REVISTA MEXICANA DE INGENIERIA QUIMICA Vol. 4 (2005) 273-287 **AMIDIQ**

ANAEROBIC BIOREMEDIATION–AN UNTAPPED POTENTIAL

BIORREMEDIACION ANAEROBICA-UN POTENCIAL SIN EXPLOTAR

N. Balagurusamy

Universidad Autónoma de Coahuila, Facultad de Ciencias Químicas, Departamento de Biotecnología, Coahuila, Saltillo. C .P. 25280.

Received May 16 2005; Accepted October 17 2005

Abstract

Recently there has been considerable interest in employing bioremediation technologies for treatment of wastes and for reclamation and restoration of contaminated ecosystems. In this technology, microorganisms or their constituents such as enzymes are used to degrade or transform the wastes. Though, contaminated ecosystems lack oxygen and favor the growth and activity of anaerobic bacteria, most of the bioremediation technology employs aerobic microorganisms. However, treatment of industrial wastewaters is the only area in which anaerobic technology is widely employed presently. Recent advances in molecular ecology helps us in understanding the diversity of anaerobic bacteria, their processes and their important role in global cycle of carbon, nitrogen, sulfur. In addition, different anaerobic bacterial groups possess the ability to use different types of electron acceptors such as nitrate, sulfate, and carbonate for degradation of organic contaminants or for biotransformation heavy metals. Still, the potential of anaerobic bacteria for bioremediation of various contaminants is not capitalized. This paper deals with application of anaerobic bioremediation for biorestoration of land and water ecosystems contaminated with hydrocarbons, chlorinated compounds and heavy metals.

Keywords: bioremediation, anaerobic bacteria, hydrocarbons, chlorinated compounds, heavy metals.

Resumen

Recientemente ha habido un interés considerable por el empleo de tecnologías del biorremediación para el tratamiento de basuras y para la restauración de ecosistemas contaminados. En esta tecnología, los microorganismos o algunos de sus componentes como las enzimas son usados para degradar o transformar las basuras. Dado que a los ecosistemas contaminados les falta oxígeno, se favorece el crecimiento y actividad de bacterias anaerobias, aunque la mayoría de la tecnología del biorremediación emplea los microorganismos aerobios. Sin embargo, el tratamiento de aguas residuales industriales representa la única área en que la tecnología anaerobia es extensamente aplicada. Logros recientes en ecología molecular nos ayudan a entender la diversidad de bacterias anaerobias, sus procesos y su papel importante en el ciclo global de carbono, el nitrógeno y el azufre. Además, los diferentes grupos de bacterias anaerobias poseen la habilidad de usar tipos diferentes de aceptores de electrones como el nitrato, el sulfato y carbonato para la degradación de contaminantes orgánicos o para la biotransformación de los metales pesados. Todavía, el potencial de bacterias anaerobias para la biorremediación de varios contaminantes no se capitaliza. Este artículo revisa la aplicación de la biorremediación anaerobia para la biorrestauración de ecosistemas de tierra y agua contaminados con hidrocarburos, compuestos clorados y metales pesados.

Palabras clave: biorremediación, bacterias anaerobias, hidrocarburos, compuestos tratados con cloro, metales pesados.

1. Introduction

Bioremediation technology uses the metabolic potential of the microorganisms to clean up the contaminated land and water. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. As bioremediation can be effective only where environmental conditions permit microbial growth and activity. Its application often involves the manipulation of environmental parameters to

---

*Corresponding author: *E-mail: bnagamani@mail.uadec.mx*
Phone: Tel. (84) 44155752 Ext.22, Fax: (84) 44159534

allow microbial growth and degradation to proceed at a faster rate (Vidali, 2001). A large number of microorganisms have been isolated in recent years that are able to degrade compounds that are previously considered to be non-degradable. This shows that under selective pressure of environmental pollution, microorganisms develop catabolic capacity either to degrade or convert them to innocuous products (Timmis and Pieper, 1999). However most of the processes do not function optimally. The design of improved biocatalysis involves different aspects of optimization, such as; creating new metabolic routes, expanding the substrate ranges of existing pathways, improving the process-relevant properties of microorganisms, etc. Until recently, practical applications of *in situ* bioremediation have focused mostly on aerobic microorganisms, which gain energy by oxidizing organic compounds to carbon dioxide with oxygen serving as the electron acceptor. However, this approach has had limited success, not least because oxygen, an absolute requirement for aerobes, is scarce in almost all contaminated environments. The scarcity of oxygen in many contaminated sub surface environments has raised interest in the bioremediation potential of anaerobes.

The most ancient of all life processes is anaerobic microbial metabolism. Billions of years ago, our entire planet was anaerobic. Living organisms that began to evolve in the warm oceans had no choice but to follow anaerobic pathways. Free atmospheric oxygen, as we know it today, simply did not exist. All molecular oxygen was bound in water, carbon dioxide, carbonates, and sulfates. The world arose anaerobically, and much of it remains so in terms of the numbers of living things rather than their size. Anaerobic bacteria are present in soil and are a part of the normal flora of humans and all other animals, as well as the insects examined so far. This microbial life in the absence of oxygen is beginning to show significant potential for solving one of the important present day problems of environmental pollution and degradation (Coates and Anderson, 2000). Lu *et al.* (1999) indicated that anaerobic processes naturally found in subsurface account for bulk of organic contaminant degradation in aquifers. Anaerobes oxidize organic compounds to carbon dioxide but use electron acceptors such as nitrate, sulfate, or Fe^{3+} oxides instead of oxygen. The diverse metabolic capabilities of anaerobes represent a potentially potent force in the fight against contamination. In sediments in which anaerobic processes are established, stimulating the anaerobic community has the advantage of enhancing an active and acclimated microbial population. If the natural rates of contaminant degradation are too slow, increased levels of alternate electron acceptors may accelerate the rate of anaerobic contaminant degradation. Electron acceptors such as sulfate and nitrate do not have these limitations because they are highly soluble and are not consumed by non-biological processes (Lovley, 2001). In this review, potential and applications of anaerobes in remediation of different contaminants is discussed.

