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Aerobic cometabolism revisited for *in situ* groundwater treatment: organisms, enzymes and remediation design considerations

Paul B. Hatzinger, David Lippincott, Graig Lavorgna and Mark E. Fuller

APTIM, Lawrenceville, New Jersey, USA

ABSTRACT

Aerobic cometabolism was first discovered more than 60 years ago and was the first biological approach to be evaluated for the treatment of chlorinated volatile organic compounds (cVOCs), such as trichloroethene (TCE). The discovery of reductive dehalogenation and subsequent anaerobic biostimulation and bioaugmentation processes supplanted cometabolism as the primary *in situ* biotreatment approaches for cVOCs beginning in the late 1990s. Anaerobic bioremediation has proven very effective for high concentration cVOC plumes. However, over the past few decades, dilute cVOC plumes (i.e., a few to a few hundred µg/L) have increasingly been recognized as an issue of concern and several contaminants have emerged that are not subject to anaerobic treatment approaches and/or are present in groundwater at very low concentrations, but above relevant regulatory or health guidance concentrations. These compounds include methyl tert-butyl ether, 1,4-dioxane, N-nitrosodimethylamine, 1,2-dibromoethane, and 1,2,3-trichloropropane, among others. For these compounds, as well as many cVOCs (when present in groundwater at low concentrations), aerobic cometabolism is reemerging as a promising and cost-effective remedial approach. This paper reviews aerobic cometabolism and provides examples and guidance concerning its field application for a number of traditional and emerging contaminants.

KEYWORDS

Aerobic; biodegradation; biostimulation; cometabolism; groundwater; remediation

Definition and history of cometabolism

Many different pollutants serve as growth substrates for aerobic bacteria and fungi. During biotransformation, the chemical is generally taken into the cell and modified by specific enzymes to one or more metabolites that then enter cellular metabolism (e.g., pyruvic acid, acetyl-CoA). In some cases, particularly for high molecular weight contaminants, extracellular enzymes also can be part of this process, cleaving the large substrates prior to cellular uptake. The intercellular metabolites are subsequently converted to energy and biomass through common metabolic pathways, with ultimate production of carbon dioxide (i.e., mineralization). Assuming contaminant concentrations are high enough (e.g., mg/L concentrations or greater) or the contaminant flux is sufficient (e.g., in groundwater), aerobic biodegradation typically results in increasing cell

numbers. This type of growth-linked biodegradation is desirable in that it can bring about a rapid loss of the pollutant from natural environments.

A second process was originally termed “co-oxidation” by Leadbetter and Foster (1959) to describe the aerobic conversion of several small alkanes into carboxylic acids and ketones (e.g., propane to propionic acid and acetone) by the organism *Pseudomonas methanica* (later renamed *Methylobacterium methanica*). The organism, a methanotroph, was unable to grow on any of these compounds, but oxidized them, nonetheless. The general concept of co-oxidation was subsequently expanded by Alexander (1967) to encompass non-growth supporting microbial reactions other than oxidation reactions of alkanes and simple hydrocarbons. This process was subsequently termed “cometabolism” and generally defined as “the transformation of an organic

CONTACT Paul B. Hatzinger ✉ paul.hatzinger@aptim.com 📍 APTIM, Lawrenceville, New Jersey, USA.

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compound by a microorganism that is unable to use the substrate as a source of energy or of one of its constituent elements...” (Alexander 1999). A general comparison of growth-linked and cometabolic biodegradation processes is provided in Figure 1.

During the next few decades after the original observation of Leadbetter and Foster (1959), it became apparent that cometabolic processes were very widespread among bacterial genera and that many different organic compounds were subject to these reactions. During this timeframe, the degradation of pesticides was at the forefront to biodegradation research, and many common pesticides, including dichlorodiphenyltrichloroethane (DDT) (Pfaender and Alexander 1973), parathion (Daughton and Hsieh 1977), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (Rosenberg and Alexander 1980) and kepone (or chlordane) (Orndorff and Colwell 1980), among others, were observed to be biotransformed by cometabolic processes. Similarly, the types of reactions reported expanded well beyond the originally described oxidations to include hydroxylations, denitrations, dechlorinations, deaminations, ether linkage cleavages, as a few examples (Alexander, 1999 and references therein). The first extensive review of cometabolism and cometabolic reactions in nature was published in 1972 (Horvath 1972).

Moving into the later 1970s to early 1980s, chlorinated volatile organic compounds (cVOCs),

including the solvents trichloroethene (TCE), perchloroethene (PCE), and 1,1-dichloroethane (1,1-DCA) among others, became an increasingly recognized as widespread groundwater contaminants in the United States (CEQ 1981; Jackson 2004). The sources of these compounds were varied, but the use of vapor degreasers in manufacturing and parts cleaning contributed heavily to their ubiquity as groundwater pollutants, as solvent disposal from these units was primarily open discharge. The absence of reliable analytical methods as well as the widespread belief that chlorinated solvents were largely of concern as workplace hazards and for smog and air quality, prevented hydrologists and other environmental professionals from recognizing the massive scope of this issue (Jackson 2004).

As the overall extent of chlorinated solvent contamination in aquifers became apparent, research commenced concerning their biological and abiotic breakdown, both of which were initially considered unlikely. Bouwer, Rittman, and McCarty (1981) first reported the anaerobic degradation of chlorinated methanes in a mixed methanogenic culture under anaerobic conditions. A number of subsequent studies then showed dechlorination of chlorinated ethenes, with the basic hypothesis that methanogenic bacteria were catalyzing these reactions, which often terminated at *cis*-dichloroethene (cDCE) and/or vinyl chloride (VC) (Barrio-Lage et al. 1986; Bouwer and McCarty 1983; Vogel and McCarty 1985). Our understanding of “reductive dehalogenation” improved over the next decade, and a new organism *Dehalococcoides ethenogenes* (now called *Dehalococcoides mccartyi*) was identified in the late 1990s, that can completely dechlorinate PCE to ethene (Maymó-Gatell et al. 1997). The laboratory studies from the 1980s, and subsequent field studies in the 1990s (e.g., Ellis et al. 2000; Major et al. 2002) led to the development of an industry that provides both substrates and cultures for the anaerobic treatment of chlorinated solvents.

Commensurate with the quest to understand reductive dehalogenation under anaerobic conditions, scientists also were looking for evidence of aerobic degradation of chlorinated solvents, largely without success (e.g., Bouwer, Rittman, and McCarty 1981; Wilson et al. 1983). However,

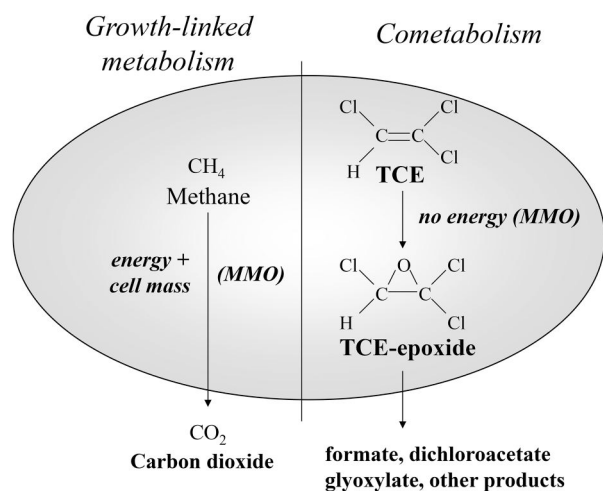


Figure 1. Representation of an individual methanotrophic bacterium degrading methane to carbon dioxide through growth-linked metabolism and degrading trichloroethylene (TCE) to TCE epoxide via cometabolism. MMO, methane monooxygenase. Modified and reprinted with permission from Hatzinger and Kelsey, 2023.

