JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2004; **47**: 903–910.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.874

Research Article

A biological method for synthesizing ^{14}C-vinyl chloride

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Summary

^{14}C-labeled vinyl chloride (VC) was produced from ^{14}C-labeled trichloroethylene by an enriched, halorespiring culture, and purified using a hydrophobic column. This technically simple and economical method generated high yields of high-purity radiolabeled VC. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: vinyl chloride; trichloroethylene; halorespiration; carbon-14

Introduction

Vinyl chloride (VC) is a known human carcinogen and an EPA priority pollutant found in at least 496 of the 1430 sites on the National Priorities List.[1] Manufacturing generates roughly 27 million ton of VC per year in the US, predominantly in the production of the polymer polyvinyl chloride. VC accumulates in polluted groundwater through the anaerobic degradation of multi-chlorinated ethenes, such as trichloroethene (TCE) and tetrachloroethene (PCE).[2,3] Under anaerobic conditions and in the presence of sufficient electron donor, the substitution of chlorine by hydrogen in PCE, TCE and, to some extent *cis*-dichloroethene (*c*DCE), occurs relatively rapidly, a process known as halorespiration.[2,3] However, VC dehalogenates to ethene more slowly. Complicating matters, very few organisms utilize VC as a sole source of carbon and energy.[4,5]

Therefore, the implementation of efficient remediation strategies depends upon a well-developed understanding of the abiotic and microbial factors involved in the various degradation processes for VC. To this end radiolabeled

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Contract/grant sponsor: National Institute of Environmental Health Sciences; contract/grant number: 5P42-ESO4696-15

Received 24 June 2004
Accepted 13 July 2004

VC would be useful. Radioactive compounds produce an accurate and sensitive mass balance, as well as aid in the identification of intermediates. Coupled with the fractionation of microbial components, radiolabeled compounds also further elucidate the details of intracellular incorporation. Finally, radioactive compounds employed in microbial techniques such as isotopically labeled microarrays and microautoradiography combined with fluorescent *in situ* hybridization (MAR-FISH), link individual microbial physiology and phylogeny within complex communities.[6–10]

Regrettably, commercially available ^{14}C-VC requires an expensive custom synthesis, thus limiting its availability. This scarcity of ^{14}C-VC has limited the number of studies of VC mineralization using radiorespirometry with ^{14}C-VC and polluted aquifer materials.[11–16] Fortunately, radiolabeled stock versions of VC's halorespiration precursors (PCE, TCE, *c*DCE) are much less expensive and more readily available than ^{14}C-VC. Significant savings would be possible if VC could be made from these less expensive precursors. In this paper, we describe a simple synthesis procedure for the production of ^{14}C-VC from ^{14}C-TCE via biological halorespiration. Using a dechlorinating enrichment culture, ^{14}C-TCE was transformed to a mixture of radiolabeled VC and ethene. VC was separated from the resulting mixture using a purge and trap system which sorbed VC, resulting in a highly purified product. The ^{14}C-VC was then thermally desorbed as needed (Figure 1).

Results and discussion

The complete transformation of TCE to VC and ethene required 73 days (Figure 2). During this time, TCE was reduced to *c*DCE with trace amounts of putative *trans*-dichloroethene (*t*DCE) (verification against a pure standard not attempted) (Figure 3, chromatograms a and b). *c*DCE was eventually transformed to VC and some ethene (Figure 3, chromatogram c). Upon complete disappearance of *c*DCE, the headspace gas was flushed and VC was trapped on the hydrophobic column. After purging off the column at 230°C, only purified VC was found in the serum collection bottle (Figure 3, chromatogram d). The total number of moles contained within both the liquid and the gaseous fractions of the enrichment culture was determined via headspace analysis and extrapolated for the entire volume using Henry's constants. Using these numbers, complete transformation of TCE to VC and ethene was achieved. The total number of moles of VC present in the enrichment culture upon collection was used as the initial value for determining the amount of VC which could be recovered after trapping on the hydrophobic column. Using headspace analysis on the collection bottle and extrapolating to the entire volume, an estimated 94% recovery of VC from TCE was achieved.

