Global NEST Journal, Vol 16, No 2, pp 316-328, 2014

ENVIRONMENTAL DYNAMICS AND ENGINEERED SYSTEMS FOR THE DEGRADATION OF TRICHLOROETHYLENE: A CRITICAL REVIEW

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Received: 16/01/2014
Accepted: 21/02/2014
Available online: 10/03/2014

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ABSTRACT

Trichloroethylene (TCE) is found in all mediums of environment in varying concentrations. Over the past 25 years, many engineered systems have been devised for its complete and sustainable degradation. This study reviews the environmental factors that influence the TCE pollution in environment and its biological mineralization via engineered systems. Although at some polluted sites natural attenuation of TCE has been found to occur but generally the natural process is very slow. The use of nanoparticles and composites provides a comparatively novel approach for the treatment of TCE contaminated waters. Biological engineered systems have been found to degrade TCE on much faster rates and higher concentrations. To identify the appropriate microorganisms in any engineered system that can effectively provide a low-cost treatment option for TCE degradation is the pressing need at the moment. Adding a second distinct organic phase to the aqueous medium for degrading fast and high concentration of TCE is recommended. The organic phase, which do not mix with the aqueous phase and can be easily separated, discharged, and reuse, should be selected based on its insolubility, volatility, non-biodegradability by the selected microorganism and the cost of the overall engineered system. Biodegradation offers the potential of cost effective treatment of TCE, however, that engineered systems should effectively use the biodegradative metabolism that nature has evolved.

Keywords: Biodegradation, engineered systems, microorganisms, nanoparticles, second phase, TCE

1. Introduction

From its invention, Trichloroethylene (TCE) has been produced on large scales and has been used for many purposes e.g. textile processing, refrigeration, lubricants and adhesives, for the production of vinyl chloride (VC), pharmaceuticals, and insecticides (Schettler *et al.*, 1999; US EPA, 2011). Mainly used as a universal degreasing and cleaning agent (Bakke *et al.*, 2007), TCE has vast trails of pollutions in all environmental mediums i.e. soil, air and water, from as low as 0.001 µg l^{-1} to as high as 100 000 µg l^{-1} (CEPA, 1993).

Although toxic effects were suspected from TCE exposure, it was not regulated until late 1980's. For example in US and Canada, environmental regulations were imposed in 1989 and 1995, respectively. Recently, strong relationship has been found between TCE exposure and cancer. More reliable results were published to relate kidney cancer due to TCE exposure (Scott *et al.*, 2011; Karami *et al.*, 2012; Chiu

et al., 2013). Several other health effects were also reported e. g. toxicity to the central nervous system, kidney, liver, immune system, male reproductive system, and the developing embryo/fetus through any type of exposure to TCE has been also found (Chiu *et al.*, 2013). Other than its own health drawbacks, it is a precursor to VC which is a strong and known carcinogen. Other daughter product like dichloroethylene (DCE) is also double bonded between two carbons like TCE and VC (Fig.1). Hence it is recommended that TCE contamination site should also be checked for VC contamination.

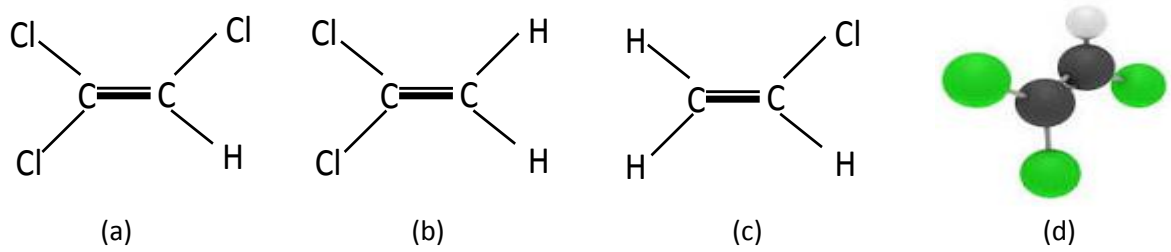

Figure 1. Molecular structure of (a) TCE; (b) 1,1-DCE; (c) VC, and (d) TCE structural model (From Wikipedia, the free encyclopedia)

TCE has unique properties with respect to contact with environment. It is heavier than water and also volatile. A spill of TCE proceeds in all dimensions. Due to heavier nature, it movies downward and adheres to soil particles, as well as volatilizes to air. Unlike many other common pollutants, it is not readily available for microbial degradation. It is stable in the sunlight and does not decompose through ordinary sunlight photo catalysis. Although it is heavier than water, yet some content of TCE is soluble in water. Major part of it is absorbed by the blood on inhalation through or ingestion through water. Bioaccumulation in plants tissue is not found. Table 1 describes some of its physical and chemical properties.