Wilson and Wilson (1985) first reported the oxidative degradation of TCE to carbon dioxide in studies using soil columns that were fed natural gas in air. Subsequently, Fogel, Taddeo, and Fogel (1986), showed that this same process could be mediated by a mixed methanotrophic culture, and then Little et al. (1988) reported the isolation of a methanotrophic bacterium (Strain 46-1), that com-
 etabolically oxidized TCE to carbon dioxide and chloride through an epoxide intermediate. Organisms that oxidize toluene were also observed to cometabolically degrade TCE to CO₂ and Cl during this same time period (Nelson, Montgomery, and Pritchard 1988; Winter, Yen, and Ensley 1989). These studies were important because they laid the foundation for field studies in which com-
 etabolic primary substrates, including methane (Piffner et al. 1997; Semprini and McCarty 1991, 1992), toluene (Hopkins and McCarty 1995), and butane (Semprini et al. 2007, 2009) were added to groundwater to stimulate aerobic TCE biodegradation.

One of the primary advantages of cometabolic bioremediation of TCE and other chlorinated solvents was that VC, a potent carcinogen, was not generated as a daughter product during the process, as it often was during reductive dehalogenation. However, there were also some issues in the practical application of this process. Firstly, some of the amendments tested at pilot scale (e.g., toluene and phenol; Hopkins and McCarty 1995; Hopkins et al. 1993) are not desirable in groundwater and others (e.g., methane, propane) represent an explosion hazard if not properly applied. Secondly, for chlorinated ethenes, such as TCE, the initial product of reaction is an epoxide (TCE-epoxide in this case), which can cause toxicity and DNA damage to the microorganisms mediating the reactions (Figure 1). The severity of these effects occurs at different levels depending on the rate at which the epoxide decomposes in the cells, with the epoxide of 1,1-dichloroethene (1,1-DCE) being particularly toxic (Anderson and McCarty 1996; Dolan and McCarty 1995; Zhang, Gedalanga, and Mahendra 2016). Thus, at sites with high concentrations of chlorinated ethenes, the cometabolic process is likely to fail due to cell

toxicity. Thirdly, competitive inhibition between the primary substrate (e.g., methane) and the contaminant of concern (e.g., TCE) could result in slow degradation rates, as contaminant degradation is limited to time periods and/or zones where the primary substrate is depleted in an aquifer (Criddle 1993; Kim et al. 2020).

Because of the limitations cited in the previous paragraph, reductive dehalogenation rather than aerobic cometabolism became the favored treatment approach for chlorinated solvents at field scale. Bio-stimulation (adding carbon substrates) and bioaugmentation (adding dehalogenating organisms with carbon substrates) became commonplace beginning in the late 1990s and continuing today, with thousands of sites treated using these processes (e.g., Stroo, Leeson, and Ward 2013). However, for many current chlorinated solvent sites of concern, the concentrations of the relevant cVOCs are exceedingly low (i.e., tens to hundreds of µg/L), well below concentrations that can support long term growth of dehalogenating bacteria. Moreover, many of these dilute cVOC sites are aerobic, and the negative impacts of adding large quantities of carbon substrates to create anaerobic conditions (e.g., sulfide and methane production, solubilization of metals including As, Fe and Mn, groundwater acidification) may outweigh the potential benefits of cVOC treatment *via* reductive dehalogenation (Mueller, Karachalios, and Fowler 2014; Mueller and Booth 2016; Wang et al. 2016). In many instances, aerobic cometabolism can be a viable treatment strategy for large dilute cVOC plumes, as well as for sites with a wide range of “newer” or “emerging” contaminants, including, but not limited to methyl tert-butyl ether (MTBE), 1,4-dioxane (1,4D), N-nitrosodimethylamine (NDMA), 1,2-dibromoethane (EDB), and 1,2,3-trichloropropane (TCP), among others. These compounds may occur alone or be mixed with dilute cVOCs (e.g., 1,4D, NDMA) or, in some cases petroleum hydrocarbons (MTBE, EDB). This paper revisits cometabolism as a groundwater treatment technology with a primary focus on aerobic cometabolic processes and provides examples of *in situ* treatment approaches that have shown success at field scale, overcoming previous implementation issues.

Organisms and enzymes

There are a wide variety of microorganisms and enzymes capable of performing cometabolic reactions. For the purposes of this review, the most common classes of aerobic bacteria and enzymes involved in the cometabolic degradation of traditional (e.g., cVOCs) and emerging (e.g., MTBE, 1,4D, EDB, NDMA) groundwater pollutants will be covered. The review will not encompass the wide variety of organisms/enzymes that cometabolize pesticides and other organics commonly found in surface soils. In general, the organisms are grouped based upon the primary substrate(s) that they oxidize, and include short-chain alkanes (methane, ethane, propane, butane), short-chain alkenes (ethene, propylene), ammonia, and small aromatics (e.g., toluene).

While a variety of different enzymes are capable of catalyzing co-metabolic reactions, those of most interest and applicability for remediation purposes are typically oxygenases, which incorporate oxygen atoms derived from O₂ into the target compound. In general, oxygenases involved in cometabolic reactions are divided into two types: monooxygenases which add a single oxygen atom to a substrate and dioxygenases which incorporate two oxygen atoms. Some of these enzymes require co-factors that include various metal atoms (e.g., Fe, Cu) (Banerjee, Jones, and Lipscomb 2019; Bugg 2003; Chiliza, Martínez-Oyanedel, and Syed 2020; van Berkel, Kamerbeek, and Fraaije 2006). A generalized scheme of monooxygenase and dioxygenase reactions with different substrates is presented in Figure 2 (adapted from Li et al. 2002; Ji et al. 2013). These enzymes can possess a very high regioselectivity with respect to where in the molecule the oxygen atoms are incorporated (Fasan 2012; Huijbers et al. 2014; Leak et al. 2009; Liang et al. 2018).

Among the different oxygenases, soluble di-iron monooxygenases (SDIMOs) are particularly important, as many of the many (but not all) common cometabolic enzymes fall into this class. The SDIMOs are typically categorized into 6 groups based on homology of amino acid sequences and primary substrates, as follows: Group 1, toluene monooxygenases; Group 2, phenol

monooxygenases; Group 3, soluble methane monooxygenases; Group 4, alkene monooxygenases; Group 5, propane/tetrahydrofuran monooxygenases; and Group 6, propane/short-chain alkane monooxygenases (Leahy, Batchelor, and Morcomb 2003; Skinner et al. 2024; Yang et al. 2025). A new Group 7 of alkene monooxygenases has also recently been proposed (Yang et al. 2024). A more detailed recent review on the genetic organization, types and reactions of SDIMOs is provided in Yang et al. (2025). Note that propane monooxygenases fall into two different groups (Group 5 and Group 6). This is discussed further in *Propane-oxidizing bacteria* below. It is important to note that individual cometabolic organisms often have and express multiple oxygenases. A nice example of this is *Mycobacterium chubuense* strain NBB4, which contains at least 6 different monooxygenase enzymes, giving it the capability of growing on C₂–C₄ alkenes and C₂–C₁₆ alkanes, and cometabolizing a variety of chlorinated ethanes and ethenes (Coleman et al. 2011; Cheung et al. 2013). Brief descriptions of key groups of cometabolic bacteria are provided below.

Short-chain alkane oxidizers (C1 – C4)

Methane-oxidizing bacteria

Methane-oxidizing or “methanotrophic” bacteria are widespread in the environment and have received extensive study over the past several decades. As noted earlier, the first reports of TCE oxidation were attributed to methanotrophic bacteria (Fogel, Taddeo, and Fogel 1986; Wilson and Wilson 1985), and these findings were substantiated when pure methanotrophs capable of oxidizing TCE were isolated from groundwater (Little et al. 1988). Methanotrophs possess two common monooxygenases which oxidize methane to methanol, particulate methane monooxygenase (pMMO), which is membrane-bound and has a copper active site, and soluble methane monooxygenase (sMMO) which is a cytoplasmic SDIMO found in Group 3 (Hanson and Hanson 1996; Koo and Rosenzweig 2021).