2. Anaerobic bioremediation of hydrocarbon contamination

Hydrocarbons are one of the most important groups of chemicals to mankind because of their natural abundance, industrial importance and their extensive use as a primary energy source. Petroleum hydrocarbon contamination is becoming a great concern due to the toxicity and recalcitrance of many of the fuel components. The majority of bioremediation strategies for removal of petroleum hydrocarbon are aerobic respiration. Prior to the 1980s, it was accepted that microbial hydrocarbon degradation occurs mainly under aerobic conditions due to favorable energetics and that anaerobic hydrocarbon degradation was negligible (Atlas, 1981). However there are several considerations in the use of aerobic bioremediation technologies. Normally the hydrocarbon contaminated soils lack oxygen. Further urea and ammonia-based fertilizers are added sometimes for biostimulation, which also can potentially exert an oxygen demand due to biological ammonia oxidation. In addition, mass transfer of oxygen may not be sufficient to replenish oxygen consumed by microbial metabolism. Under such conditions anaerobic hydrocarbon degradation may be of relevance. Recently anaerobic hydrocarbon metabolism and the importance of anaerobic hydrocarbon metabolism in contaminated anoxic environments is being widely reported (Coates *et al.*, 1997; Caldwell *et al.*, 1998; Wilkes *et al.*, 2002, Chakraborty and Coates, 2004). Different mechanisms for microbial utilization of aromatic compounds are presented in Fig. 1. Several denitrifying, manganese-iron and sulfate-reducing bacteria that have the ability to degrade simple aromatic or aliphatic hydrocarbons under anoxic conditions have been isolated (Leahy and Olsen, 1997; Rabus and Widdell, 1995; Rueter *et al.*, 1994; Zhou *et al.*, 1995). Gibson and Harwood (2002) reviewed the microbial diversity of anaerobic bacteria utilizing aromatic hydrocarbons.

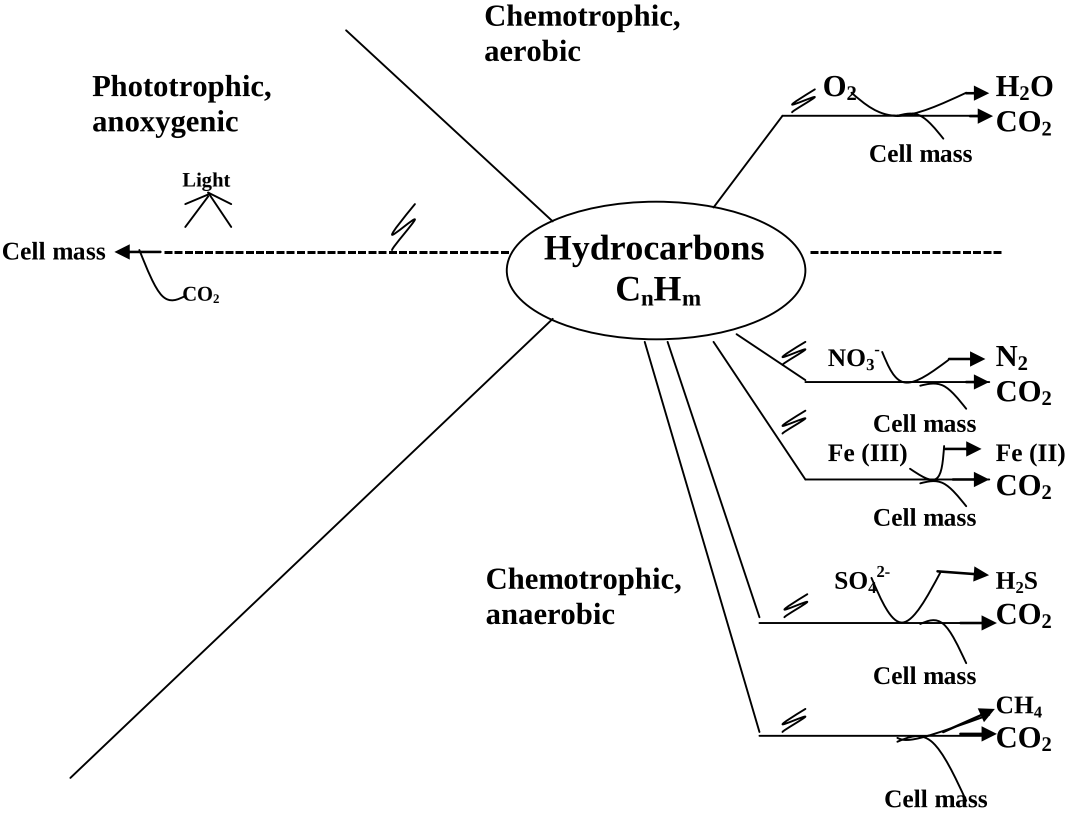

Fig. 1. Microbial utilization of hydrocarbons with different terminal e$^-$ acceptors. Reprinted with permission from Widdel and Rabus (2001), Copyright 2001. Elsevier Science Ltd.

Several studies have also demonstrated that monoaromatic hydrocarbons such as benzene, toluene, and xylene (BTEX) and hexadecane can be biodegraded in the absence of oxygen (Coates *et al*., 1996a; Coates *et al*., 1996b; Edwards and Grbic-Galic, 1992; Kazumi et al., 1995; Rabus and Widdell, 1995). Anaerobic degradation of aliphatic hydrocarbon has also been reported and has been linked to denitrification (Bregnard *et al.*, 1997; Chayabutra and Ju, 2000; Wilkes *et al*., 2002), sulfate reduction (Coates *et al*., 1997; Kropp *et al.,* 2000; Rueter *et al.*, 1994; So and Young, 1999) and methanogenesis (Anderson and Lovley, 2000; Zengler *et al*., 1999). Most of the reports related to the anaerobic mineralization of aliphatic hydrocarbons are with enrichments or pure cultures in laboratory scale (Chayabutra and Ju 2000; So and Young 1999; Zengler *et al.*, 1999). Metabolic pathways of degradation of aromatic compounds by different groups of anaerobic bacteria can be referred in these review articles (Chakraborty and Coates, 2004; Diaz, 2004; Gibson and Harwood, 2002; Heider and Fuchs, 1997; Widdel and Rabus, 2001). Significance of these results in the bioremediation of contaminated soils and sediments is not yet completely investigated and only in few cases the anaerobic degradation of alkanes in environmental samples have been reported (Anderson and Lovley, 2000).

Polycyclic aromatic hydrocarbons (PAHs) are of most concern due to their toxicity, low volatility, resistance to microbial degradation, and high affinity for sediments. Although several authors have reported that PAHs are not degraded under strict anaerobic conditions (Heitkamp *et al.*, 1987; Mihelcic and Luthy, 1988), degradation of PAHs in the absence of oxygen with nitrate as the apparent electron acceptor has also been demonstrated (Leduc *et al*., 1992). Though degradation of polycyclic aromatic hydrocarbons has been confirmed, the rate of anaerobic hydrocarbon degradation was generally lower (Coates *et al*., 1997; Caldwell *et al*., 1998) than equivalent aerobic degradation rates. Nonetheless, removal of the contaminant hydrocarbons was just as extensive under anoxic conditions (Coates *et al*., 1997; Caldwell *et al*., 1998) and substantial degradation of high molecular weight *n*-alkanes and the isoprenoid hydrocarbons pristane and phytane was also observed (Caldwell *et al*., 1998).

