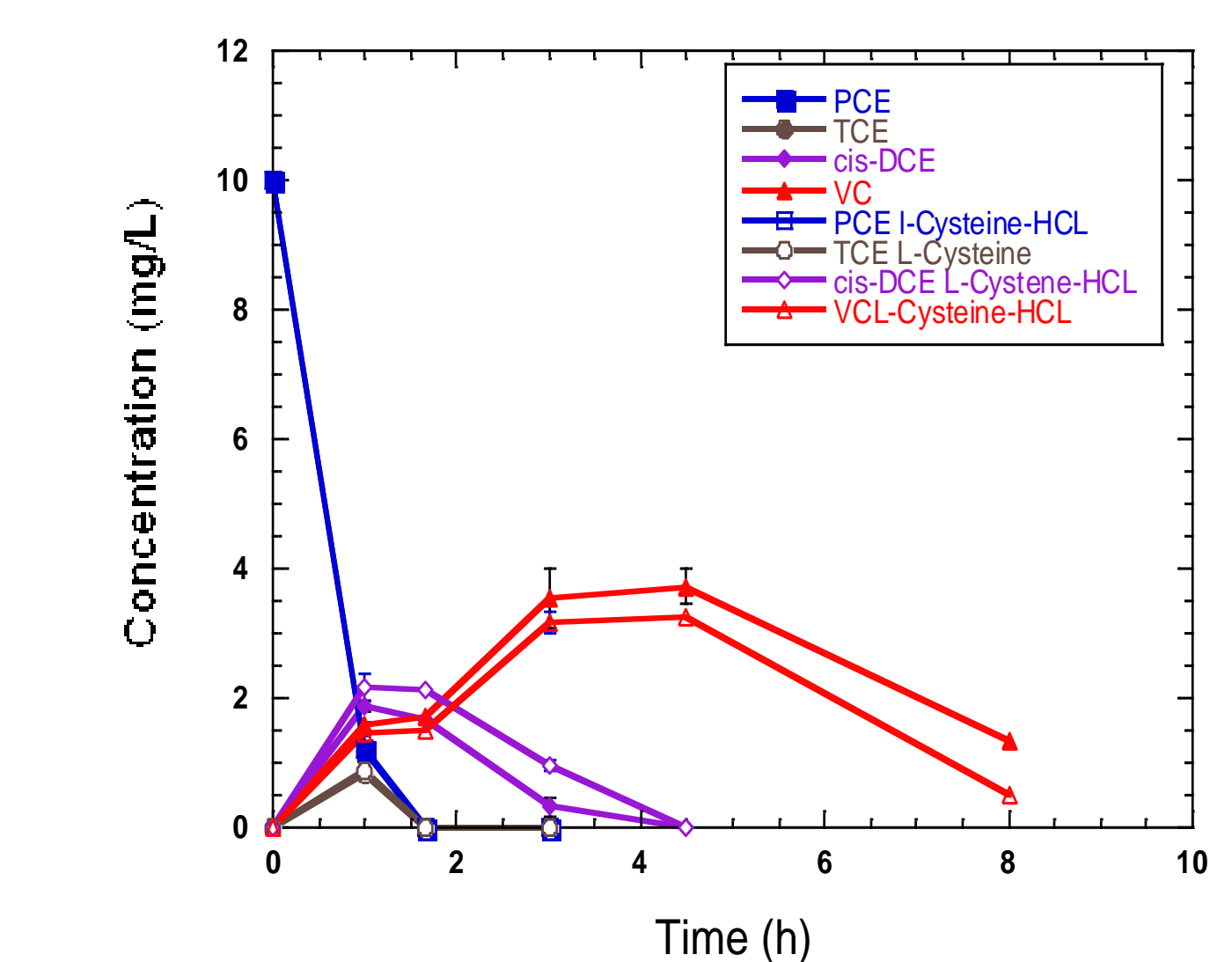
Effect of Ethanol on SDC-9 Degradation Activity

Ethanol (%)	Concentration after 8 h of incubation at 28°C (mg/L)			
	PCE	TCE	c-DCE	VC
0	0	0	0	0
5	0	0	0	0.29
10	0	0	0	3.69
15	8.9	0	0	0.69
20	10.6	0	0	0

Vials with SDC-9 and added ethanol were incubated for 24 h before PCE was added to vials.