MMOs oxidize a variety of chlorinated ethenes, ethanes and methanes, as well as several other common groundwater contaminants, including TCP, MTBE and NDMA (Bosma and Janssen

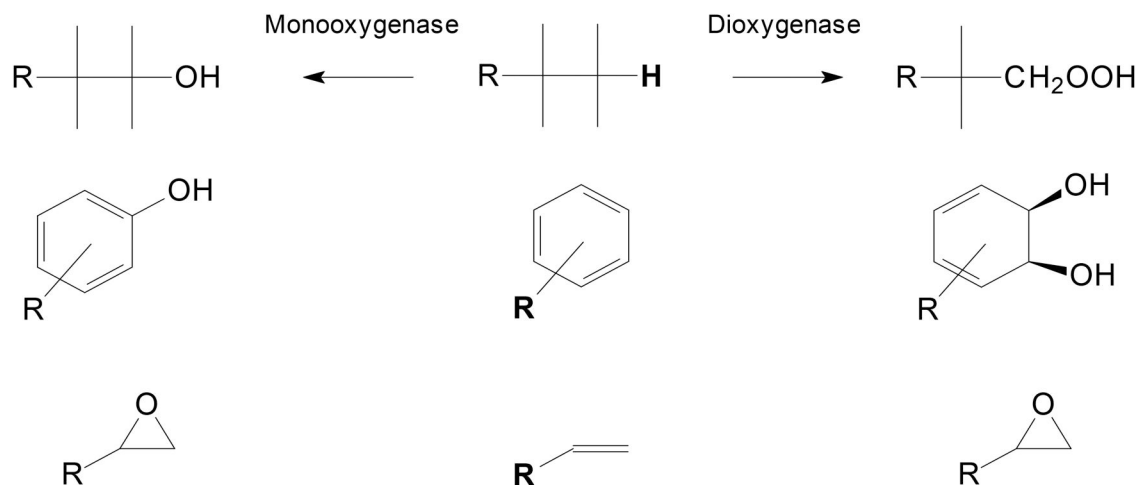


Figure 2. Examples of monooxygenase and dioxygenase reactions with different substrates. Adapted from Li et al. (2002) and Ji et al. (2013).

1998; Hand, Wang, and Chu 2015; Im and Semrau 2011; Malachowsky et al. 1994; Wackett et al. 1989; Wang and Chu 2017). 1,4D was originally reported to be oxidized by sMMO in *Methylosinus trichosporium* OB3b (Mahendra and Alvarez-Cohen 2006). However, a later study with microcosms, pure cultures, and pure sMMO enzyme showed no degradation of 1,4D at environmentally relevant concentrations (Hatzinger et al. 2017). The compounds oxidized by methanotrophs as well as rates of oxidation vary somewhat based on which MMO is expressed, with sMMO typically oxidizing a wider variety of compounds at faster rates than pMMO (Anderson and McCarty 1997; Hanson and Semrau 1996; Lee et al. 2006; Lontoh and Semrau 1998; Oldenhuis et al. 1991; Tsien et al. 1989). In laboratory studies, excluding Cu from growth media promotes the expression of sMMO vs pMMO, as the latter requires Cu as a co-factor, and its presence represses sMMO expression and inhibits activity (Semrau 2011). In the field, however, it is difficult to control Cu concentrations (particularly to reduce Cu to promote sMMO vs pMMO), so the key organisms/enzymes stimulated by methane addition will depend, in part, on this variable.

Propane-oxidizing bacteria

Propane-oxidizing bacteria or “propanotrophs” can oxidize a broad suite of traditional and emerging environmental contaminants, including many cVOCs, MTBE (and other gasoline oxygenates),

NDMA, 1,4D, TCP, and EDB (Chen and Hyman 2023; Fournier et al. 2006, 2009; Frascari, Zonaroli, and Danko 2015; Steffan et al. 1997, 1999; Wang and Chu 2017). A majority of these bacteria, as well as those that grow on ethane and butane (see subsequent sections) are Gram-positive actinomycetes, belonging to one of several different genera, including *Rhodococcus*, *Mycobacterium*, and *Nocardia*, among others (Shennen 2006). As noted above, propane monooxygenases are found in both the Group 5 and Group 6 SDIMOs. The Group 5 propane monooxygenase (PrMO), which is found characteristically in the organism *Rhodococcus jostii* RHA1 is capable of oxidizing propane, NDMA, and perhaps TCP (Sharp et al. 2007; Wang and Chu 2017; Chen 2021), whereas the Group 6 enzyme (also known as short-chain alkene monooxygenase; SCAM) oxidizes a wide range chlorinated alkenes and ethanes as well as 1,4-D and likely EDB (Chen and Hyman 2023; Skinner et al. 2024). Many bacteria possess both PrMO and SCAM, and likely express these enzymes simultaneously (M. Hyman, personal communication). Interestingly, however, the branched chain compound isobutane, selectively induces SCAM vs traditional PrMO, at least in one of the organisms evaluated to date, *Rhodococcus rhodochrous* ATCC 21198 (Chen 2021). Given that SCAM oxidizes a much broader array of COCs than PrMO, and at relatively fast rates, the use of isobutane in field studies (e.g., with a mixture of 14D and chlorinated ethenes, such as VC and cis-DCE), may provide a selective advantage over propane (Hyman 2023).

Ethane-oxidizing bacteria

Ethane-oxidizing bacteria “ethanotrophs” have not received the same level of attention as either methanotrophs or propanotrophs, either physiologically or in terms of cometabolic capabilities. There is also no specific “ethane-oxidizing” SDIMO at present. Rather, it is likely that a subset of SCAM and PrMO enzymes oxidize ethane to ethanol, as some but not all propanotrophs also can oxidize ethane (e.g., Redmond, Valentine, and Sessions 2010; Shennen 2006). Despite the general questions regarding microbial ethane utilization, this alkane has been used as a cometabolic substrate for successful *in situ* treatment of EDB (Hatzinger et al. 2018), and *ex situ* treatment of chlorinated ethenes (Chi et al. 2023). Ethane oxidizers also have been observed to cometabolize 1,4-D (Hatzinger et al. 2017; Tesfamariam et al. 2024; Xiong et al. 2020).

Butane-oxidizing bacteria

Numerous butane-oxidizing bacteria have been described over the past several decades, including the Gram-negative *Thauera butanivorans* (formerly *Pseudomonas butanovora*) (Cooley et al. 2009; Takahashi et al. 1980) and a variety of Gram-positive strains, such as *Arthrobacter* sp. PG-3-2 (Zhang et al. 2013). Butane-oxidizers are often capable of using C2 to C9 alkanes (but not C1 compounds) for growth. The enzyme butane monooxygenase, a Type 3 SDIMO encoded by the *bmoX* gene, has been identified as one key enzyme involved in butane oxidation to butanol in these organisms (Sluis, Sayavedra-Soto, and Arp 2002). Interestingly, much like sMMO, BMO can be repressed in the presence of copper ions (Doughty et al. 2008). This indicates the strong similarity between these Group 3 SDIMOs. Butane has been used as a substrate an early field demonstration of *in situ* cometabolic cVOC oxidation conducted at Moffit Federal Airfield, CA, where biostimulation and bioaugmentation were compared (Semprini et al. 2007, 2009).