Though there are several pure culture examples of nitrate-reducing, Fe (III)-reducing, and sulfate-reducing bacteria that are capable of completely oxidizing some of these hydrocarbon contaminants to carbon dioxide (Coates *et al*., 1996a; Rabus and Widdell, 1995; Rueter *et al*., 1994), there are only few reports on the efficiency of these cultures in the degradation of complex hydrocarbon mixtures under anaerobic conditions. In marine environments, the most important terminal electron-accepting processes are iron, manganese and sulfate reduction (Canfield *et al*., 1993), and the limited number of studies indicated that the process of anaerobic hydrocarbon degradation in marine environments is associated primarily with sulfate reduction (Coates *et al*., 1997; Caldwell *et al*., 1998). In contrast nitrate was observed to stimulate hydrocarbon degradation in terrestrial and freshwater environments. Coates *et al.* (1997) demonstrated that a wide variety of hydrocarbon contaminants can be degraded under sulfate-reducing conditions, and they suggested that it is possible to use sulfate reduction rather than aerobic respiration as a treatment strategy for hydrocarbon-contaminated dredged sediments. In addition, the authors observed that inoculation of high activity sediment samples to low activity sediments stimulated anaerobic hydrocarbon degradation significantly.

3. Anaerobic bioremediation of chlorinated compounds

Chlorinated aliphatic hydrocarbons (CAH) are manmade organic compounds. CAH are used in a wide variety of applications, such as solvents and degreasers and in the manufacturing of raw materials. Some of the most frequently occurring CAHs in soil and groundwater are tetrachloroethene (PCE), trichloroethene (TCE), carbon tetrachloride (CT), chloroform (CF), methylene chloride (MC), trichloroethane (TCA), dichloroethane (DCA), chloroethane (CA), dichloromethane (DCM), chloromethane (CM), *etc*. Of the various CAHs, TCE is the most prevalent contaminant.

Under anaerobic conditions, direct metabolism by reductive dechlorination, cometabolism and fermentation are the three mechanisms employed by anaerobic bacteria for effective biodegradation of CAHs. Reductive dechlorination generally involves the sequential replacement of a chlorine atom on a CAH with a hydrogen atom and has been observed to occur both directly and cometabolically. Reductive dechlorination theoretically is expected to occur under most anaerobic conditions, but has been observed to be most effective under sulfate-reducing and methanogenic conditions (USEPA, 1998). As in the case of aerobic oxidation, the direct mechanisms may biodegrade CAHs faster than cometabolic mechanisms (McCarty and Semprini, 1994). In cometabolism, often the amount of primary substrate required is a factor of 100 to 1,000 times the amount of the CAH. In direct metabolism (respiration with only the chlorinated solvent as the electron acceptor), the stoichiometry is much more favorable, and a much smaller amount of supplemental chemical is required (Bouwer, 1994). In the case of fermentation, the chlorinated hydrocarbon serves as an electron donor, electron acceptor and carbon source. Mägli *et al*. (1996) isolated a strict acetogenic anaerobic bacterium capable of growth on dichloromethane and reported that the fermentation of dichloromethane to formate and acetate. Fermentative dechlorination is an energetically favorable process (Table 2). The diversity of dechlorinating anaerobic bacteria (El Fantroussi *et al*., 1998) and their energy metabolism (Holliger *et al*., 1999) have been reviewed earlier.

4. Direct anaerobic reductive dechlorination

In direct anaerobic reductive dechlorination bacteria gain energy and grow as one or more chlorine atoms on a chlorinated hydrocarbon are replaced with hydrogen (Gerritse *et al*., 1999). In that reaction, the chlorinated compound serves as the electron acceptor, and hydrogen serves as the direct electron donor (Fennel *et al*., 1997). Hydrogen used in the reaction typically is supplied indirectly through the fermentation of organic substrates. The reaction when coupled with growth is also referred to as *halorespiration* or *dehalorespiration* (Gossett and Zinder, 1997). The Gibbs free energy change for all major half-reactions of the reduction of chlorinated methanes, ethanes and ethenes and electron acceptors such as sulfate, nitrate and carbon dioxide are given in Table 1. From these data, it can be seen that the reductive dechlorination of nearly all CAHs is an energetically favorable (exergonic) reaction under standard conditions. This implies that biological reductive dechlorination processes may occur in nature.

The anaerobic reductive dechlorination of the more chlorinated CAHs (PCE and TCE) occurs more readily than the dechlorination of CAHs that already are somewhat reduced, *viz*., dichloroethene (DCE) and vinyl chloride (VC).

Table 1. Half reactions of reductive dechlorination of some chlorinated hydrocarbons in comparison with other commonly used terminal electron acceptors.

Electron acceptor	Half reaction of reductive transformations	$\Delta G^{o\prime}$/ electron (kJ)*
O_2	$O_2 + 4\,H^+ + 4e^- \longrightarrow 2\,H_2O$	-78.7
MnO_2	$MnO_2 + HCO_3^- + 3H^+ + 2e^- \longrightarrow MnCO_3 + 4H_2O$	-58.9
NO_3^-	$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$	-41.7
$Fe(OH)_3$	$Fe(OH)_3 + 3H^+ + e^- \longrightarrow Fe^{2-} + 3H_2O$	-11.4
SO_4^{2-}	$SO_4^{2-} + 9H^+ + 8e^- \longrightarrow HS^- + 4H_2O$	+20.9
HCO_3^-	$HCO_3^- + 9H^+ + 8e^- \longrightarrow CH_4 + 3H_2O$	+23.0
PCE	$C_2Cl_4 + H^+ + 2e^- \longrightarrow C_2HCl_3 + Cl^-$	-55.3
TCE	$C_2HCl_3 + H^+ + 2e^- \longrightarrow$ *cis*-$C_2H_2Cl_2 + Cl^-$	-53.0
TCE	$C_2HCl_3 + H^+ + 2e^- \longrightarrow$ *trans*-$C_2H_2Cl_2 + Cl^-$	-50.9
TCE	$C_2HCl_3 + H^+ + 2e^- \longrightarrow$ 1,1-$C_2H_2Cl_2 + Cl^-$	-50.8
cis-DCE	*cis*-$C_2H_2Cl_2 + H^+ + 2e^- \longrightarrow C_2H_3Cl + Cl^-$	-38.3
trans-DCE	*trans*-$C_2H_2Cl_2 + H^+ + 2e^- \longrightarrow C_2H_3Cl + Cl^-$	-40.4
1,1-DCE	1,1-$C_2H_2Cl_2 + H^+ + 2e^- \longrightarrow C_2H_3Cl + Cl^-$	-40.5
VC	$C_2H_3Cl + H^+ + 2e^- \longrightarrow C_2H_4 + Cl^-$	-43.4
DCM	$CH_2Cl_2 + H^+ + 2e^- \longrightarrow CH_3Cl + Cl^-$	-47.5
CM	$CH_3Cl + H^+ + 2e^- \longrightarrow CH_4 + Cl^-$	-45.2
TCA	$C_2H_3Cl_3 + H^+ + 2e^- \longrightarrow C_2H_4Cl_2 + Cl^-$	-54.1
1,2-DCA	$CH_2Cl\text{-}CH_2Cl + 2e^- \longrightarrow C_2H_4 + 2Cl^-$	-71.3
DCA	$C_2H_4Cl_2 + H^+ + 2e^- \longrightarrow C_2H_5Cl + Cl^-$	-38.3
1,2-DCA	$CH_2Cl\text{-}CH_2Cl + H^+ + 2e^- \longrightarrow C_2H_5Cl + Cl^-$	-36.2
CA	$C_2H_5Cl + H^+ + 2e^- \longrightarrow C_2H_5 + Cl^-$	-44.5
CT	$CCl_4 + H^+ + 2e^- \longrightarrow CHCl_3 + Cl^-$	-65.0
CF	$CHCl_3 + H^+ + 2e^- \longrightarrow CH_2Cl_2 + Cl^-$	-54.0