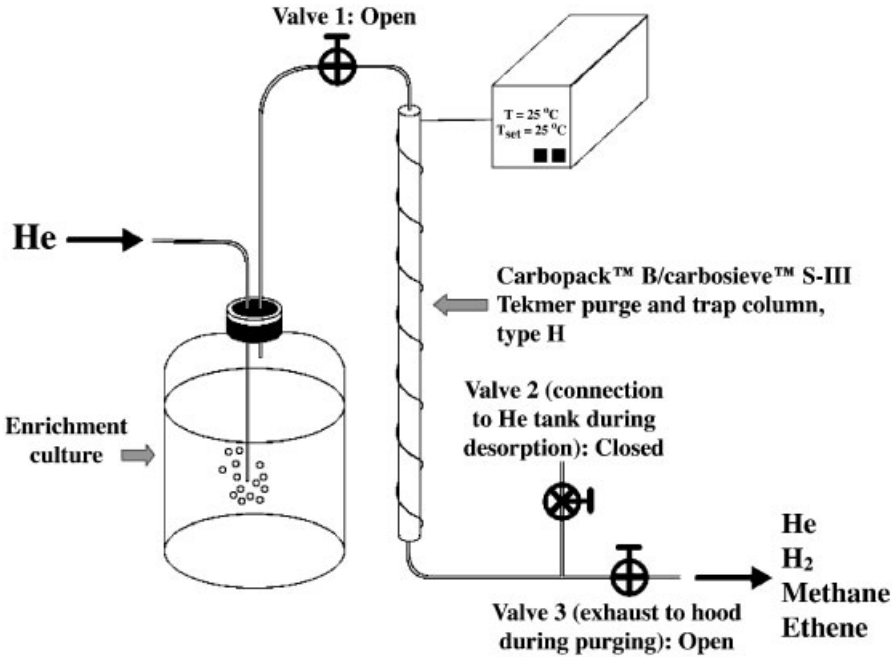

Figure 1. Purge and trap system for harvesting and purification of radiolabeled VC. Set up shown is in the configuration used to purge the culture following reductive dechlorination of TCE to VC. During desorption, the enrichment culture was replaced with an evacuated 160 ml serum bottle. Valve 3 was shut and the gas flow reversed by connecting a helium tank to the line leading from valve 2. Valve 2 was then opened and the column heated to 230°C

Using unlabeled VC, several experiments were conducted exploring different collection and storage efficiencies. While collection of VC in methanol and dry-ice was successful, the least complicated and efficient storage method involved leaving the VC on the hydrophobic column until needed. In this procedure, both column ends were plugged with stainless-steel screw caps and the entire apparatus stored at 4°C. This method may also minimize polymerization of VC, although we conducted no experiments to test this hypothesis.

This small-scale biological VC synthesis system is an economical, efficient and technically simple method for producing ^{14}C-VC. No other gaseous products were detected using GC-FID analysis, assuaging initial concerns regarding the purity of the final product. Our approach also produces VC free of solvent or other chemicals – a substantial advantage over commercial VC, which often contains polymerization inhibitors (such as phenol) and solvents that may have unpredictable effects on the microorganisms being studied.[17] The high yields for both the reductive dechlorination and the purge-and-trap