Toluene-oxidizing bacteria

Three different toluene monooxygenases have been described, toluene-2 monooxygenase (T2MO), toluene-3-monooxygenase (T3MO), and toluene-4-

monooxygenase (T4MO), based on their abilities to oxidize toluene in the ortho-, meta- and para-positions, thereby producing creating o-creosol, m-creosol, and p-creosol, respectively (Parales et al. 2008). These SDIMOs fall into Group 1 (T2MO) and Group 2 (T3MO, T4MO), respectively. A toluene dioxygenase also exists, which forms a *cis*-dihydrodiol product. These enzymes are capable of cometabolizing a variety of different xenobiotics, including various cVOCs, 1,4-D, NDMA, and EDB among others (e.g., Chen and Hyman 2023; Danko et al. 2012; Fournier et al. 2006; Hori et al. 2005; Mahendra and Alvarez-Cohen 2006). For the most part, these organisms and enzymes are of interest due to their potential for enhancing natural attenuation of compounds, such as cVOCs in mixed plumes that contain gasoline constituents (e.g., benzene, toluene, ethylbenzene, and xylenes; BTEX), because intentional injection of toluene into groundwater is unlikely to be approved by relevant regulatory agencies.

There were, however, two demonstrations conducted in the 1990s where toluene and/or phenol were supplied at cometabolic substrates to promote the biodegradation of a range of cVOCs. These studies, which were conducted at Moffit Federal Airfield (CA) (Hopkins et al. 1993; Hopkins and McCarty 1995) and Edwards Air Force Base (Ca) (McCarty et al. 1998), proved to be relatively successful for reducing the concentrations of several chlorinated ethenes, including TCE, *cis*-DCE and VC (i.e., > 90% removal), and represent among the earliest large-pilot scale applications of cometabolism for cVOCs. One of the advantages of using toluene and phenol for cometabolic remediation is that they are liquids (rather than gases) at room temperature, but as noted, their relative toxicity has excluded larger scale application.

Other cometabolic organisms and enzymes

Besides the main categories listed above, there are a variety of other organisms and enzymes that cometabolically oxidize common pollutants, including ammonia monooxygenase, a Cu -containing enzyme of nitrifying bacteria such as *Nitrosomonas europaea* that was one of the earliest studied for degradation of cVOCs, including

TCE, cis-DCE, 1,1-DCE, and TCP among others (Hyman et al. 1995; Vannelli et al. 1990). There are, however, few examples of ammonia being used to enhance cometabolic degradation in the field because the substrate is readily assimilated by many organisms (without ammonia monooxygenase) as a source of inorganic N for growth and cell yields by these organisms are very low. Competition for this substrate is likely to favor assimilation rather than dissimilatory energy production (i.e., *via* ammonia monooxygenase). Other enzymes that can mediate cometabolic oxidation include additional enzymes that oxidize alkanes, including cytochrome P450s, and alkane hydroxylases (e.g., AlkB), as well as a number of Group 4 alkene monooxygenases that oxidize substrates such as ethene, and propene, and isobutene (Cheung et al. 2013). Ensign, Hyman, and Arp 1992; Mattes, Alexander, and Coleman 2010; Alkene-oxidizers have received study for cometabolic biodegradation and treatment of chlorinated compounds (e.g., Ensign, Hyman, and Arp 1992; Freedman, Danko, and Verce 2001; Freedman and Herz 1996; Mattes, Alexander, and Coleman 2010), and full-scale field application of ethene has been used to enhance the cometabolic degradation of vinyl chloride at a field site (Begley et al. 2012), as detailed further in [Supplemental Information, Section S.3](#) *Passive addition of ethene and oxygen for vinyl chloride treatment*.

Primary growth substrates

The primary growth substrates used to enhance cometabolic processes are largely based on target organisms/oxygenase enzymes as described in detail in the previous section. These substrates (e.g., methane, propane, butane) are typically gases at room temperature and often flammable. A nontoxic liquid or solid substrate to stimulate cometabolism of contaminants, particularly one that could last for months or years (akin to emulsified oils for stimulating reductive dehalogenation; Borden 2007) would provide a significant advantage over current approaches using select gases. One interesting recent example includes alcohols from orthosilicates (Bealessio et al. 2023; Murnane et al. 2021) to stimulate contaminant degradation by isobutane-oxidizing *Rhodococcus*

spp. Other studies by this group included encapsulating the organisms in gellan gum with the slow release substrate to provide a selective advantage to the bacterium (Rasmussen et al. 2020). The primary challenge with candidate liquid or solid substrates is that they are often not sufficiently selective (i.e., they stimulate organisms capable of cometabolizing a substrate as well as those that do not). Moreover, the enzyme responsible for cometabolizing a given contaminant may not be induced by the nongaseous substrate, so even if the correct organisms grow *in situ*, they may not biodegrade the contaminant, as the correct enzyme is not present within the cells. Typically, the gases used as primary substrates for cometabolic bioremediation (e.g., methane, ethane, propane, isobutane) are selective for organisms capable of carrying out cometabolic reactions as these enzymes are relatively well conserved among different microbial species. In other words, if propane is added to an environment, most bacteria capable of using that substrate have the requisite oxygenase(s) to degrade both propane and the contaminants of interest (e.g., 1,4D, NDMA). Thus, the substrate is selective for this activity, even if the organisms carrying out the reactions differ by site.

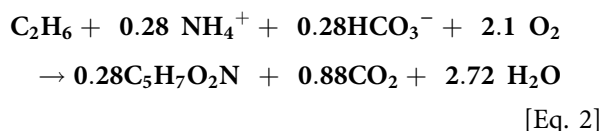
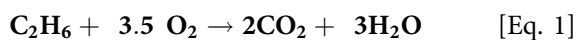
Important considerations

There are several important considerations when planning and designing a cometabolic treatment remedy. In terms of amendments, these considerations include application of oxygen and inorganic nutrients along with the organic cometabolic substrate(s), physicochemical characteristics and purity of the primary substrates, and control of potential biofouling.

Oxygen and inorganic nutrients

One important factor for successful application of cometabolic substrates is understanding the quantities of oxygen and inorganic nutrients (particularly nitrogen) required to support microbial growth on a given primary substrate. As an example, the calculated ratios of ethane, oxygen, and nitrogen required for growth of ethane-degrading bacteria are provided below.

The required molar ratio of ethane to oxygen (O_2) for complete oxidation of ethane (C_2H_6) to carbon dioxide is $\sim 3.5 \text{ mol } O_2$ to $1 \text{ mol } C_2H_6$ [Equation 1]. If one incorporates microbial cell growth on a portion of the carbon from ethane (assuming 40% of ethane utilized for cell growth and 60% for respiration), then the molar ratio declines to $\sim 2.1 \text{ mol } O_2$ to $1 \text{ mol } C_2H_6$ [Equation 2]. Cell biomass in Equation 2 is represented by $C_5H_7O_2N$, which roughly represents the molar ratio of these elements in bacterial cells (Rittman and McCarty 2001). This equation also predicts the cellular requirement for N as $0.28 \text{ mol } NH_4^+$ per $\text{mol } C_2H_6$. When converted to a mg/L basis, the above stoichiometry suggests that the oxygen and ammonium requirement for bacteria to biodegrade 1 mg/L of C_2H_6 is $2.2 \text{ mg/L } O_2$ (3.7 mg/L if biomass growth is not included) and $0.17 \text{ mg/L } NH_4^+$.



For field applications, oxygen is typically supplied to groundwater *via* either sparging with air or through the use of pure oxygen. Examples of these two approaches are provided in subsequent sections. In a few instances, hydrogen peroxide also has been used as a source of dissolved oxygen (e.g., McCarty et al. 1998).