Table 1. Physical and chemical properties of TCE (Russel *et al.*, 1992; Chiao *et al.*, 1994; Jacoby *et al.*, 1998)

Parameter	Unit	Value
Molecular weight	g mol^{-1}	131.39
Melting point	°C	-86.5
Boiling point	°C	87.3
Vapor pressure	Pa	9700
Diffusion coefficient in pure air	m^2 day^{-1}	0.68
Diffusion coefficient in pure water	m^2 day^{-1}	0.00009
Water solubility	g l^{-1}	1

Due to recalcitrant nature, TCE remains in all media in most of the cases. However, when it is absorbed by animal and humans, biological processes occurring in human body makes it hazardous causing many adverse health effects as stated earlier. Hence it is reported as priority pollutant by many agencies like Environmental Protection Agency (EPA) and Agency for Toxic Substances and Disease and Registry (ATSDR) (ATSDR, 2009). Most importantly, it is urged that ground water plumes of TCE must be remediated to avoid hazards to masses using groundwater as drinking and household use.

2. Strategies of TCE Remediation

Since TCE hazards were comprehended, efforts to remediate TCE started. Several types of techniques have been reported to date for TCE remediation. With respect to nature, physical, chemical and biological processes have been employed. For example, pump and treat, soil flushing, in-well aeration,

air stripping, pump and treat, permeable reactive barrier, electro kinetics (EK), oxidation and reduction with reagents, thermal treatment and biodegradation etc. have been documented with pros and cons of each technique (Huang *et al.*, 2013).

A number of techniques like biological processes (Dong *et al.*, 2009)., in situ chemical oxidation and reduction (Al-Abed and Fang, 2007; Waldemer *et al.*, 2007; Katsanou *et al.*, 2008; Cope and Benson, 2009; Tsai *et al.*, 2009; Phillips *et al.*, 2010), and electrochemical transformation processes (Petersen *et al.*, 2007; Mishra *et al.*, 2008; Carter and Farrell, 2009; Lohner *et al.*, 2011; Mao *et al.*, 2011, 2012) have been used for TCE removal from groundwater. The application of nanoparticles for TCE degradation is increasing rapidly. The biological degradation of TCE was studied by using nanoparticles which remain reactive in soil and water for up to several weeks (Zhang, 2003; Macé *et al.*, 2006; Zhang and Elliott, 2006). The application of nanoparticles has not only reduced the operating cost but also the time of exposure to the contaminated sites (Karn *et al.*, 2009).

The application of bimetallic nanoparticles like Ni/Fe and Pd/Fe serves a useful method for achieving a complete dechlorination of the TCE (Barnes *et al.*, 2010a; Bhattacharyya *et al.*, 2010; Zhang *et al.*, 2011; Vijayakumar *et al.*, 2013). The zero-valent iron (nZVI) and bimetallic (for example, palladium-doped) nZVI immobilized in a support and electrospun nanofibrous mats were also applied for TCE degradation without the accumulation of chlorinated intermediates (Kim *et al.*, 2010; Ma *et al.*, 2012). Modified Fe nanoparticles were also tried for improving the TCE degradation. Nanoscale emulsified zero-valen iron (nEZVI) was used for TCE degradation both from soil and ground water (Quinn *et al.*, 2005; Macé *et al.*, 2006; O'Hara *et al.*, 2006; US EPA, 2008; CLU-IN, 2009).

The application of F_e nanoparticles creates conditions for abiotic degradation of TCE followed by biological degradation (Henn and Waddill, 2006). A combination of bimetallic nanoparticles and indigenous dechlorinating bacteria, however, requires employing a two-step process. An initial stimulated biodegradation of TCE followed by the addition of bimetallic nanoparticles to degrade the remaining cis-1,2-DCE and VC (Henn and Waddill, 2006; Barnes *et al.*, 2010b). For effective nanoremediation of TCE, however, few site-specific requirements must be met including porosity, hydraulic conductivity, flow velocity, geochemical properties etc. Furthermore, the effect of soil sorption on the effectiveness of bimetallic nanoparticles must be taken into account in process design for the effectiveness of surfactant uses.