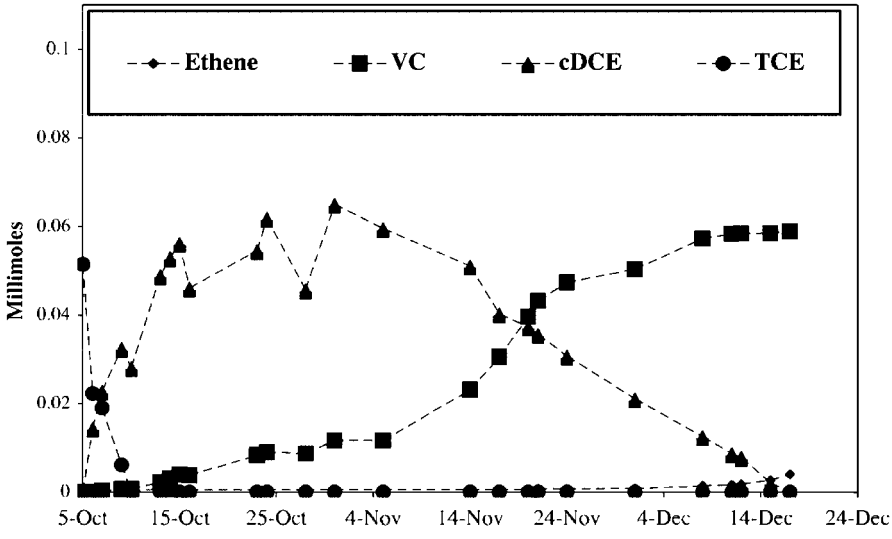

Figure 2. Time course of chloroethene transformation by the reductive dechlorinating culture

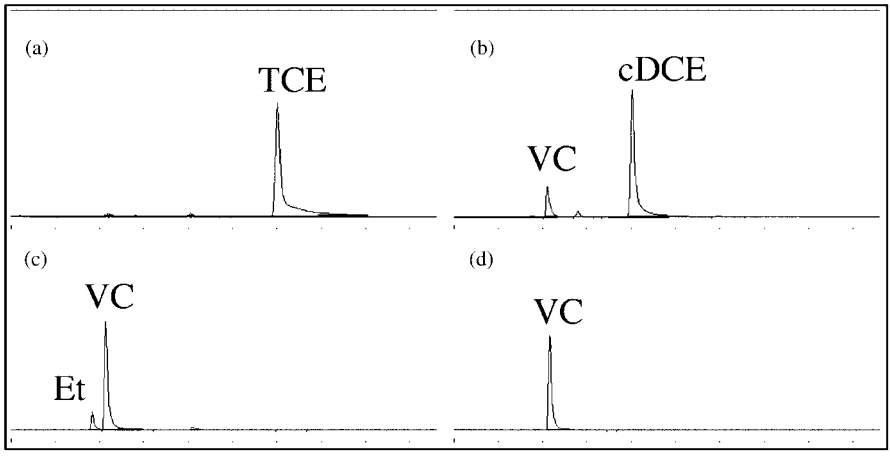

Figure 3. Chromatograms of various stages in synthesis procedure: (a) Headspace of reductive dechlorinating culture, day 0 (after equilibrium); (b) day 10 (15-October); (c) day 71 (15-December); and (d) desorbed product

collection system further validate this technique. Finally, storage of the ^{14}C-VC on the trapping column at 4°C prevents any substantial product loss and may inhibit polymerization.

A smaller amount of unlabeled TCE could be used if a shorter transformation time is desired, while still providing sufficient concentrations

of chloroethenes for monitoring. Alternatively, *c*DCE could be used instead of TCE, minimizing the presence of *t*DCE, which may be dechlorinated significantly more slowly by some dehalorespiring cultures. In separate experiments, unlabeled *c*DCE was converted to VC and ethene within 5 days by a methanogenic halorespiring enrichment culture, and VC recovered from the trapping column with VC recovery efficiencies ranging from 90 to 93% of the initial *c*DCE (data not shown). However, as can be seen in Figure 2, the additional time required to convert TCE to *c*DCE was not significant compared to the time required to transform *c*DCE to VC. Thus, for a particular enrichment culture, replacing TCE with *c*DCE may provide less benefit than reducing the total amount of chloroethenes.

While this study employed an enrichment culture, future applications might utilize a pure culture, such as *Dehalococcoides ethenogenes* strain 195, which accumulates large amounts of VC before producing ethene.[18–20] Use of a pure culture would likely reduce variability in the procedure and perhaps decrease the transformation time.