Amendment addition and distribution system designs

With the exception of locations with high nitrate in groundwater (e.g., $> 5 \text{ mg/L}$ nitrate-N), adding bioavailable nitrogen is typically necessary for successful cometabolic treatment. An example of this requirement is provided for a site on Cape Cod (MA) in Figure 3. In this case, aquifer microcosms amended with ethane only or inorganic nutrients only (50 mg/L diammonium phosphate; DAP) resulted in no enhanced biodegradation of EDB, while addition of both amendments resulted in complete biodegradation of EDB during several weeks of incubation. A successful field demonstration was conducted

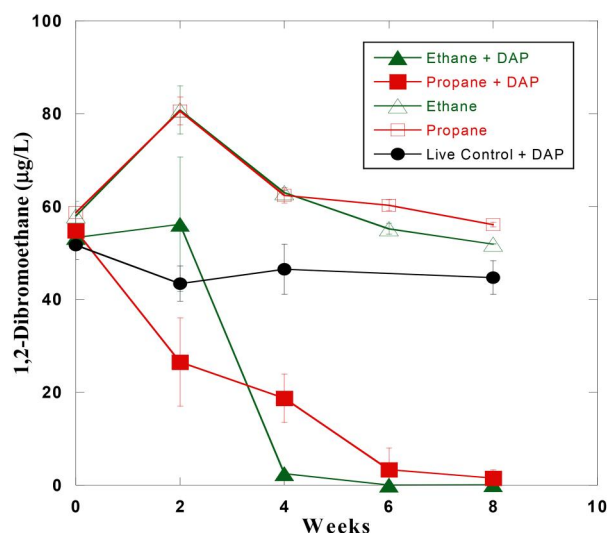


Figure 3. Aquifer microcosms showing the biodegradation of 1,2-dibromoethane over time in treatments amended with diammonium phosphate (DAP) only (Live Control + DAP), ethane gas only (Ethane), propane gas only (Propane), ethane gas with DAP (Ethane + DAP) or propane gas with DAP (Propane + DAP). All samples received oxygen gas. Error bars are from duplicate samples.

based on the results of the laboratory microcosms in which oxygen, ethane and DAP were added to a groundwater recirculation system to treat EDB in groundwater (Hatzinger et al. 2018). Further details are provided in [Supporting Information, Section S.2 Groundwater recirculation system with ethane amendment for 1,2-dibromoethane](#).

Besides nitrogen, phosphorus is perhaps the second most important inorganic nutrient for bacterial cells, as it is a component of many different cellular macromolecules (e.g., phospholipids, nucleotides, adenosine triphosphate (ATP)). The cellular requirement for P, however, is substantially lower than for N, with a rough estimate on a molar basis of C:N:P of 50:10:1 (Fagerbakke, Heldal, and Norland 1996). As such, it is unclear how often P becomes a limiting factor in aerobic cometabolic, or anaerobic bioremediation processes, as enough may naturally be present (albeit in insoluble forms) to meet cellular needs. Regardless, DAP, which is commonly used as a source of inorganic N in remediation applications, is also provides more than adequate P for cell growth.

The addition of sufficient DAP to an aquifer generally requires that groundwater be recirculated (see Section “Groundwater recirculation with amendment addition”). In this case, the DAP is

mixed into site groundwater and typically added into one or more injection wells, often using recirculation of the groundwater for distribution. However, in some instances, gas-sparging (without groundwater recirculation) is the most desirable option to add necessary amendments, as this approach is often simpler and less prone to issues such as biofouling. However, in this case, volatile sources of N and P (or compounds that can be readily vaporized that contain these elements) are required. One commercialized approach is termed the PHOSter[®] technology, which injects a mixture of air, methane, nitrous oxide (N source) and triethyl phosphate (P source) to stimulate microbial cometabolism of relevant contaminants, such as TCE (e.g. Eggers et al. 2008). Studies in our laboratory have shown ammonia to be a much more readily utilized N source than nitrous oxide, and an effective N amendment for field use in biosparging systems (Lippincott, Lavorgna, and Hatzinger 2022).

Competitive inhibition

One significant challenge with practical application of cometabolic processes is that one enzyme often catalyzes the oxidation of both the substrate and the contaminant of concern as indicated in Figure 1. Depending on the affinity of that enzyme for each of the compounds as well as their relative concentrations, the rates of contaminant biodegradation can be negatively impacted by the presence of the growth substrate, a phenomenon that has come to be termed “competitive inhibition” (Alvarez-Cohen and McCarty 1991a,1991b; Criddle 1993). The extent to which competitive inhibition impacts the rate of biodegradation of a contaminant can vary appreciably by organism, as illustrated by Sharp, Sales, and Alvarez-Cohen (2010) for the degradation of NDMA by two different propanotrophs. *Rhodococcus ruber* RR1 has a much higher affinity for propane than NDMA, so NDMA biodegradation was inhibited significantly at very low dissolved propane concentrations (tens of µg/L). Conversely, *Mycobacterium vaccae* JOB 5 continued to degrade NDMA at mg/L concentrations of dissolved propane, showing only a 50% reduction

in NDMA biodegradation rate at 7.7 mg/L of dissolved propane.

From a practical perspective, competitive inhibition can often be readily overcome in the field. This can be accomplished by adding the growth substrate in pulses rather than continuously, thus promoting periods where substrate is exhausted and the organisms are primarily degrading the contaminant of concern (e.g., Hopkins et al. 1993; Lippincott et al. 2015, Lippincott, Lavorgna, and Hatzinger 2022; Semprini and McCarty 1992; Toovanbootr et al. 2001). Cometabolic biosparging systems (Section “Biosparging”), are particularly amenable to this approach, as the duration and spacing of sparge cycles (where substrates and oxygen are added) can be easily modified. The rates of primary substrate biodegradation at the field scale can be quantified by pulsing the substrate (e.g., initiating a sparge cycle for a biosparge system) and then measuring substrate increase and decline in local monitoring wells (Lippincott et al. 2015).

For groundwater extraction-reinjection systems (see Section “Groundwater recirculation with amendment addition”), substrate pulsing can also be used to optimize contaminant degradation rates (e.g., Chu et al. 2018). However, even if the primary substrate is added continuously (assuming no other imitations in inorganic nutrients or oxygen), microbial biomass will typically increase in the treatment area to the point where all the primary substrate is depleted along the flow path of the system and high rates of contaminant degradation occur (e.g., Hatzinger et al. 2018). However, one disadvantage of continuous substrate addition in this type of system is the potential for injection well biofouling over time (see Section “Biofouling”).

Gas characteristics

Purity

Using a lower purity gas may seem like a good approach to minimize costs during application of aerobic cometabolic bioremediation in the field. For instance, the cost of liquified natural gas or propane used for residential cooking and heating (e.g., consumer grade) is usually lower than the cost for high purity (99+%) gases. However, the

consumer grade gases are usually dosed with odorous compounds a safety measure to allow rapid detection of gas leaks and often contain other alkanes and alkenes. For example, consumer grade wet natural gas is composed of approximately 85% methane, 6% ethane, 5% of propane, <2% of other alkanes (isobutane, butane, isopentane, pentane), along with <5% CO₂, <5% H₂S, and the balance primarily as N₂ with traces of noble gases (Faramawy et al. 2016). Odorants consisting of various sulfur containing compounds (tert-butyl mercaptan (TBM), isopropyl mercaptan (IPM), tetrahydrothiophene (THT), n-propyl mercaptan (NPM), and dimethyl sulfide (DMS) (Michanowicz et al. 2023) are also present at concentrations of 1% (Speight 2019). Consumer grade propane, often designated as liquified petroleum gas (LPG) of heavy duty 5% (HD-5) is formulated to contain 90% propane, 5% propylene, and no more than 5% of other gases included butane and butylene (Alternative Fuels 2025).

These additional compounds may either not promote the desired cometabolic process (e.g., not induce the correct enzymes), or may inhibit the process, as is the case with propylene (Meza 2019). During previous research in our group, the growth of propanotroph *Rhodococcus ruber* ENV425 was evaluated using either consumer grade propane or 99% laboratory grade (or “chemically pure”, CP) propane. As shown in Figure 4, the bacterium grew readily with the pure propane, while limited growth was observed with consumer grade propane. The greater cost of using the highest purity gases is outweighed by the potential downsides of using lower purity gases, and the total gas substrate cost is a small percentage of the overall remediation costs.

