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Bioaugmentation of Chlorinated Solvents in Groundwater Using Dehalococcoides

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ABSTRACT

With the era of industrialization and excessive dependence on chemicals, there is increased indiscriminate disposal of especially chlorinated solvents causing a variety of environmental problems. Chlorinated solvents including trichloroethylene (TCE) and perchloroethylene are significant groundwater contaminants causing severe health effects. Various technical strategies have been developed and applied, often with limited success. Time frames for remediation tend to be long, usually measured in decades. In this review we focus on remediation of chlorinated solvents in groundwater by the addition of exogenous microorganisms, specifically members of the genus Dehalococcoides (DHC) referred to as bio augmentation. Many potential technologies for remediation of chlorinated solvents, its past, present and future as well as how bio augmentation is superior to these techniques are discussed.

Keywords: *Bio augmentation; Chlorinated Solvents; Groundwater Contamination; Dehalococcoides.*

1. INTRODUCTION

Groundwater is of of most importance to human life as it is the greatest source of drinking water. (M. Giordano, 2009) It is also useful in several other domains such as agriculture and industrial purposes. (Kulkarni et al., 2015).However, with the advent of industrialization and urbanization, more and more resources of groundwater have been polluted by increasing anthropogenic emissions (Zhanxue Suna et al, 2014). India incorporates some of the world's largest fluvial systems in the world (River Ganges, Brahmaputra and Indus Basins), yet its distribution of usable groundwater in the region varies considerably and the presence of contaminants has constrained the continued availability of safe water (A. Mukherjee et al, 2015). Due to the lack of awareness of protection in groundwater, the situation is worsening day by day. (Simpson et al, 2002).According to 2010 statistics, about 38% of groundwater was found to be with chlorinated solvents. The most recurrent organic contaminants in groundwater consist of chlorinated solvents such as tetrachloroethene (PCE) and trichloroethene (TCE), methyl chloroform, 1,2- dichloropropane, 1,2-dichloroethylene and chloroform. These chlorinated solvents were used formerly in a widespread manner due to its high solvent power and lack of flammability. Although these solvents were of great use, it had a hazardous impact on the ecology and insidiously affects the human and environmental health.

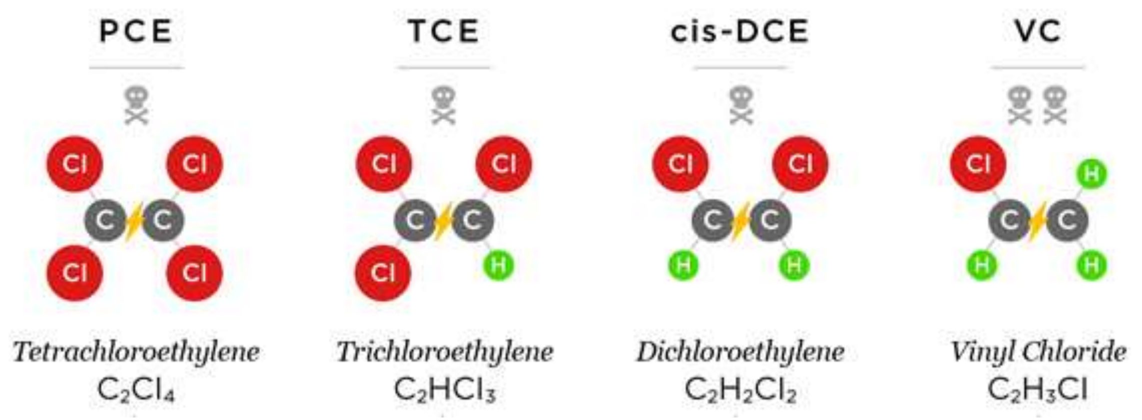

Fig 1.Structures of Different Chlorinated Solvents

As per the 2012 U.S.E.P.A Toxic Release Inventory, a total of approximately 10,000,000 chlorinates aliphatic hydrocarbons (CAH) were reported to be released in the U.S. The CAH most frequently released are trichloroethylene (TCE), dichloromethane (DM), carbon tetrachloride (CT), chloroform (CF), and tetrachloroethylene (PCE). Furthermore, the examination of the CAH material safety data sheets (MSDS) indicates that 11 out of the 17 most common CAH's are classified as known or suspected carcinogens (Xuhui Maoa et al, 2012). Nations are rapidly increasing in either population or chemical use, for instance, China and India realize that their natural resources cannot support the burden of uncontrolled chemical disposal. While pollution prevention and sustainable development measures are preferred in many cases damage has already been done. Remediation offers the chance to reduce the polluted levels. There are numerous proposed remediation technologies incorporating physical, chemical, and biological processes. Although the existing microorganisms in the environment can degrade organic pollutants due to their metabolic processes, their performance in an environmental matrix (e.g groundwater) might not be adequate to degrade target contaminants at a reasonable rate (Dario Frascari, 2015). Thus, bioaugmentation comes into play.