* Calculated on the basis of data from Thauer *et al*. (1977), Dolfing and Janssen (1994) and Vogel *et al*. (1987) under the following standard conditions; $H^+ = 10^{-7}$ M; $Cl^- = 10^{-3}$ M; T=25ºC.

Table 2. Fermentative dechlorination of few chlorinated hydrocarbons and Gibb´s free energy of the reactions.

Compound	Reaction	$\Delta G^{o\prime}$ (kJ)
TCM	$CH_3CCl_3 + 2H_2O \longrightarrow CH_3COOH + 4HCl$	-379.5
DCM	$3CH_2Cl_2 + CO_2 + 4H_2O \longrightarrow CH_3COOH + 2HCOOH + 6HCl$	-685.2
CM	$4CH_3Cl + 2CO_2 + 2H_2O \longrightarrow 3CH_3COOH + 4HCl$	-455.5
CT	$CCl_4 + 2H_2O \longrightarrow CO_2 + 4HCl$	-619.7
CF	$4CHCl_3 + 6H_2O \longrightarrow CH_3COOH + 2CO_2 + 12HCl$	-416.6

Accumulation of DCE and VC are observed in anaerobic environments. It also has been observed that, while VC can be effectively dechlorinated, the presence of PCE in groundwater may inhibit the anaerobic reductive dechlorination of VC (Tandoi *et al*., 1994). VC is more commonly remediated using aerobic mechanisms than anaerobic mechanisms. In anaerobic environments in which VC accumulates, enhanced aerobic bioremediation can be implemented to degrade the VC. Recent studies have demonstrated significant anaerobic oxidation of VC to carbon dioxide under Fe (III)-reducing conditions and of DCE to VC and VC to carbon dioxide under humic acid-reducing conditions (Bradley and Chapelle, 1998). These studies suggest the possibility of alternative biotransformation mechanisms under anaerobic conditions.

Hydrogen has been observed to be an important electron donor in anaerobic reductive dechlorination (Fennell *et al*., 1997). The presence of hydrogen establishes a competition between the bacteria that mediate the anaerobic reductive dechlorination (such as *Dehalococcus ethenogenes* and *Dehalospirillium multivorans*) and methanogenic bacteria that also use hydrogen as an electron donor. However, it has been observed that the dechlorinating bacteria can survive at a partial pressure of hydrogen ten times lower than that at which the methanogenic bacteria can survive (Smatlak *et al*., 1996), thus providing an opportunity to support the dechlorinating bacteria by providing hydrogen at a slow rate (Fennell *et al*., 1997). In addition, in some subsurface environments, competition from nitrate or sulfate-reducing bacteria may limit both methanogenic activity and the extent of anaerobic reductive dechlorination. Studies have shown that anaerobic reduction of CAHs can occur by reductive dechlorination in a variety of environmental conditions (Beeman *et al*., 1994; Semprini *et al*., 1995). However, the efficiency of the anaerobic dechlorination processes at high redox potential values is limited; efficiency improves as the redox potential decreases. Pilot studies have been conducted at a variety of sites to examine the feasibility of stimulating *in situ* anaerobic reductive dechlorination by providing to the subsurface simple organic substrates, such as lactate, butyrate, methanol, ethanol, and benzoate (Harkness *et al*., 1999).

5. Cometabolic anaerobic reductive dechlorination

In cometabolic anaerobic reductive dechlorination, a chlorinated hydrocarbon is fortuitously degraded by an enzyme or cofactor produced during microbial metabolism of another compound. In such a case, biodegradation of the chlorinated compound does not appear to yield any energy or growth benefit for the microorganism mediating the reaction (Gossett and Zinder, 1997). In addition, enzyme systems such as iron-sulfur clusters, cobalamins, factor F430 or hematin can take part in side reactions that yield cometabolic transformation of CAHs. All these enzyme systems contain redox-active metal centers and are referred to as transition-metal cofactors. These transition-metal cofactors act as electron carriers in anaerobic bacteria. As electron transfer by these carriers is not very specific, a wide range of CAHs can be transformed (Gantzer and Wackett, 1991). Cometabolic anaerobic reductive dechlorination has been observed for PCE, TCE, DCE, VC, DCA, and CT under anaerobic conditions (Fathepure *et al*., 1987; Yager *et al*., 1997). In pilot- and full-scale applications, it is generally difficult to distinguish between direct and cometabolic anaerobic reductive dechlorination reactions. Both biodegradation mechanisms are referred to more generally as anaerobic reductive dechlorination. In laboratory-scale applications, direct and cometabolic anaerobic reductive dechlorination reactions can be distinguished. Several investigators have suggested that the most efficient bioremediation of CAHs will occur in aquifers that are characterized by an up-gradient anaerobic zone and a down-gradient aerobic zone (Bouwer, 1994). In the up-gradient zone, anaerobic reductive dechlorination of PCE might degrade to TCE, and eventually to VC. VC could then be degraded in the down-gradient aerobic zone of the CAH plume.

6. Anaerobic bioremediation of metal contamination

In case of toxic metals and metalloids, they are often soluble, and thus mobile in aerobic systems. However, under anoxic conditions, microorganisms reduce them to insoluble forms and immobilize them as precipitates. Different mechanisms used by anaerobic bacteria in the metals biotransformation are given in Fig. 2. Elements that may be immobilized in this way include chromium, uranium, technetium, cobalt, and selenium. Until recently, investigations of terrestrial bioremediation primarily focused on the treatment of soils and waters contaminated with organic pollutants (Blackburn, 1998; Head, 1998).

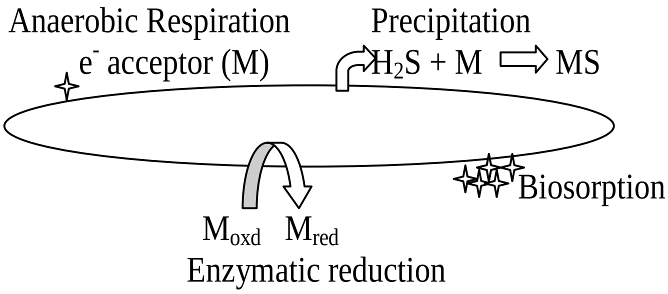

Fig. 2. Schematic representation of the mechanisms of metal transformation by different anaerobic bacteria (M-Metal; MS-Metal sulfide; M_{oxd} – Metal oxidized form; M_{red} – Metal reduced form).