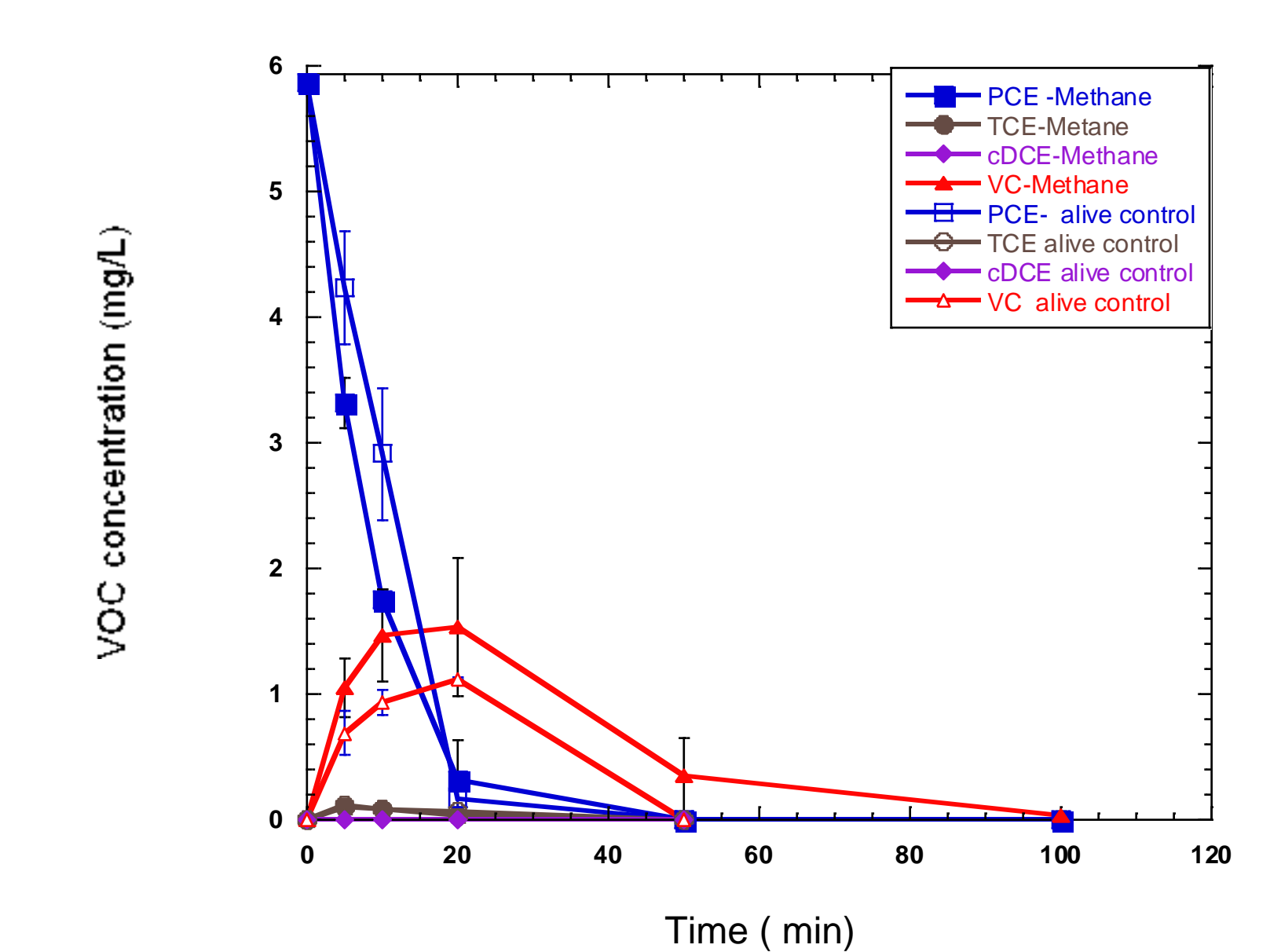
Effect of L-Cysteine-HCl on Dechlorination by SDC-9

L-cysteine-HCl was added at 0.69 g/L (2.6 g/gallon) of medium



Effect of exposure of SDC-9 to high concentration of Methane on dehalogenation activity.

Exposure time 17h. Methane pressure in headspace of vials was 20 psi.



Effect of different Acetone concentrations on TCE Dechlorination rate by SDC-9

DHC concentration 5E10 cells/L Sodium Lactate 12mM