Bioaugmentation is the addition of specialized microbial strains into a niche environment to enhance the degradation of toxic compounds that results in an innocuous state. (Agnieszka Mrozika, 2010) A good remediation strategy has to be effective, efficient and economical and bioaugmentation provides the same. Different successful cases of bioaugmentation for dechlorination of chlorinated organics have been stated. A specialized microorganism Burkholderia cepacia ENV435 decreased the total concentration of chlorinated ethenes in groundwater by 78% within 2 days after the bacteria injection (R.J. Steffan et al, 1999). An enhancement of dechlorination was observed for tetrachloride and 1,1,1- trichloroethane when methanogenic enrichment was used in conjugation with iron. (K.B. Gregory et al,2000) .A successful bioaugmentation using KB-1 culture was reported in an *in-situ* groundwater remediation decreasing concentrations of PCE , TCE and cis- 1,2 – dichloroethene from several hundred micrograms per liter to below 5 grams per liter within 200 days after injection (D.W. Major et al,2002)

2. CONTAMINATION OF GROUNDWATER BY CHLORINATED SOLVENTS

Groundwater quality deterioration and supply of safe drinking water is a major concern throughout the world. There are different ways in which groundwater can be contaminated. (a)Some groundwater pollution occurs naturally. Several studies have reported arsenic contamination in many regions of the world especially the deltas and flood plains in the south and south East Asia. (S. Wang et al, 2000). As of 2009, more than 140 million people worldwide were drinking arsenic contaminated ground waters. (Ravenscroft et al, 2009). There have been also reporting of fluoride contamination (Amlan Banerjee, 2015) in groundwater characterized by the presence of crystalline basement rocks / volcanic bed rocks with the dissolution of fluoride ions. (V.K. Saxena, 2001) According to WHO more than 260 million people around the world are affected by high fluoride concentration in groundwater exceeding 1.5 mg/L. (Amini et al., 2008).

(b) Secondly, we have petroleum based fuels such as gasoline and diesel that contribute to the contamination of groundwater. Nationally, the US Environmental Protection Agency (EPA) has recorded that there have been over 400,000 confirmed releases of petroleum based fuels leaking from underground storage tanks (E. Martinez, 2004). Gasoline is a mixture of various hydrocarbons that evaporate easily, dissolved to some extent in water and often toxic. Benzene, a component of gasoline is considered to cause cancer in humans. (Dalva A. Souzaa et al, 2009)

(c) Another frequent class of groundwater contaminants comprises of chlorinated solvents. These chemicals resemble petroleum hydrocarbons in that they are made up of carbon and hydrogen atoms, but the molecules also have chlorine atoms in their structure. Presence of chlorine makes this class of compounds more lethal than fuels. Unlike petroleum based fuels, solvents are usually heavier than water and thus tend to sink to the bottom of aquifers. This makes solvent contaminated aquifers much more difficult to clean up than those polluted by fuels. (N. Sonoyama, 2001).

The chlorinated hydrocarbons play its role either as reactants or solvents for the synthesis of herbicides and plastics, dry cleaning solvents in the garment industry, meticulous solvent cleaning in electronic industries, petroleum refining, chlorine bleach comprising household products, industrial automotive and aerospace solvent lubricating(B. de Rivas,2012). Consumption of chlorinated groundwater has been proved to be hazardous to human health.(Ambuja et al,2011)These were found to cause lightheadedness, bradycardia, arrhythmia, nervous system damage (W.M. Oshiro et al,2008), cognitive impairment and abnormalities in sleep cycle(G. Winneke,1981).Due to their high volatility in ambient condition, noxiousness, strong recalcitrance to deprivation and oncogenic effects, CHC's have been listed as one of the significant pollutants by the United States Environmental Protection Agency and European Commission.(ATSDR,2000) Its hazardous impact affects not only the human health but also the niche environment. These hydrocarbons can cause ozone layer depletio01

11n and formation of photochemical fog. Due to the direct and indirect detrimental effects associated with CHC's it is of great prominence to develop efficient methods for the removal of such pollutants and toxins. (McCarty, 2010).