There has been little investigation into the use of microorganisms for the remediation of metal contamination. In recent years there is considerable interest on the exploitation of microorganisms to ameliorate toxic metal contamination in terrestrial environments. Though, use of anaerobic microorganisms for remediation of toxic metal contamination is mostly laboratory-based research (Schmieman *et al*., 1997; Tucker *et al*., 1998), field level studies utilizing microorganisms for toxic metal remediation are becoming more prevalent. Lovley and Coates (1997) observed that remediation is due to the result of changes in the redox state of metal ions. Microorganisms can remove toxic metals and metalloids by converting them to forms that are precipitated or volatilized from solution. In other instances, microbial alteration of the redox state of either the contaminants or the Fe3+ and Mn4+ oxides, which bind most heavy metals, can make metals and metalloids more soluble. This can aid in the leaching of these contaminants from soils. The adsorption of metals and metalloids onto microbial biomass can also prevent further migration of these contaminants. Furthermore, microbe–metal interactions can play an important role in the remediation of organic contamination because microorganisms that use Fe3+ or sulfate as the terminal electron acceptor can remove organic contaminants from the environment (Lovley and Coates, 1997).

Chromium is recognized as a serious pollutant among heavy metals in the environment. Indeed, it is one of the most widely used metals in industry. Hexavalent chromium is considered to be much more toxic than trivalent chromium. Current treatment techniques for chromium-containing wastes involve aqueous reduction of Cr (VI) to Cr (III) by means of a chemical reducing agent to precipitate the less soluble Cr (III) (Eary and Rai, 1988). In case of bioremediation of chromium, a wide variety of microorganisms, such as *Escherichia coli*, *Enterobacter cloacae* (Shen and Wang, 1993), *Deinococcus radiodurans* (Frederickson *et al*., 2000), *Pseudomonas fluorescens* (DeLeo and Ehrlich, 1994), iron and manganese reducing *Pyrobaculum islandicum* (Kashefi and Lovley, 2000), denitrifying bacterial consortia (Schmieman *et al*., 1997) and anaerobic sulfate reducing bacteria (SRB), are able to reduce Cr (VI) and thereby able to detoxify the polluted environments (Gadd, 2000; Lovley, 1994, 1995; Lovley and Coates, 1997; White *et al*., 1997). Marsh and McInerney (2001) developed a consortium dependent on hydrogen for growth and Cr (VI) reduction. This technique appears to be efficient, environmentally friendly and cheaper than currently used processes such as treatment with lime (Barkay and Schaefer, 2001; Sen and Johnson, 1999). Due to their resistance to high heavy metal concentrations, SRB appear to be the best candidates for this process (Lovley, 2001). Cr (VI) is a known oxidizing agent (Mahan, 1967) and causes stress to the bacteria. The length of the lag phase increases with Cr (VI) concentration. But when substrate is not limiting, growth occurred in presence of chromium, leading to the removal of Cr (VI) from chromium-polluted effluents (Michel *et al*., 2001). Turick *et al*. (1996) reported that 92% of anaerobic Cr (VI)-reducing bacteria from soils were capable of greater than 30% Cr (VI)-reduction.

The use of sulfate as terminal electron acceptor, associated with the presence of very low redox potential cytochromes (cytochromes c3), produces sulfide, which can easily reduce a large number of heavy metals and precipitate them as metallic sulfides (Kim *et al*., 2001; White and Gadd, 1996). White *et al*. (1997) leached metals from the contaminated soil and stripped the toxic metals by a mixed culture of sulfate-reducing bacteria, which precipitated the metals as solid metal sulfides. Using this integrated procedure, 69% of the toxic metals present in an industrially contaminated soil were removed in 75 days. The procedure produced a liquid effluent of sufficiently low metal concentration that it could be safely discharged into the environment. This integrated procedure will probably be more commonly applied to *ex situ* applications (e.g. slurry reactors) but its successful use in soil is a prime example of advances in this field.

Chromate reduction is controlled not only by chemical reduction but also by an enzymatic process. The enzymatic reduction of Cr (VI) by cytochrome c_3 and hydrogenase has recently been demonstrated in *Desulfomicrobium norvegicum* and *Desulfovibrio vulgaris* strain Hildenborough (Lovley and Phillips, 1994; Tucker *et al*., 1998). It has also been demonstrated that the metal reductase activity of *Desulfuromas acetoxidans* and *Desulfovibrio vulgaris* is associated with the polyhemic *c*-type cytochrome (Assfalg *et al*., 2002).

Further heavy metals are used as terminal electron acceptors and the ability to use this reaction for energy conservation and growth is strain-dependent. For instance, *Dv*H can reduce Cr (VI) using several enzymes involved in the electron chain transfer, but reduction of this metal does not support growth (Chardin *et al*., 2002).

Ganesh *et al*., (1999) reported that there was effective precipitation of uranium from U (VI)-containing waste streams by *D. desulfuricans*. Technetium (^{99}Tc), a fission byproduct of ^{235}U, is a contaminant in waste streams of the nuclear-fuel cycle. The highly soluble pertechnetate anion (TcO_4^-) has been reduced and precipitated from solution in a flow-through bioreactor using *Desulfovibrio desulfuricans* (Lloyd *et al*., 1999). Tc(VII) precipitation rates by this organism were higher than Fe(III)-reducing *Shewanella* and *Geobacter* species (Lloyd and Macaskie, 1996). This implies that sulfate-reducing organisms are the optimal candidates for Tc (VII) removal from waste streams. Thus, direct and indirect anaerobic microbial reduction offers potential mechanisms for immobilizing these contaminants because many metals, such as uranium (U) and chromium, are less soluble in the reduced valence state (Lovley and Coates, 1997). In addition, bioremediation technologies using anaerobic bacteria are feasible alternatives to physical cleansing of soils and chemical or physical concentration of metals in polluted waters. Since the toxic metal seemed to undergo a degree of precipitation around the bacterial cells (Valls *et al*., 2000), metal immobilization in soil using bacteria (and the corresponding decrease in bioavailability) could have a longer term effect, not unlike addition of chemical amendments, such as zeolite, beringite and hydroxyapatite (Oste *et al*., 2001; Seaman *et al*., 2001). Microorganisms could therefore be employed to immobilize metals in moderately polluted fields, thus allowing their use in agriculture (Valls *et al*., 2000). It has also been shown that inoculation of metal-resistant bacteria into soils protected the indigenous bacterial community from the effects of heavy metals (Stephen *et al*., 1999).