Furthermore, a hybrid electrolysis and Pd-catalytic oxidation process was also applied for TCE degradation in groundwater (Yuana *et al.*, 2013) and oxidation was shown to be dominant pathway for degradation instead of hydrodechlorination (Yuan *et al.*, 2012). Titanium nanotubes loaded with Pd-Ti were also used effectively for TCE degradation in groundwater (Xie *et al.*, 2013) and Pd-Ti/TiO_2 cathode shows superior performance for TCE reduction as compared with other common cathodes. The decreased electrocatalytic activity of the cathode with repeated treatments, however, needs structural improvement.

Finally, a number of comprehensive reviews are reported in literature so far addressing TCE issues and remediation covering many critical aspects. Arp *et al.* (2001) discussed in detail the molecular and cellular fundamental of TCE aerobic cometabolism. They thoroughly discussed the types of enzymes and their related reactions. Pant and Pant (2010) established a review on microbial remediation of TCE. They presented the discussion on advances in cometabolic reductive dechlorination, co-metabolism, direct oxidation of TCE, Microbiology of TCE degradation and possibilities of bio augmentation. Suttinum and Luepromachai, (2013) thoroughly discussed the concepts, limitations and available strategies for sustained biodegradation of TCE. For abiotic degradation of chlorinated ethanes and ethenes in water, Tobiszewski and Namiesnik, (2013) reviewed the available literature reports. There are some other reviews on topics like phytoremediation of TCE and molecular techniques (Furukawa, 2000a,b; Furukawa *et al.*, 2002; Wood, 2008). However, the degradation efficiency and sustainability of TCE depends directly or indirectly upon the environmental conditions and on the design of engineered system. The objective of this review is to assess the problems related with TCE dynamics in the

environment and to present a comprehensive review of the engineered systems utilizing the biological degradation ability of microorganisms.

3. Dynamics and implications of TCE occurrence and removal

There are two distinct features in TCE (and other similar chlorinated hydrocarbons) pollution scenarios. Firstly, TCE is not only volatile but also possess good adsorption to soil and organic matter and moderate water solubility (Karami *et al.*, 2012). Secondly TCE is generally toxic, persistent and cause bioaccumulation with respect to natural attenuation as compared to many common pollutants (Smidt *et al.*, 2000; Rittmann and McCarty, 2001). On the other hand, TCE contamination level also varies in different media. For example, TCE levels between 0.001µg l^{-1} to 100µg l^{-1} have been reported in Canadian surface water while higher levels ranging from under 1000 µg l^{-1} to almost 10^6 µg l^{-1} in the groundwater at various contaminated sites (CEPA, 1993) are reported. High levels (10 to 100 000 µg l^{-1}) have also been reported following a spill in the contaminated sediment of St. Clair River, Canada (CEPA, 1993). In the sediment at 6% of the 388 US observation stations, TCE has been detected at a median level of 5 µg per kg of dry sediment weight (Staples *et al.*, 1985; ATSDR, 1995). It has been estimated that a typical daily intakes inhalation by the general U.S. population is 13 µg day^{-1}. United Stated EPA reported an intake of about 5µg day^{-1} via food although it was based on limited data. TCE exposure through air is quickest depending upon whether it is occupation site or open air. In a review by Bakke *et al.* (2007), occupational exposure to TCE was assessed and the measurements across all industries and decades were 38.2 ppm (arithmetic mean). The level could be the hundreds of ppm (>500,000 µg m^{-3}) at short term exposures in industrial sites e.g. aircraft industry. Similar contamination of TCE have been reported in other industrial economies e.g. Japan, Korea, Vietnam, Thailand etc. Hence, if TCE is to low it may not be available for degradation and if it is too high it may be toxic and may cause pollution over huge areas persistently for long time spans.

The "Molecular Recalcitrance" or persistence of organic compounds like TCE in natural systems has been summarized by Alexander (1965) and Rittman and McCarty (2001). They listed following factors:

1. Molecular structure of the pollutant: if the molecular structure of the organic pollutant is not inherently biodegradable biodegradation will not occur because structural characteristic of the pollutant molecule prevents the degradation enzyme from acting.
2. Environmental conditions i.e. either the compound is inaccessible or unavailable, microorganism cannot grow due to some deficiency, the environment is toxic to microbes or the concentration of the pollutant is very high, or once the enzyme produced it is inactivated
3. Appropriate organisms are not present in the environment.

Hence to bring natural attenuation to TCE and similar organic compounds, these factors must be addressed. Many engineered systems have been devised to get complete or enhanced removal/degradation of TCE via physical, biological and chemical unit processes. Selection of the remediation (engineered) process falls in two major but basic targets which are partial degradation/removal from water and soil to air and complete mineralization and control of toxic byproducts during a degradation process of any type.