Experimental

Culture & media

Frank Löffler kindly provided a methanogenic reductive dechlorinating enrichment culture capable of reducing TCE to VC and eventually, ethene. The culture was transferred into fresh anaerobic media, prepared according to Löffler *et al.*[21], flushed with N_2 gas, and dosed with 10 µl of ^{14}C-TCE (35 nmol, 430 000 cpm; Sigma-Aldrich). In addition, the culture was provided with 0.20 mmol H_2 and 59 mg sodium pyruvate (bringing the initial liquid concentration to approximately 5 mM pyruvate) to serve as electron donor and carbon source, respectively. An additional 5 µl of TCE (approximately 56 µmol, Sigma-Aldrich) was added in order to bring the total TCE concentration up to a level easily monitored by GC-FID (as described below). The culture was incubated at room temperature on a shaker (300 rpm). Over a period of several weeks the headspace of the culture was periodically monitored in order to determine whether any of the VC precursors (TCE and dichloroethenes) remained.

Analytical methods

Headspace analysis was performed using a SRI 8610B gas chromatograph equipped with a flame ionization detector (GC-FID) and a capillary column (Supelco SPB624, 60 m × 0.5 mm ID). Helium was used as the carrier gas with a flow rate of 11.2 ml/min, and an isothermal oven temperature of 75°C, which provided sufficient separation of ethene, VC, *t*DCE, *c*DCE and TCE. Total concentrations were calculated using external standards by assuming liquid–

gas phase equilibrium and the following Henry's Law constants (M/atm, 20°C): 4.7×10^{-3} for ethene, 3.8×10^{-2} for VC, 0.27 for *c*DCE and 0.11 for TCE.[22,23]

Purge & trap system

VC and ethene were purged from the enrichment culture using a trapping apparatus (Figure 1). The system was equipped with valves configured to allow gas to pass from the reductively dechlorinating enrichment culture into a hydrophobic column or to allow gas to thermally strip trapped components from the column to a collection bottle. Gaseous compounds were purged with helium from the reductive dechlorinating culture onto a hydrophobic column (Carbopack™ B/carbosieve™ S-III Tekmar purge and trap column, type H, Supelco). The column trapped VC, while all but trace amounts of gases such as methane, ethene and hydrogen passed through unaffected. Commercial grade helium flowed through a needle inserted through the serum vial septum such that the tip of the needle was below the liquid surface of the culture. A relief needle was inserted through the septum into the headspace of the vial. Effluent gas passed through the second needle through stainless-steel tubing to the hydrophobic column, which was at room temperature. The culture was purged at room temperature for 30 min (100 ml/min flow rate), in order to completely strip all VC from the culture.

VC was removed from the hydrophobic column into an evacuated collection bottle (160 ml with a Teflon-lined stopper) by heating the column after all the trap valves were shut and the purge valves opened (see Figure 1). The helium flow rate was adjusted to 5 ml/min and the hydrophobic column heated to 230°C using electrical heat tape. The column was purged for 30 min, producing approximately 150 ml of gas in the collection bottle.

Radioactivity measurement

Radioactivity was measured using a Packard Liquid Scintillation counter (LSC) with the following program: 0–156 keV with a counting time of 10 min. 10 ml of Ultima Gold (Perkin Elmer) liquid scintillation (LS) fluid was used. Background radioactivity measurements were determined by running samples containing either 10 ml of LS fluid or 10 ml of LS fluid and 3 ml of TCE. The initial activity of ^{14}C-TCE was determined by triplicate injections of 10 µl TCE directly into the LS fluid. The activity of the VC was determined by injecting 100 µl of VC into a N_2-flushed, sealed 4 ml serum bottle filled with 3 ml of non-labeled TCE. It was assumed that all of the VC would partition into the TCE. The TCE containing ^{14}C-VC was then pipetted into 10 ml of LS fluid and measured using the same program as before.

Conclusion

Ultimately, the ^{14}C-VC produced by this method can be usefully employed in a variety of ways, such as mapping the carbon flux in dechlorinating communities. Since the limited availability of ^{14}C-VC precluded testing large numbers of aquifers, mineralization of VC may be underreported currently. Thus, this method can reduce one of the barriers to a better understanding of the fate of VC in the environment.

Acknowledgements

We thank Frank Löffler and Youlboong Sung for generously supplying the dechlorinating cultures used in this study. We also thank John F. Ferguson, Russell P. Herwig and David A. Stahl for their insights and suggestions. Funding for this work was provided by a grant from the National Institute of Environmental Health Sciences (5P42-ESO4696-15).

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