Conclusions

Anaerobic strategies for *in situ* bioremediation are promising, but substantial research remains to be done before they can be widely adopted. Studies of *in situ* bioremediation of hydrocarbons coupled to Fe^{3+} reduction, reductive dechlorination, and stimulated metal reduction suggest that the anaerobic microorganisms involved in these processes in the contaminated aquifers are closely related to those that carry out these bioremediation reactions in pure culture in the lab. But some reports suggest that the anaerobic processes are effective at some sites, but not so in other cases. It is not clear whether this is due to the variability or heterogeneity in the distribution of the anaerobes or in environmental factors controlling their activity. A clear understanding on the diversity, distribution of anaerobic bacteria in diverse natural environments and their metabolic mechanisms will shed more light on the above observed discrepancies. These studies will provide better knowledge on the natural attenuation and promote rational design of strategies for accelerating bioremediation using anaerobes. In addition, understanding the physiology and genetics of anaerobic populations will be very useful in assessing their potential and to give a thrust on bioremediation using anaerobic bacteria.

References

Anderson, R.T. and Lovley, D.R. (2000). Hexadecane decay by methanogenesis. *Nature 404*, 722–723.

Assfalg, M., Bertini, I., Bruschi, M., Michel, C. and Turano, P. (2002). The metal reductase activity of some multiheme cytochrome *c*: chromium (III) by cytochrome c_7. *Proceedings of the National Academy of Science USA 99,* 9750–9754.

Atlas, M. (1981). Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiological Reviews* 45, 180-209.

Barkay, T. and Schaefer, J. (2001). Metal and radionuclide bioremediation: issues, considerations and potentials. *Current Opinion in Microbiology 4,* 318–323.

Beeman, R.E., Howell, J.E., Shoemaker, S.H., Salazar, E.A. and Buttram, R. (1994). A field evaluation of in situ microbial reductive dehalogenation by the biotransformation of chlorinated ethenes. In: *Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbon Compounds.* (R.E.Hinchee, A. Leeson, L. Semprini and S.K. Ong, eds.). Pp. 14-27. Lewis Publishers. Boca Raton. Blackburn, J.W. (1998). Bioremediation scale-up effectiveness: a review. *Bioremediation Journal 1,* 265-281.

Blackburn, J.W. (1998). Bioremediation scale-up effectiveness: a review. *Bioremediation Journal* 1, 265-282.

Bouwer, E.J. (1994). Bioremediation of chlorinated solvents using alternate electron acceptors. In: *Handbook of Bioremediation*. (R.D. Norris, ed.). Lewis Publishers, Inc. Boca Raton, Florida.

Bradley, P.M. and Chapelle, F.H. (1998). Effect of contaminant concentration on aerobic microbial mineralization of DCE and VC in stream-bed sediments. *Environmental Science and Technology 32,* 553-557.

Bregnard, T.P-A., Häner, A., Höhener, P. and Zeyer, J. (1997). Anaerobic degradation of pristane in nitrate-reducing microcosms and enrichment cultures. *Applied and Environmental Microbiology 63*, 2077–2081.

Caldwell, M.E., Garrett, R.M., Prince, R.C. and Suflita, J.M. (1998). *Environmental Environmental Science and Technology 63,* 2077–2081.

Caldwell, M.E., Garrett, R.M., Prince, R.C. and Suflita, J.M. (1998). Anaerobic biodegradation of long-chain *n*-alkanes under sulfate-reducing conditions. *Environmental Science and Technology 32*, 2191-2195.

Canfield, D., Thamdrup, B. and Hansen, J.W. (1993). The anaerobic degradation of organic matter in Danish coastal sediments: Fe reduction, Mn reduction and sulfate reduction. *Geochimica et Cosmochimica 57,* 3867-3883.

Chakraborty, R. and Coates, J.D. (2004). Anaerobic degradation of monoaromatic hydrocarbons. *Applied Microbiology Biotechnology 64*, 437-446.

Chardin, B., Dolla, A., Chaspoul, F., Fardeau, M.L., Gallice, P. and Bruschi, M. (2002). Bioremediation of chromate: thermodynamic analysis of the effects of Cr(VI) on sulfate-reducing bacteria. *Applied Microbiology Biotechnology 60,* 352–360.

Chayabutra, C. and Ju, L. (2000). Degradation of *n*-hexadecane and its metabolites by *Pseudomonas aeruginosa* under micro aerobic and anaerobic denitrifying conditions. *Applied and Environmental Microbiology 66*, 493–498.

Coates, J. D., Anderson, R. T. and Lovley, D. R. (1996a). Anaerobic hydrocarbon degradation in petroleum-contaminated harbor sediments under sulfate reducing and artificially imposed iron-reducing conditions. *Environmental Science and Technology 30,* 2784–2789.

Coates, J. D., Anderson, R. T. and Lovley, D. R. (1996b). Anaerobic oxidation of polycyclic aromatic hydrocarbons under sulfate-reducing conditions. *Applied and Environmental Microbiology 62*, 1099–1101.

Coates, J.D. and Anderson, R.T. (2000). Emerging techniques for anaerobic bioremediation of contaminated environments. *Trends in Biotechnology 18,* 408-412.

Coates, J.D., Woodward, J., Allen, J., Philp, P. and Lovley, D.R. (1997). Anaerobic degradation of polycyclic aromatic hydrocarbons and alkanes in petroleum-contaminated marine harbour sediments. *Applied and Environmental Microbiology 63,* 3589-3593.

DeLeo, P.C. and Ehrlich, H.L. (1994). Reduction of hexavalent chromium by *Pseudomonas fluorescens* LB300 in batch and continuous cultures. *Applied Microbiology and Biotechnology 40*, 756–759.

Diaz, E. (2004). Bacterial degradation of aromatic pollutants: a paradigm of metabolic diversity. *International Microbiology 7,* 173-180.

Dolfing, J. and Janssen, D.B. (1994). Estimates of Gibbs free energies of formation of chlorinated aliphatic compounds. Biodegradation *5*, 21-28.

Eary, L. E. and Rai, D. (1988). Chromate removal from aqueous wastes by reduction with ferrous iron. *Environmental Science and Technology 22,* 972–977.

Edwards, E.A. and Grbic-Galic, D. (1992). Complete mineralization of benzene by aquifer microorganisms under strictly anaerobic conditions. *Applied and Environmental Microbiology 58,* 2663–2666.

El Fantroussi, S., Naveau, H. and Agathos, S.N. (1998). Anaerobic dechlorinating bacteria. *Biotechnology Progress 14,* 167-188.

Fathepure, B.Z., Nengu, J.P. and Boyd, S.A. (1987). Anaerobic bacteria that dechlorinate perchloroethene. *Applied and Environmental Microbiology 53,* 2671-2674.