Initially from any surface/groundwater and sediments etc. evaporation was found to be the primary route of removal of TCE which is merely shifting the TCE from one medium to another without actually destroying it, into simple harmless end products. Physical processes/technologies such as pump-and-treat, air sparging coupling with vapor extraction, in well aeration, soil excavation and venting, (activated carbon) adsorption etc. have been employed in this regard, which have been found relatively slow, costly, and inefficient, along with being environmentally disruptive in nature (Lorah *et al.*, 1997; Bankston, 2002; Han *et al.*, 2002). In addition, many factors affect the efficiency of physical removal processes e.g. the complex nature of aquifers, sorption onto soil media, and entrapment in the porous media (Beeman and Bleckmann, 2002).

Low efficiency and no treatment of TCE lead the researchers to shift towards more advance and sustainable engineered systems to mineralize TCE to simpler end products. Engineered processes based on sole principle like biological or chemical and hybrid like physical-biological and biological–chemical have been extensively reported to get high efficiency and complete degradations of TCE. In biological or chemical treatment systems of TCE, transport of reactant/substrates to target sites is a crucial step in process efficiency, for example in cometabolism of TCE (Goltz *et al.*, 2001). How efficient the process is, it is only valuable when the required ingredients meet in appropriate concentrations and conditions. It is especially important during in-situ remediation of polluted soils, sediment and aquifers. Even if all the required conditions (listed earlier in this section described by Alexander (1965) and Rittmann and McCarty (2001) are provided, there are still many performance related issues with engineered systems. TCE is highly chlorinated compound and its degradation is not completed in one step, both in biological and chemical processes. Hence before the complete mineralization, it takes number of routes and intermediates. In many cases these intermediates or daughter products are more toxic than TCE itself. Chemical dechlorination or anaerobic dechlorination and chemical reductive dechlorination, for example produces VC in first step (Horber *et al.*, 1999; Middeldorp *et al.*, 1999; Keppler *et al.*, 2002; Zhang and Bennett, 2005; Tobiszewskiand and Namiesnik, 2012; Huang *et al.*, 2013). This following section will present the evaluation of different biological engineered systems for TCE degradation, reported in literature with respect to the control of such performance related parameters.

4. Biological mineralization of TCE via engineered systems

There are different approaches of biological degradation of TCE. So far studies on aerobic cometabolism, anaerobic dehalogination, direct oxidation and phytoremediation have been reported using biological processes to degrade TCE. TCE is considered persistent with respect to biological degradation due to its highly chlorinated structure. Microbial degradation of chlorinated compounds depends on chlorine substitution and is highly strain dependent. Specific microbial cultures can utilize most of the mono-halogenated compounds. Compounds having more than one halogen atoms (e.g. TCE) are more recalcitrant in nature (Janssen and Witholt, 1992). Biological degradation of TCE through aerobic and anaerobic bacteria is possible and has been reported in lab scale studies and in natural environments. It is stated that TCE can be degraded by enzymatic reaction through a process termed as cometabolism. Cometabolism is "the fortuitous transformation of a compound by enzymes or co-factors designed for other purposes" (Rittmann and McCarty, 2001). In TCE aerobic cometabolism, nonspecific enzymes (e.g. methane mono-oxygenase, toluene mono-oxygenase, and toluene di-oxygenase) forms an epoxide that is chemically unstable and degrades to simpler end products. There are three crucial ingredients for initiation and completion of cometabolism that is microorganism that can produce non-specific enzyme, a co-substrate that can induces the non-specific (e.g. methane, toluene, ammonia) and oxygen (Rittman and McCarty, 2001). Proper working environment and conditions are also required for the cometabolism.

Toxicity of TCE (and its cometabolic byproducts) to microorganism, viability of microorganisms, competition for enzymes and substrates, inactivation of enzymes, slower kinetics as compared to normal metabolism, low utilization for substrate are few of the many factors that affect TCE cometabolic mineralization (Arp *et al.*, 2001). Many engineered systems have been reported in literature to address these issues and to get the complete, sustained and highly efficient TCE degradation through biological cometabolism. Following section will review the literature for different engineered systems.