Physicochemical properties

Besides purity, physicochemical characteristics of gaseous substrates are also important for application as primary substrates. In particular, vapor pressure and lower explosive level (LEL), need to be considered when selecting a gaseous substrate, in addition to efficacy at promoting contaminant biodegradation. The vapor pressure of a gas at the typical operational ranges of the system, particularly at the lowest ambient temperatures expected, is critical to system design, particularly for biosparging

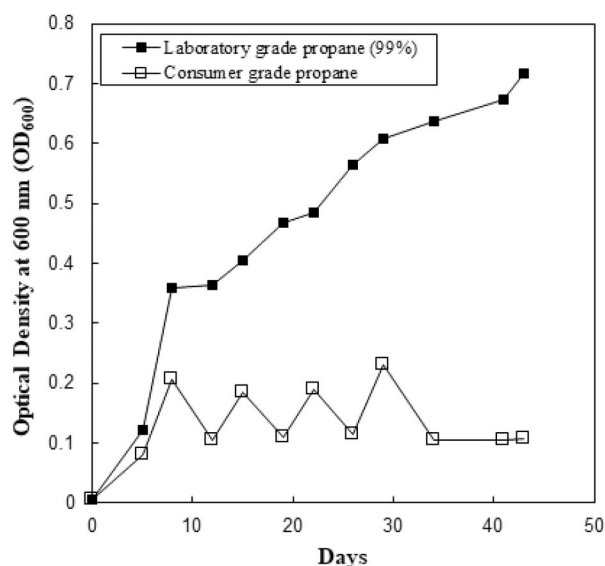


Figure 4. Effect of propane purity on the growth of propanotroph ENV425. Both gases added at a concentration of 3% (v:v) of the bottle headspace.

applications. Vapor pressures of common hydrocarbon gases at different temperatures are available (The Engineering Toolbox 2025). If the vapor pressure of a substrate gas is too low, quantities supplied to an aquifer may vary with ambient temperature (i.e., decreasing in cold weather if the system is not heated) and/or the material can liquify in supply lines connecting a system to the sparge wells. Both of these outcomes are undesirable. It is possible to use gases with relatively low vapor pressures as cometabolic substrates (e.g., toluene, butane) or nutrients (TEP), but the system must be designed to add the substrates as vapors rather than pure gases (e.g., Eggers et al. 2008).

The LEL is also an important consideration when choosing a gaseous substrate for a cometabolic system. Because alkane/alkene gases are flammable, specific safety measures must be considered when designing, installing, and monitoring an *in situ* cometabolic biosparging system. However, it is very easy for a competent engineer to design a system that is safe for operation. All electrical equipment and wiring in the system enclosure supplying a flammable gas should be intrinsically safe, and any cylinders/tanks should be stored outside of the trailer, and separated (e.g., compressed oxygen separated from hydrocarbon gas and/or ammonia). Any enclosed space where gases enter (e.g., in lines to be proportioned) also should be monitored with LEL gas

detectors that shut-down the system and notify appropriate personnel in the case of an alarm condition. Venting can also be activated in case of an alarm condition. Finally, when gases are added to biosparging wells, the LEL for any flammable gas should not be exceeded, and ideally should not go above $\sim 50\%$ of the LEL. Additional discussion of system design is provided in subsequent sections.

Biofouling

One issue that can arise when introducing a cometabolic substrate and inorganic nutrients into groundwater is biofouling. This is particularly problematic for groundwater extraction-recirculation systems (where groundwater is pumped to the surface, amended with substrates, and reinjected into an aquifer) compared to strictly biosparging systems without groundwater recirculation (see Section “Amendment addition and distribution system designs”). Biofouling occurs when the bacterial biomass grows to such an extent that it fills the pore space in the aquifer matrix and/or the screened portion of the injection/extraction wells, thereby reducing or stopping water flow. Often the cellular biomass is combined with extracellular polymeric substances (EPS), forming biofilms (Kierek-Pearson and Ece Karatan 2005). Depending on the redox gradients, precipitated metals (e.g., Fe and Mn oxides) can also contribute to the fouling. Biofouling can sometimes be managed by backflushing (e.g., switching the flow in the injection/extraction wells). However, it is often necessary to periodically apply biocidal chemicals such as bleach or hydrogen peroxide to maintain groundwater flow (Kierek-Pearson and Ece Karatan 2005). It is also possible to use hydrogen peroxide as a source of oxygen in recirculation designs; in this case, it can act as a biocide near the injection well and as an oxygen source further into the aquifer as it readily decomposes to oxygen in water (McCarty et al. 1998). Additional details are provided in Section “Groundwater recirculation with amendment addition” and [Supporting Information, Section S.2](#). For systems that operate over long timeframes, well redevelopment may be necessary periodically to maintain system operation.

Amendment addition and distribution system designs

As noted previously, typical amendments added *in situ* to groundwater to promote cometabolic biodegradation of a contaminant are a gaseous substrate, oxygen gas or air, and one or more inorganic nutrients. On occasion, a bioaugmentation culture also will be required. Critical considerations with these amendments include both concentration and distribution. For gaseous substrates, there are three basic approaches for addition (1) biosparging; (2) groundwater recirculation with ex situ amendment addition; and (3) passive gas addition *via* in well diffusion ([Figure 5](#)). It is also possible to engineer hybrid systems (e.g., *in situ* biosparging wells with groundwater recirculation for enhanced distribution and non-gaseous nutrient addition). The application of one approach over another is typically based on several different factors, including (1) amendments required; (2) site geology and hydrogeology; (3) infrastructure needs and restrictions; and (4) treatment goals. In many instances, more than one of the above options is feasible. The different approaches, and design considerations for each are described in the subsequent three subsections and field examples of each are provided in the Supporting Information.

Biosparging

Sparging of gaseous growth substrates to enhance cometabolic biodegradation of a contaminant of concern is termed *biosparging*. Often, the growth substrate (e.g., propane, methane) is added to the aquifer in a stream of air supplied by a compressor. The air contains the oxygen required for growth of the cometabolic organisms. In some instances (e.g., if power is not available or accumulation of N_2 from air in a tight geologic formation is of concern), pure oxygen gas from cylinders can be used instead to supply O_2 in place of air. As noted previously, gaseous nutrients in the form of nitrous oxide or ammonia (N sources) and TEP (P source) can also be added using a biosparging approach, although the TEP is only moderately volatile, and must be added as a vapor.

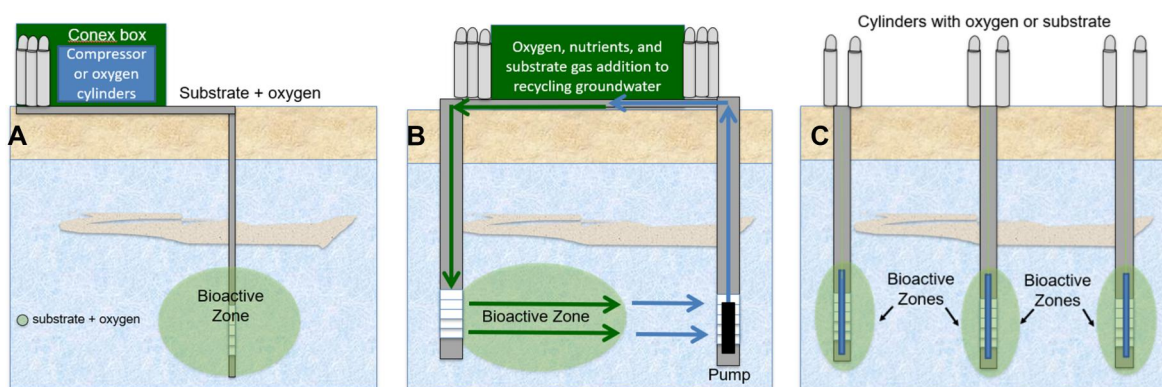


Figure 5. Diagram of three basic approaches for adding gaseous substrates to groundwater for cometabolic remediation. (A) bio-sparging, (B) groundwater extraction and recirculation; (C) passive addition.