Table 1.Physichemical Factors of Chlorinated Solvents and Its Environmental Impact

Attribute	Environmental Challenges
Volatile	Readily form vapor plumes in soils
Chemically stable under typical aerobic conditions	Often slow to degrade in aerobic soils and groundwater systems
Many are nonflammable	Stable under natural aerobic conditions
soluble in water	Small releases can contaminate large amounts of water and persist as sources for long periods of time
Densities much greater than water	Can sink through water-saturated media (e.g., aquifers and aquitards), contaminating water deep underground
Low viscosity	Can move quickly through subsurface environments

3. HISTORICAL DEVELOPMENTS OF BIO AUGMENTATION

The notion of adding microbes to perform reactions is an ancient technology such as the usage of microbial inoculum to create fermented dairy products such as cheese and yoghurt. Using this concept, various remediation methods have been developed as a cost effective clean up technology to treat the pollutants. Bio augmentation and bio stimulation are two methods that enhance the biodegradation of hydrocarbons, thereby increasing the efficiency of bioremediation. Bio augmentation is the introduction of exogenous degrading microorganisms to the site of contagion.

Bio augmentation for treating contaminated soils and groundwater were initially deliberated during early 1990's with the growing recognition of bioremediation to treat petroleum hydrocarbons and wood preserving waste. By 1992, there were at least 75 bioaugmentation cultures available commercially for in-situ bioremediation, according to a survey on microbial inoculants for bioremediation published Ontario Ministry of the Environment (MOE)(Major et al,1992)This survey indicated that inoculants were composed of common soil microorganisms (e.g., various species of Pseudomonas, Bacillus, Mycobacterium, Micrococcus, Phanaerochaetes, Alcaligenes, Nocardia, Thiobacillus, Arthrobacter, Flavobacterium) grown under aerobic conditions.

Lack of knowledge on biodegradation pathways for many subject chemicals poses a threat to the researchers who are unable to select the best microbe for its degradation. The early development of bioaugmentation technology for in situ remediation may also have been hindered by the common misunderstanding that bioaugmentation necessarily involved the release of genetically engineered microorganisms (GEMs) into the environment. (Lajoie et al., 1994) Indeed, with the advent of new genetic engineering tools in the early 1990s led to the development of a variety of GEMs with specialized capabilities for biodegrading recalcitrant compounds (Krumme et al., 1994). A lack of understanding regarding the risks of GEMs to human health ultimately led to public and regulatory opposition to bioaugmentation using GEMs (Pipke et al., 1992).

With the introduction of molecular techniques for microbial identification and a better understanding of success and failure of bioremediation under wide-ranging environmental conditions led to the various researches and field study in successful Bio augmentation. (Ellis et al, 2000).