Fennell, D.E., Gossett, J.M. and Zinder, S.H. (1997). Comparison of butyric acid, ethanol, lactic acid, and propionic acid as hydrogen donors for the reductive dechlorination of trichlorothene. *Environmental Science and Technology 31,* 918-925.

Frederickson, J.K., Kostandarithes, H.M., Li, S.W., Plymale, A.E. and Daly, M.J. (2000). Reduction of Fe(III), Cr(VI), U(VI), and Tc(VII) by *Deinococcus radiodurans* R1. *Applied and Environmental Microbiology 66,* 2006–2011.

Gadd, G.M. (2000). Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. *Current Opinion in Biotechnology 11,* 271–279.

Ganesh, R., Robinson, K.G., Chu, L., Kucsmas, D. and Reed, G.D. (1999). Reductive precipitation of uranium by *Desulfovibrio desulfuricans*: evaluation of cocontaminant effects and selective removal. *Water Research 33,* 3447-3458.

Gantzer, C.J. and Wackett, L.P. (1991). Reductive dechlorination catalyzed by bacterial transition-metal coenzymes. *Environmental Science and Technology 25,* 715-722.

Gerritse, J., Drzyzga, O., Kloetstra, G., Keijmel, M., Wiersum, L.P., Hutson, R., Collins, M.D. and Gottschal, J.C. (1999). Influence of different electron donors and acceptors on dehalospiration of tetrachloroethene by *Desulfitobacterium Frappiere* TCE1. *Applied and Environmental Microbiology 65,* 5212-5221.

Gibson, J. and Harwood, C.S. (2002). Microbial diversity in aromatic compound utilization by anaerobic microbes. *Annual Review of Microbiology 56,* 345-369.

Gossett, J.M. and Zinder, S.H. (1997). Microbiological aspects relevant to natural attenuation of chlorinated ethenes. *Proceedings of the US EPA Symposium in Natural Attenuation of Chlorinated Organics in Ground Water*. Pp. 12-15. Dallas, Texas. EPA/540/R-97/504/1997.

Harkness, M.R., Bracco, A.A., Brennan Jr., M.J., DeWeerd, K.A. and Spivack, J.L. (1999). Use of bioaugmentation to stimulate complete reductive dechlorination of trichloroethene in Dover soil columns. *Environmental Science and. Technology 33,* 1100-1109.

Head, I.M. (1998). Bioremediation: towards a credible technology. *Microbiology 144,* 599-608.

Heider, J. and Fuchs, G. (1997). Microbial anaerobic aromatic metabolism. *Anaerobe 3,* 1-22.

Heitkamp, M. A., Freeman, J. P. and Cerniglia, C. E. (1987). Naphthalene biodegradation in environmental microcosms: estimates of degradation rates and characterization of metabolites. *Applied and Environmental Microbiology 53,* 129–136.

Holliger, C., Wohlfarth, G. and Diekert, G. (1999). Reductive dechlorination in the energy metabolism of anaerobic bacteria. *FEMS Microbiology Reviews 22,* 383-398.

Kashefi, K. and Lovley, D.R. (2000). Reduction of Fe(III), Mn(IV), and toxic metals at 100°C by *Pyrobaculum islandicum*. *Applied and Environmental Microbiology 66,* 1050–1056.

Kazumi, J., Häggblom, M.M. and Young, L.Y. (1995). Degradation of mono-chlorinated and non-chlorinated aromatic compounds under iron-reducing conditions. *Applied and Environmental Microbiology 61,* 4069–4073.

Kim, C., Zhou, Q., Deng, B., Thornton, E.C. and Xu, H. (2001). Chromium(VI) reduction by hydrogen sulfide in aqueous media:

stoichiometry and kinetics. *Environmental Science and Technology 35,* 2219–2225.

Kropp, K.G., Davidova, I.A. and Suflita, J.M. (2000). Anaerobic oxidation of n-dodecane by an addition reaction in a sulfate-reducing bacterial enrichment culture. *Applied and Environmental Microbiology 66,* 5393–5398.

Leahy, J.G. and Olsen, R.H. (1997). Kinetics of toluene degradation by toluene oxidizing bacteria as a function of oxygen concentration, and the effect of nitrate. *FEMS Microbiology Ecology 23,* 23-30.

Leduc, R., Samson, R., Al-Bashir, B., Al-Hawari, J. and Cseh, T. (1992). Biotic and abiotic disappearance of four PAH compounds from flooded soil under various redox conditions. *Water Science and Technology 26,* 51–60.

Lloyd, J.R. and Macaskie, L.E. (1996). A novel phosphoimager based technique for monitoring microbial reduction of technetium. *Applied and Environmental Microbiology 62,* 578-582.

Lloyd, J.R., Ridley, J., Khizniak, T., Lyalikova, N.N. and Macaskie, E. (1999). Reduction of technetium by *Desulfovibrio desulfuricans*: biocatalyst characterization and use in a flowthrough bioreactor. *Applied and Environmental Microbiology 65,* 2691-2696.

Lovley, D.R. (1994). Microbial reduction of iron, manganese and other metals. *Advances in Agronomy 54,* 175–229.

Lovley, D.R. (1995). Bioremediation of organic and metal contaminants with dissimilatory metal reduction. *Journal of Industrial Microbiology 14,* 85–93.

Lovley, D.R. (2001). Anaerobes to the rescue. *Science* 293, 1444–1446.

Lovley, D.R. and Coates, J.D. (1997). Bioremediation of metal contamination. *Curr. Opin. Biotechnol. 8,* 285–289.

Lovley, D.R. and Phillips, E.J.P. (1994). Reduction of chromate by *Desulfovibrio vulgaris* and its c_3 cytochrome. *Current Opinion in Biotechnology 60,* 726–728.

Lu, G., Clement, T.P., Zhen, C. and Wiedemeier, T.H. (1999). Natural attenuation of BTEX compounds model development and field application. *Groundwater 37,* 707-717.

Mägli, A., Wendt, M. and Leisinger, T. (1996). Isolation and characterization of *Dehalobacterium formicoaceticum* gen. nov. sp. nov., a strictly anaerobic bacterium utilizing dichloromethane as source of carbon and energy. *Archives of Microbiology 166,* 101-108.

Mahan, B.H. (1967). The chromium family. In: University chemistry, 2nd edn. Pp 557–559. Addison Wesley, London.

Marsh, T. and McInerney, M.J. (2001). Relationship of hydrogen bioavailability and chromate reduction in aquifer sediments. *Applied and Environmental Microbiology 67,* 1517-1521.

McCarty, P.L. and Semprini, L. (1994). Ground-water treatment for chlorinated solvents. In: *Handbook of Bioremediation*. (R.D. Norris, ed.). Pp. 87-116. Lewis Publishers. Boca Raton, Florida.

Michel, C., Brugna, M., Aubert, C., Bernadac, A. and Bruschi, M. (2001). Enzymatic reduction of chromate: comparative studies using sulfate-reducing bacteria. *Applied Microbiology and Biotechnology 55,* 95–100.