When designing a biosparge system, as with almost any *in situ* remediation system, a thorough understanding of site geology and hydrogeology are necessary. Typically, for a biosparge application, soil cores are initially collected and logged throughout the contaminated zone. The application of a hydraulic profiling tool (HPT) fitted with an Electric Conductivity (EC) sensor and a discrete zone groundwater sampler is also recommended. This tool allows estimation of horizontal hydraulic conductivity, which is important for sparging applications, and depth-dependent groundwater samples can be taken simultaneously and analyzed for relevant COCs, anions (especially NO_3^- -N), and typical field parameters, including dissolved oxygen and pH. These data can then be used to determine (1) the feasibility of biosparging within the COC plume based on aquifer permeability and (2) initial system design assuming that the approach is feasible based on the core logs, HPT data and groundwater analyses.

A single-well gas-sparging test can be performed to assess the overall potential for COC biotransformation prior to installation of a full-scale treatment system (Kim, Istok, and Semprini 2008). For this test, one or more cometabolic gases (e.g., methane, propane) and inorganic nutrients (e.g., ammonia), if required, are sparged into a single groundwater well fitted with a packer, and groundwater samples are collected over time to assess rates of loss of the substrate gases and the relevant COCs. Ethylene and/or propylene (which are converted to ethylene oxide and propylene oxide, respectively, by

monooxygenases), can be added to quantify monooxygenase activity, and acetylene can be used to inhibit all activity and confirm that relevant reactions are cometabolic (Kim, Istok, and Semprini 2006, 2008).

The next recommended step prior to full-scale system design is to install one or more vertical sparge wells into the formation with screen intervals throughout the contaminated zone as well as a series of monitoring wells at increasing distances from the sparge well(s). If the aquifer has significant heterogeneity, nested wells with varying screen depths should be considered as detailed in Lippincott, Lavorgna, and Hatzinger (2022). This well network should then be used for an initial set of sparge tests whereby tracer gases, such as oxygen and/or helium are sparged into the test well. The objective of this testing is to determine both the breakout pressure for gas flow in the formation (i.e., at what pressure gas flows into the saturated formation) and the distribution of gas both vertically and horizontally in the aquifer under different flow regimens (e.g., continuous flow at different injection pressures as well as pulsed flow). A sparge testing design from Lippincott, Lavorgna, and Hatzinger (2022) is provided in Figure 6. During the tests, sparge well pressure should be measured to determine breakout pressure, and groundwater samples should be collected periodically from the monitoring well network and analyzed for the sparged gases (oxygen can be field measured with a water meter and helium by gas chromatography (GC) in the laboratory) and water table elevations are obtained to determine the extent of groundwater mounding. A detailed

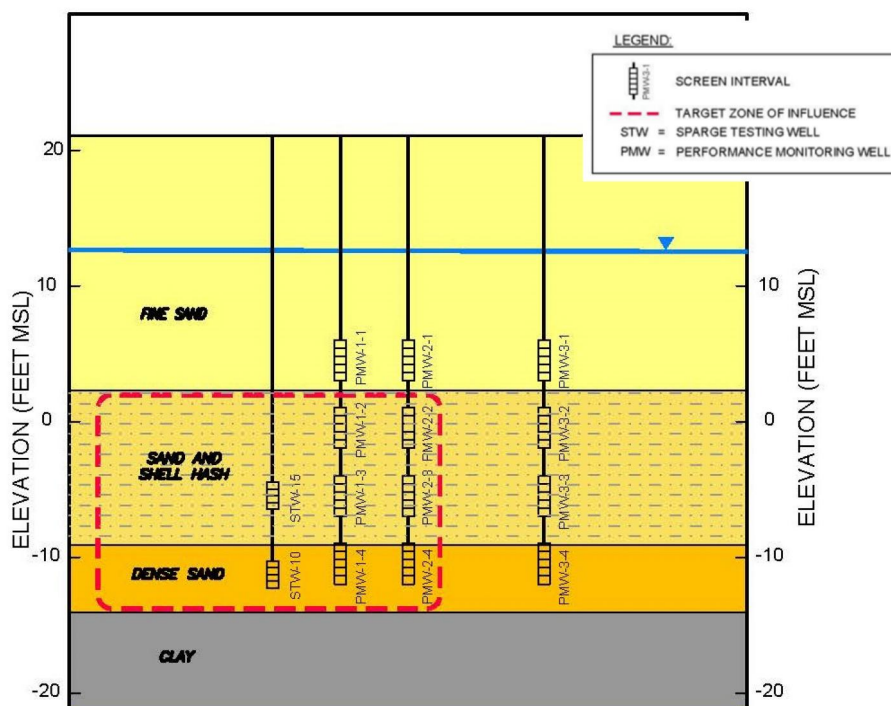


Figure 6. Photographs (top) and diagram (bottom) of sparge testing setup. During each of the sparge tests, oxygen and helium (cylinders in top left photo) were sparged into the shallow (STW-10) or deep (STW-15) sparge wells, with pressure monitoring (pressure gauge on STW in top right photo) and gas concentration measurements at the various PMWs over time. Sparge rates were varied in a step-wise fashion (modified from Lippincott, Lavorgna, and Hatzinger 2022).

description of this type of sparge testing as well as application of the data in system design is provided in Lippincott, Lavorgna, and Hatzinger 2022.

The sparge test gas data yields a general radius of influence (often in the vicinity of 3–5 m in alluvial aquifers) necessary for development of an injection and monitoring well network to provide gas distribution and performance monitoring, respectively. In some instances, gas sparge wells

must be placed at different depths in the plume to provide adequate vertical distribution because of aquifer anisotropy, which can result in far greater horizontal than vertical gas transport in an aquifer.

Systems to supply necessary gaseous amendments *in situ* typically either blend the substrate and nutrient gases into air supplied by a compressor or utilize sequential addition of individual

gases with O₂ supplied by cylinders rather than an air compressor system. An example of an air-compressor driven system is provided in Hatzinger and Lippincott (2018) for *in situ* treatment of NDMA *via* propane biosparging, and Lippincott, Lavorgna, and Hatzinger (2022), provides an example of a system designed for sequential gas addition *via* cylinders. With either type of system, automated gas monitoring and shutdown features are required and substrate gas concentrations should be maintained well below relevant LELs.

Groundwater recirculation with amendment addition

A second technique to supply cometabolic amendments to groundwater is to pump the water to the surface, add the required amendments and then reinject the water to the contaminated aquifer. This approach is often, but not always, used in a recirculation design as shown in Figure 5(B). One of the advantages of this approach is the ability to easily add liquids (e.g. DAP solution) as well as gases to the aquifer. This system design also more readily allows the addition of bioaugmentation cultures than a biosparging system, although cultures have been applied to biosparging wells in a few instances (Lippincott et al. 2015, Bell et al. 2022).

As noted above for biosparging, an understanding of site geology and hydrogeology are also necessary to implement a successful groundwater recirculation system for cometabolic treatment. The techniques and analyses recommended above (e.g., continuous core collection and lithological analysis, HPT and EC logging, discreet groundwater sampling) are also recommended for this approach. In addition, it is important to understand the aquifer response to pumping so that appropriate extraction and injection rates can be determined to maximize amendment distribution in injected groundwater. Pump testing should generally be performed to determine horizontal and vertical hydraulic conductivities, estimate aquifer transmissivity and storativity, and evaluate pumping well radius of influence. Depending on the site, pump tests can be done with existing wells, or a subset of the wells that require installation for

the recirculation system (e.g., one injection well (IW) and 2–4 monitoring wells (MWs) at varying distances from the IW). Examples of pump test options are provided in Osborne (1993).