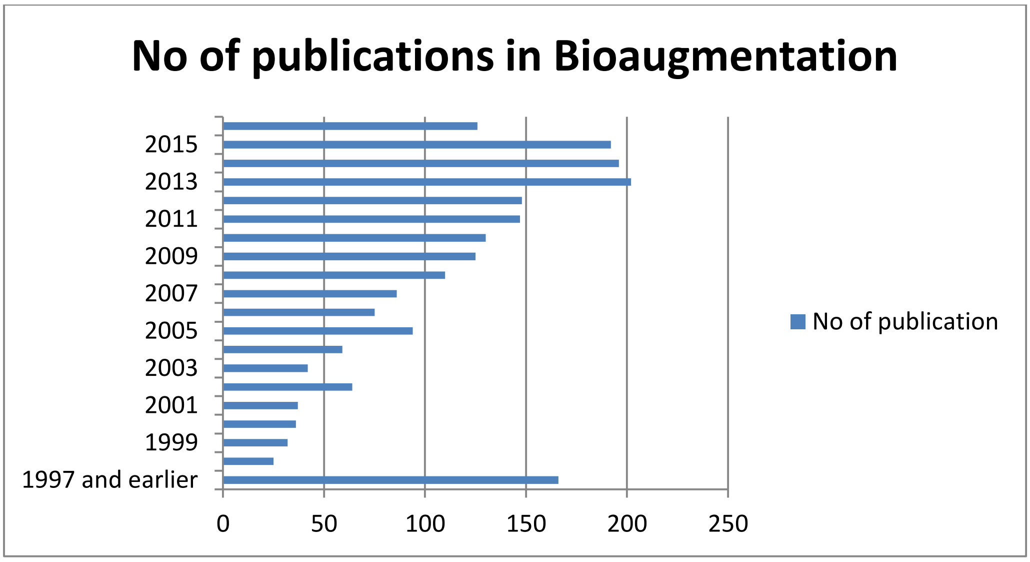

Fig 2. No of publication in the field of bioaugmentation
Adapted from science direct (1996-2016)

4. CHALLENGES

Identification and isolation of suitable microbial strains, and their continual survival and activity, once released into the target habitat are the sole key factors on which the bioaugmentation techniques depends on(Ian P.Thompson et al,2005).For the past 100 years, the microbial strains have been accomplished by selective enrichment which involves collecting samples from the polluted area and enrich them to grow, relative to the background community, in culture, using the target contaminant as the sole elevating carbon or nitrogen source (Beijerinck, 1901). However, such enriched populations are not certainly typical or representative of indigenous communities in the target habitat and by chance, can equally, be derived from transient populations (Recorbet et al., 1992).

Though there have been much success reports it's often overlooked with controversy because of its unreliable past performance records (Bouchez et al., 2000). Often, failures are due to a plethora of factors, such as fluctuating environmental conditions which includes moisture , pH, redox, osmotic factors, the presence of toxic co-contaminants, concentration, competition from indigenous microbial populations, the absence of key co substrates are few among them. (Goldstein et al., 1985, Barriault and Sylvestre, 1993 ;) It are pointed out that selection step, and explicitly poor knowledge of the composition and population dynamics of source communities, as well as those in the target habitats (competitors in waiting), is the major reason for inocula failures. (England et al., 1993).

5. BIOAUGMENTATION

Bio augmentation is the addition of vigorously growing, specific microbial strains into a microbial community in an effort to augment the ability of the microbial community to respond to process fluctuations or to degrade certain compounds, resulting in improved treatment. Some see it as an expensive and ineffective equivalent to voodoo medicine, while others eulogize its virtues and even stem a living from its commercialization (El Fantroussi and Agathos, 2005). The rationale underlying bio augmentation in bioremediation is simple: the augmentation of catabolically-relevant organisms to hasten remediation.

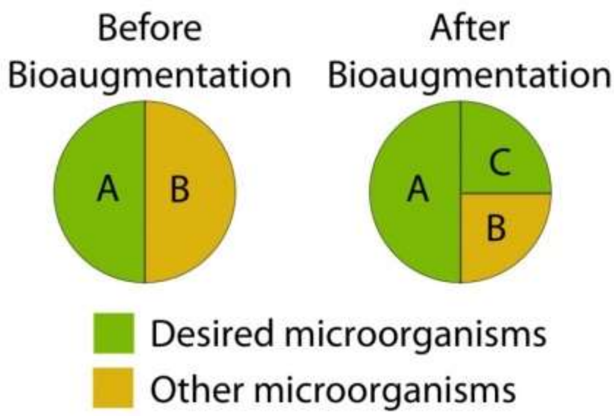

Fig 3. An overview of Bioaugmentation

Bioaugmentation should be applied in soils (i) with a lesss or non-detectable number of contaminant-degrading microbes, (ii) enclosing compounds requiring multiprocessor remediation, including processes harmful or toxic to microbes and (iii) for small-scale sites on which rate of non-biological methods exceed the cost of bio augmentation(Forsyth et al,1995).Moreover, the introduction of microorganisms into the soil is particularly recommended for areas polluted with compounds requiring a long period of time to adapt. Bioaugmentation finds its relevance in case of failure or unsatisfying results from approaches such as biostimulation and bioattenuation. (Vogel 1996; Iwamoto and Nasu, 2001;).The addition of nutrients to a contaminated site to mandate encouraged the growth of naturally occurring chemical-degrading microorganisms is called Bio stimulation while Bio attenuation is an enhancement of natural processes to disintegrate contaminants through biological alteration(Hamdi et al,2007).One of the studies reported that bioaugmentation was the most operative method in comparison with biostimulation and bioattenuation in the removal of petroleum hydrocarbons(Bento et al,2005).