Mihelcic, J. R. and Luthy, R. G. (1988). Microbial degradation of acenaphthene and naphthalene under denitrification conditions in soil-water systems. *Applied and Environmental Microbiology 54,* 1188–1198.

Oste, L.A., Dolfing, J., Ma, W.C. and Lexmond, T.M. (2001). Effect of beringite on cadmium and zinc uptake by plants and earthworms: more than a liming effect? *Environmental Toxicology and Chemistry 20,* 1339-1345.

Rabus, R. and Widdel, F. (1995). Anaerobic degradation of ethylbenzene and other aromatic hydrocarbons by new denitrifying bacteria. *Archives of Microbiology 163,* 96–103.

Rueter, P., Rabus, R., Wilkes, H., Aeckersberg, F., Rainey, F.A., Jannasch, H.W. and Widdel, F. (1994). Anaerobic oxidation of hydrocarbons in crude oil by new types of sulphate reducing bacteria. *Nature* 372, 455-4458.

Schmieman, E.A., Petersen, J.N., Yonge, D.R., Johnstone, D.L., Bereded-Samuel, Y., Apel, W.A. and Turick, C.E. (1997). Bacterial Reduction of Chromium. *Applied Biochemistry and Biotechnology 63,* 855–864.

Seaman, J.C, Meehan, T. and Bertsch, P.M. (2001). Immobilization of cesium-137 and uranium in contaminated sediments using soil amendments. *Journal of Environmental Quality 30,* 1206-1213.

Semprini, L., Kitanidis, P.K., Kampbell, D. and Wilson, J.T. (1995). Anaerobic transformation of chlorinated aliphatic hydrocarbons in a sand aquifer based on spatial chemical distributions. *Water Resources Research 31,* 1051-1062.

Sen, A.M. and Johnson, B. (1999). Acidophilic sulphate-reducing bacteria: candidates for remediation of acid mine drainage. In: *Biohydrometalurgy and the environment toward the mining of the 21st century. Part A: Bioleaching, microbiology*. (R. Amils and A. Ballester, eds.). Pp. 709-718. Elsevier, Amsterdam.

Shen, H. and Wang, Y.T. (1993). Characterization of enzymatic reduction of hexavalent chromium by *Escherichia coli* ATCC 33456. *Applied and Environmental Microbiology 59,* 3771–3777.

Smatlak, C.R., Gossett, J.M. and Zinder, S.H. (1996). Comparative kinetics of hydrogen utilization for reductive dechlorination of tetrachloroethene and methanogenesis in an anaerobic enrichment culture. *Environmental Science and Technology 30,* 2850-2858.

So, C.M. and Young, L.Y. (1999). Isolation and characterization of a sulfate-reducing bacterium that anaerobically degrades alkanes. *Applied and Environmental Microbiology 65,* 2969–2976.

Stephen, J.R., Chang, Y.J., Macnaughton, S.J., Kowalchuk, G.A., Leung. K.T., Flemming, C.A. and White, D.C. (1999). Effect of toxic metals on indigenous soil beta-subgroup proteobacterium ammonia oxidizer community structure and protection against toxicity by inoculated metal-resistant bacteria. *Applied and Environmental Microbiology 65,* 95-101.

Tandoi, V., DiStefano, T.D., Bowser, P.A., Gossett, J.M. and Zinder, S.H. (1994). Reductive dehalogenation of chlorinated ethenes and halogenated ethanes by a high-rate anaerobic enrichment culture. *Environmental Science and Technology 28,* 973-979.

Thauer, R.K., Jungermann, K. and Decker, K. (1977). Energy conservation in chemotrophic anaerobic bacteria. *Bacteriological Review 41,* 100-180.

Timmis, K.N. and Pieper, D.H. (1999). Bacteria designed for bioremediation. *Trends in Biotechnology 17,* 201-204.

Tucker, M.D., Barton, L.L. and Thomson, B.M. (1998). Reduction of Cr, Mo, Se and U by *Desulfovibrio desulfuricans* immobilized in polyacrylamide gels. *Journal of Industrial Microbiology and Biotechnology 20,* 13–19.

Turick, C.E., Apel, W.A. and Carmiol, N.S. (1996). Isolation of hexavalent chromium reducing anaerobes from hexavalent chromium contaminated and non-contaminated environments. *Applied Microbiology and Biotechnology 44,* 683–688.

U.S. Environmental Protection Agency. (1998). *Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water*. Office of Research and Development. Washington, D.C.

Valls, M., Atrian, S., de Lorenzo, V. and Fernandez, L.A. (2000). Engineering a mouse metallothionein on the cell surface of *Ralstonia eutropha* CH34 for immobilization of heavy metals in soil. *Nature Biotechnology 18,* 661- 665.

Vidali, M. (2001). Bioremediation. An overview. *Pure and Applied Chemistry 73,* 1163-1172.

Vogel, T.M., Criddle, C.S. and McCarty, P.L. (1987). Transformations of halogenated aliphatic compounds. *Environmental Science and Technology 22,* 722-736.

White, C. and Gadd, G.M. (1996). A comparison of carbon/energy and complex nitrogen sources for bacterial sulphate-reduction: potential applications to bioprecipitation of toxic metals as sulphides. *Journal of Industrial Microbiology 17,* 116–123.

White, C., Sayer, J.A. and Gadd, G.M. (1997). Microbial solubilization and immobilization of toxic metals: key biogeochemical processes for treatment of contamination. *FEMS Microbiology Reviews 20*, 503–516.

Widdel, F. and Rabus, R., (2001). Anaerobic biodegradation of saturated and aromatic hydrocarbons. *Current Opinion in Biotechnology 12,* 259-276.

Wilkes, H., Rabus, R., Fischer, T., Armstroff, A., Behrends, A. and Widdel. F. (2002). Anaerobic degradation of n-hexane in a denitrifying bacterium: Further degradation of the initial intermediate (1-methylpentyl) succinate via C-skeleton rearrangement. *Archives of Microbiology 177,* 235–243

Yager, R.M., Bilotta, S.E., Mann, C.L. and Madsen, E.L. (1997). Metabolic and *in situ* attenuation of chlorinated ethenes by naturally occurring microorganisms in a fractured dolomite aquifer near Niagara Falls, New York. *Environtal Science and Technology 31,* 3138-3147.

Zengler, K., Richnow, H.H., Rossello-Mora, R., Michaelis, W. and Widdel, F. (1999). Methane formation from long-chain alkanes by anaerobic microorganisms. *Nature 401,* 266–269.

Zhou, J., Fries, M.R., Chee-Sanford, J.C. and Tiedje, J.M. (1995). Phylogenetic analyses of a new group of denitrifiers capable of anaerobic growth on toluene and description of *Azoarcus tolulyticus* sp. nov. *International Journal of Systematic Bacteriology 45,* 500-506.