The combined data from site characterization and pump testing should subsequently be used to construct a numerical three-dimensional groundwater model (e.g., MODFLOW) of the treatment area. The model should be designed to simulate groundwater recirculation (extraction and reinjection) using different well configurations and pumping rates. Results of the simulations will allow various scenarios to be evaluated in terms of groundwater flow patterns and residence time in the treatment area. Different extraction-injection designs can be evaluated with flow model simulations (e.g., central injection well with surrounding extraction wells to create a treatment cell or upgradient injection and down-gradient extraction wells along the flowpath of the contaminant plume). The model should also be used to determine pumping rates and final placement of all necessary monitoring wells for the treatment system.

A typical design for a groundwater recirculation system includes submersible pump(s) installed in extraction wells, which pump water to a Conex box or other structure designed to house necessary equipment and controls. Submersible pressure transmitters should also be placed in the extraction and injection wells and tied into the controls (PLC/SCADA system) to shut down pumping if low or high set-point levels are reached. The pumped groundwater should typically be passed through a filtration system (e.g., cartridge filters) to remove sediments and/or biomass prior to passage through flow meters to measure volumes and rates of flow to each of the injection wells. The filters protect the flow meters as well as the injection wells from clogging.

Necessary gases (e.g., alkane gas, ammonia, oxygen) can be delivered using regulated cylinder gas pressures with specified addition rates controlled by individual mass flow controllers. The water with added gases should then be passed through static injection mixers to enhance dissolution prior to reinjection into the IW(s). In instances where more than one gas is necessary, each can be added sequentially rather than in a continuous mixture.

Pressure and flow indicators and transmitters are designed throughout the system to monitor system operational conditions, trigger alarms, or shut-down the system, if necessary. Flame arrestors/non-return valves should be incorporated upstream of the mass flow controllers to protect the system from potential flashbacks and stop the unwanted backflow of gases. Individual gas monitors, (e.g., for oxygen and any flammable gas) should also be incorporated into the system enclosure to monitor the atmosphere and shut-down the system if LEL alarm set-points are reached.

In addition to gas delivery, liquids can be delivered (e.g., DAP in solution) *via* one or more chemical feed pumps in short pulses from a tank placed within or outside of the Conex box as needed. To minimize fouling, nutrient solutions should be added periodically rather than continuously, and not in excess. Bioaugmentation cultures can also be added *via* the same liquid deliver system (downgradient of any system filters).

A biofouling mitigation system should be considered with any groundwater recirculation system to reduce performance degradation due to potential biofouling of the various system components and/or the IWs. One type of system design employs a separate pumping/injection system that adds a small amount of oxidant (35% hydrogen peroxide or equivalent) prior to designed system shutdowns. In most cases, the recirculation systems do not need to be operated continuously but rather can be used primarily to mix in the amendments to the treatment area and shut down until those amendments are consumed. This approach reduces biofouling, particularly when combined with addition of an antifouling agent. The volume of oxidant should be enough to fill any process piping, filter housings, flow meters and injection well screen intervals (and surrounding sand pack), but not a large enough volume to appreciably distribute the oxidant out into the formation.

Passive gas diffusion devices

A third approach to supplying gaseous amendments for cometabolic treatment is the use of

passive diffusion devices placed in groundwater wells. These devices, such as the “Waterloo Emitter” (Solinst, Georgetown, Ontario, Canada) or the iSOC (Innovation Remediation Technologies, Inc., Taneytown, Maryland, USA) generally include gas permeable materials that slowly emit oxygen and substrate gas to groundwater when attached to a gas cylinder under pressure. This type of design has been most often applied for adding oxygen only to groundwater, but field applications of this design for oxygen plus cometabolic substrates have also been performed (Begley et al. 2012; Hatzinger and Lippincott 2018). In general, this type of system will utilize cylinders in a central location for a small site) or at each gas addition well (for a larger site) to provide oxygen (as compressed air or oxygen gas) and the desired hydrocarbon substrate. In that it is not possible to readily control substrate gas concentrations with this design as noted below), custom-mixed dilute substrate gases may be required (e.g., 0.25% alkane gas in air) to avoid adding substrate to groundwater that exceeds available oxygen based on solubility. The gases (air, oxygen and/or hydrocarbon) are added by applying pressure on the Waterloo Emitter, and the emitter systems require venting of low amounts of gases to the atmosphere.

The advantages of this type of system include simplicity and no requirement for power, making passive treatment ideal for remote areas. Passive gas distribution devices can be installed into existing monitoring wells or a line of wells to create a barrier (See [Supporting Information, Section S.3](#)). The infrastructure required is also straightforward. However, the main disadvantages of this approach are (1) poor overall substrate distribution and (2) an inability to readily control substrate concentrations *in situ*. The distribution of the added gases (e.g., oxygen and propane) is largely based on diffusion through the emitters, diffusion in groundwater, and groundwater flow rate. Thus, the added gases typically impact a relatively narrow zone downgradient of the injection wells, requiring them to be relatively closely spaced for a cutoff barrier design, typically closer than for a typical biosparging design. Secondly, the concentration of the gases in groundwater are not readily modified. In some cases, pressure on

the gas diffusion devices can be adjusted to increase or decrease gas addition rates to the aquifer, or the gas concentration in a cylinder can be premixed as noted above to achieve the desired concentrations *in situ*, but the design is generally not as flexible as either of the previous approaches.

Conclusions

For the past 25 years, reductive dehalogenation has typically been the favored biological treatment approach for chlorinated solvents at field scale. However, we are currently faced with many sites where cVOC concentrations are exceedingly low (i.e., tens to hundreds of µg/L) and/or where low concentrations of lesser chlorinated compounds, such as *cis*-DCE and VC, persist in site groundwater either after anaerobic bioremediation or due to partial attenuation by indigenous dehalogenating bacteria. There are also a variety of other compounds that have emerged which are present in groundwater at concentrations that are generally too low to support microbial growth, but well above levels of regulatory concern, such as MTBE, 1,4-D, NDMA, EDB, and TCP, among others. Dilute plumes with these compounds, alone or, more often, comingled together represent a significant remedial challenge.

Aerobic cometabolic bioremediation approaches were first tested in the field more than 30 years ago for treatment of cVOCs, but were largely displaced by anaerobic bioremediation due to several factors, including (1) the relative complexity of dosing and distributing oxygen and gaseous hydrocarbon substrates in groundwater (compared to molasses, soybean oil or other bulk liquids for anaerobic bioremediation); (2) the necessity to use flammable gases; (3) competitive inhibition between the substrate and the cVOC being treated; and (4) the microbial toxicity of high concentrations of chlorinated ethenes to cometabolic organisms. However, as the problem definition has changed over time, from high concentration cVOC sites to dilute plumes of cVOCs and a variety of newer contaminants, co-metabolic approaches deserve reconsideration. Many of the issues or perceived issues with this technology are either not a problem for current dilute plumes

(e.g., toxicity only typically occurs with high concentration chlorinated ethenes) or have largely been resolved through system engineering (e.g., handling flammable gases). Numerous field tests and full-scale applications of different system designs have been performed that allow precise addition of gases and other amendments based on site conditions. These systems include simple passive gas diffusion devices, biosparging systems, and groundwater recirculation designs. Some of the current compounds of concern, such as NDMA and EDB, require remedial approaches that achieve low ng/L concentrations, which has now been shown to be feasible *via* cometabolism. Few other current *in situ* technologies are likely to meet these stringent requirements. The objective of this review is to highlight the potential for applying aerobic cometabolism to resolve current issues with both traditional and emerging contaminants and to provide guidance concerning the application of this technology at field scale.

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