Many studies have shown that both abiotic and biotic factors influence the effectiveness of bioaugmentation. The key abiotic factors are temperature, moisture (Cho et al. 2000), pH (Wolski et al,2006)and organic matter content, aeration, nutrient content and soil type(Ronen et al,2000).Biotic factors, including competition between exogenous and indigenous microorganisms for restricted carbon sources as well as antagonistic interactions and predation by protozoa and bacteriophages, also play critical roles in the final results of bioaugmentation(England et al,1993).All these interactions possibly decrease the number of introduced cells. Hong et al. (2007) Since successful bioaugmentation depends on the use of the active inoculants, either single culture or consortium, governed by various biotic and abiotic factors, use of autochthonous bioaugmentation is highly recommended for the enhancement of efficiency of wastewater system to avoid competition with normal flora(Nastee Kornochalert,2014).

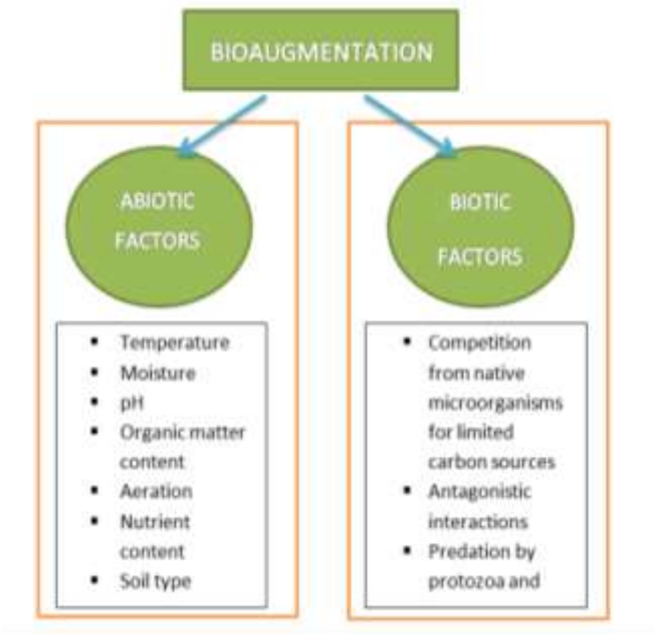

Fig 4. Factors Affecting Bioaugmentation

6. SELECTION OF METHOD AND MICROORGANISM

Not all microorganisms are capable of degradation of contaminants (Gentry et al. 2004). Hence several features such as fast growth, easy cultured, to withstand high concentrations of contaminants and the capability to survive in a broad spectrum of environmental conditions should be taken into consideration(van der Gast et al.2003).Several strategies are utilized for selection of microorganisms useful for bio augmentation. (Singer et al. 2005).The first approach involves isolation of bacteria from the contaminated area and culturing them under laboratory condition and returning them back to that area. This strategy is called inoculation of the site with native microorganisms (Cordova-Rosa et al. 2009). For example, the use of pure culture of Pseudomonas putida ZWL73 in soil polluted with 4-chloronitobenzene (4CNB) enhanced the 4CNB degradation in soil microcosms (Niu et al. 2009). The latter includes selection of suitable microorganisms from sites with similar contaminants submitted to clean-up the target site(Goux et al,2003) for example microbial consortia of Mycobacterium fortuitum, Bacillus cereus, Microbacterium sp., Gordonia polyisoprenivorans, Microbacteriaceae bacterium and Fusarium oxysporum was applied to degrade and mineralize anthracene, phenanthrene, and pyrene in soil. (Jacques et al,2008).Many studies have indicated that the use of consortia of aromatic-degrading bacteria has been more effective in removing pollutants as compared with selected single strains(Ghazali et al,2004).

Dehalorespiration is the key process underlying bioaugmentation of chlorinated solvents in groundwater. Dehalorespiration undergoes a process called reductive dechlorination of chlorinated ethenes, in which individual chlorine atoms are replaced with hydrogen. Reductive dechlorination of PCE and TCE was recognized as early as 1983 (Bouwer and McCarty, 1983), but the application of anaerobic biodegradation seems to be more harmful due to the accumulation of VC, a compound more toxic and carcinogenic than the parent compounds. At the same time (the early 1980s), it was also discovered that chlorinated ethenes could be cooxidized by aerobic bacteria. Cometabolism (co-oxidation) is the transformation of a compound by an enzyme synthesized by the cell for metabolism of another compound. The chlorinated ethenes can degrade via cometabolic dechlorination (e.g., Fathepure et al., 1987), for example, PCE cometabolism by Pseudomonas putida OX1 by Ryoo et al. (2000). Unfortunately, despite the promise of the aerobic cometabolism approach, field implementation was found to be very challenging and was met with a series of incremental hindrances
(Steffan et al., 1999).

Later studies demonstrated the complete dechlorination through cis-1,2-dichloroethene (cis-DCE) and VC to ethene of PCE and TCE in the lab(Freedman and Gossett, 1989) as well as in field (Major et al., 1991).However, after more than a decade of intense researches, it appears that complete conversion does not occur due to stalling at DCE where DCE gets accumulated for various reasons including 1) DCE is almost 4 times more soluble than TCE and can “emerge” and be retained in ways that would simulate a build-up related to poor metabolic response in the aquifer; and 2) that high levels of bioavailable iron and conversions from ferric to ferrous forms can interfere with electron flow to DCE (Evans and Koenigsberg, 2001; Koenigsbergetal.2002).The only microorganism belonging to the genus Dehalococcoides have demonstrated the capacity to dechlorinate cis-DCE and VC to ethene (e.g., Maymo-Gatell et al.,
1997).

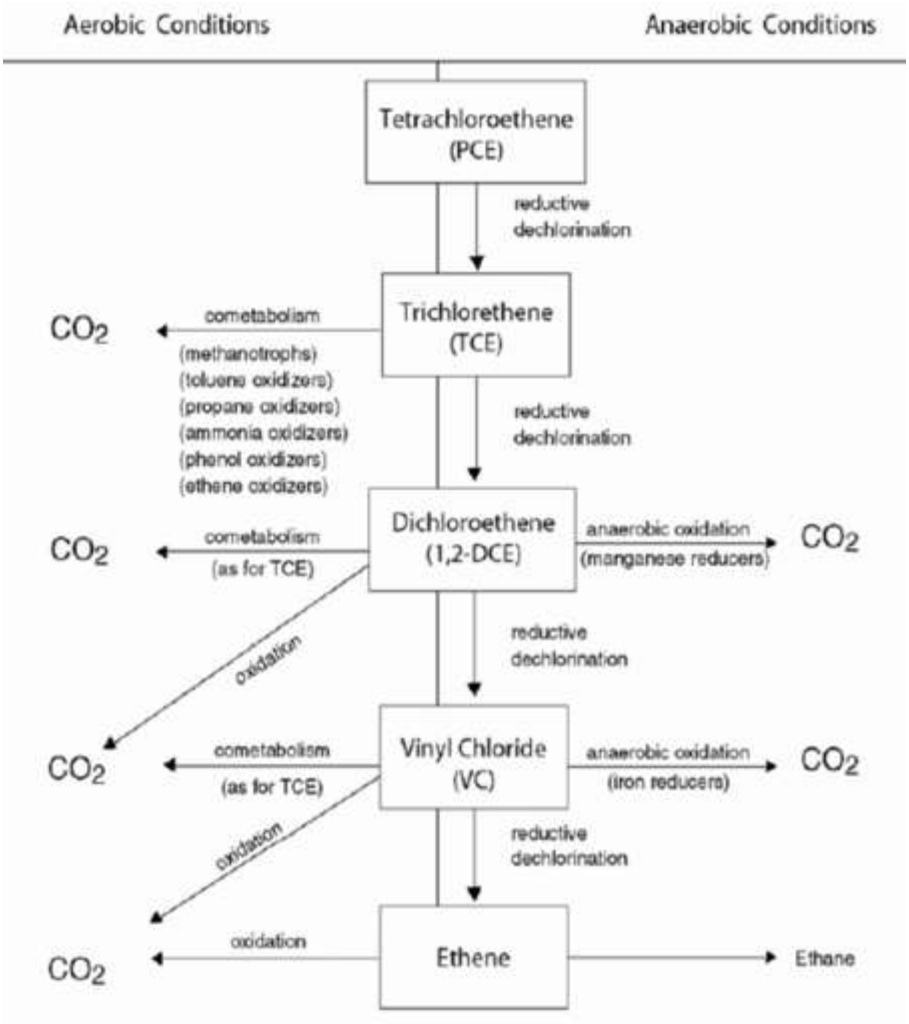

Fig 5: Degradation of chlorinated Ethenes

7. DEHALOCOCCOIDS

The Dehalococcoides belong to an isolated branch of the Bacteria phylogenetic tree containing organisms of the phylum Chloroflexi. The known Dehalococcoides sequences aredivided into two groups, putatively defined as the Ethenogenes group and the Alameda group. The first member of the "Dehalococcoides" (DHC) group, "Dehalococcoides ethenogenes", now D. mccartyi[2] strain 195 (DET) is noted for its potential use in the bioremediation of tetrachloroethene (PCE) and trichloroethene (TCE) contaminated ground water sites.

Table 2: Classification of Dehalococooides extracted from NCBI (accessed 4/3/2016)

Dehalococcoides -Classification	
Family	Dehalococcoidaceae
Order	Dehalococcoidales
Class	Dehalococcoidia
Division or phylum	Chloroflexi
Domain or empire	Bacteria

Dehalococcoides have proven its capacity to dechlorinate cis-DCE and VC to ethane. Convincing evidence for the critical role of Dehalococcoides in chlorinated ethane bioremediation was provided by Hendrickson et al. (2002), who conducted a survey of the occurrence of this microorganism at 24 contaminated sites using a 16S rRNA molecular genetic method. It was witnessed that dechlorination proceeded beyond cis-DCE to VC and ethene in the presence of dehalococcoids (21 of 24 sites) whereas in its absence (3 of 24 sites) dechlorination stalled, resulting in the accumulation of cis-DCE. The presence of Dehalococcoides does not essentially indicate that complete chloroethene reduction to ethene will occur. However, the inverse appears to be true: if Dehalococcoides is absent, then dechlorination past cis-DCE and VC to ethene does not happen (Hendrickson et al., 2002).

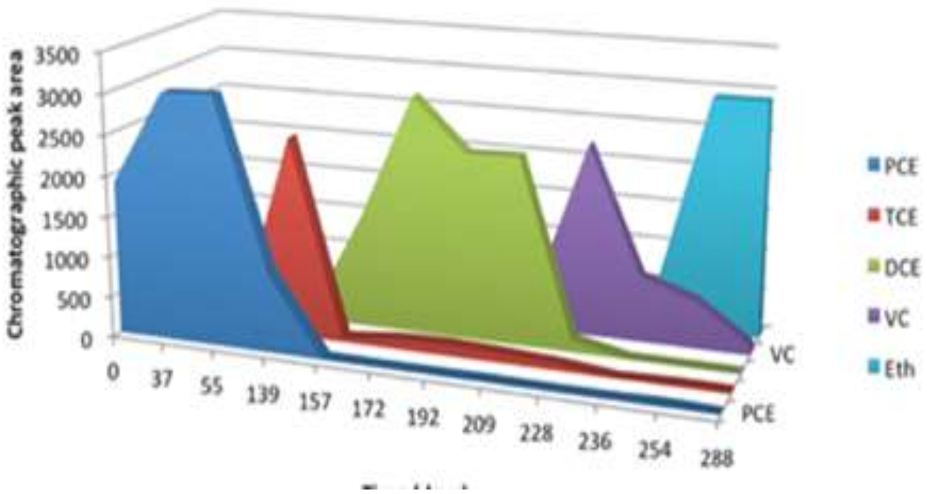

Fig 6.Case Study Of Bioaugmentation Of Chronited Solvents In Grounwater In Italy

8. CONCLUSION

Bioaugmentation has progressed from the realm of "snake-oil salesmen" selling unnecessary cultures into an ecologically credible and economically attractive technology. Bioaugmentation is currently overwhelmingly focused on the dehalorespiration of chlorinated ethenes by Dehalococcoides organisms although, in the future, anaerobic bioaugmentation with dehalorespiring bacteria will undoubtedly be applied to compounds other than the chloroethenes. For example, there appears to be significant potential for utilizing Dehalococcoides organisms for in situ biodegradation of chlorinated benzenes, ethanes, and propanes; polychlorinated biphenyls; dioxins; and brominated ethenes and ethanes. Also, various researches are being done in genetic augmentation and use of viral augmentation which is goine to change the phase of bioremediation strategies involved in remediation of chlorinated solvents in ground water.

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