Aerobic cometabolism for TCE degradation was evaluated by Wilson and Wilson (1985), Little *et al.* (1985), and Alvarez-Cohen and McCarty (1991) on early stages using methane Mono-oxygenase (MMO). Soon it was clear that there are substrates and microorganism other than methane oxidizing bacteria that can also help to express non-specific enzymes. Soluble methane monooxygenase , Toluene 2-monooxygenase, Toluene dioxygenase,Soluble butane monooxygenase,Isopropylbenzenedioxygenase, Isopropyl benzene/toluene dioxygenase, Alkene monooxygenase,Butane monooxygenase,Butane monooxygenaseare the examples of enzymes that can cause TCE cometabolism (Oldenhuis *et al.*, 1989;

Wackett and Householder, 1989; Fox *et al.*, 1990; Dabrock *et al.*, 1992, 1994; Landa *et al.*, 1994; Hyman *et al.*, 1995; Chu and Alvarez-Cohen, 1996; 1998; 1999; Pflugmacher *et al.*, 1996; Newman and Wackett, 1997; Lange and Wackett, 1997; Saeki *et al.*, 1999; Yang *et al.*, 1999; Milintawisamai *et al.*, 2001; Yeager *et al.*, 2001;2004; Morono *et al.*, 2004, 2006; Suttinun *et al.*, 2004; Halsey *et al.*, 2005; Kocamemi and Cecen, 2007, 2010; Suttinun *et al.*, 2010).

Phelps *et al.* (1990) used expanded bed bioreactors to degrade TCE in continuous cycles using mixed bacterial cultures that utilized methane, propane, and tryptone-yeast extract as aerobic carbon and energy sources. They achieved 95% TCE removal in 5 days. However the pH shift from 7 to 7.5 decreased the efficiency by 85%. Landa *et al.* (1994) used *Burkholderia cepacia* G4 to degrade TCE in a chemostate using toluene as energy and growth substrate. It was found that increasing the loading of TCE, effects the efficiency of TCE conversion. However, specific TCE degradation activity of the cells and the volumetric activity increased. TCE loading as high as 3,400 µmol per liter per h for 12 h caused inhibition of toluene and TCE conversion, which recovered to within two days after reduction of the TCE load to the original nontoxic level. In a study by Uchiyama *et al.* (1994), immobilization of *Methylocystis sp.* Strain M in Different Matrices resulted in different results. Cells immobilized in Ca-alginate, z-carrageenan, and agarose showed higher or almost the same degradation activity in comparison with that of free cells, while low activity was observed in the cells immobilized in photocross linkable resin, polyurethane, and polyelectrolyte complex. The optimum pH and temperature was found to be 7 and 35 degrees Celsius, respectively. Shimomura *et al.* (1997) developed a fluidized-bed bioreactor (FBB) containing *Methylocystis sp.* strain M (strain M) immobilized in 2% calcium alginate gel beads for treating synthetic groundwater containing TCE which was supplied with a methane/air gas mixture. About 80-90% of the influent TCE was degraded in the reactor. However, the efficiency of the gel-immobilized cells to degrade TCE declined rapidly during the TCE degradation process. They operated the reactor on alternate days for TCE degradation and reactivation of the immobilized strain M. Sun and Wood (1996) developed a fixed film, aerobic, single-pass, bioreactor for the continuous degradation and mineralization of gas-phase TCE using *Burkholderia cepacia* PR123 (TOM23C) which was immobilized on sintered glass and activated carbon. Repeated starving of the cells was found to restore TOM activity in the bioreactor with activated carbon and extended TCE mineralization by 34%. Smith and McCarty (1997) evaluated a two-stage (first mixed culture growth and secondly, a plug flow TCE degrading reactor. When methane was added to the TCE degrading reactor, it inhibited TCE cometabolism at low TCE concentrations and enhanced TCE cometabolism at high TCE concentrations. The results indicated that the TCE cometabolism in the presence of methane does not follow simple competitive inhibition kinetics. Radway *et al.* (1998) used polyurethane foam embedded *Burkholderia cepacia* G4 to degrade TCE using benzene as growth substrate. They found that foam embedded *Burkholderia cepacia* G4 degradation efficiency enhanced but there was adsorption of TCE onto polyurethane foam which may not be available for degradation. It was also noticed that TCE and benzene also increased the binding of the foam.

Sipkema *et al.* (1999) evaluated TCE degradation in a two stage system by *Methylosinus trichosporium* OB3b using formate and methane. In first stage the organism *Methylosinus trichosporium* OB3b was grown; in the second reactor, the cells degraded TCE. They described that the system presented is flexible (oxygen/methane addition) and can easily be scaled up for field application with 90% reduction in operating cost of chemicals. Nakano *et al.* (2000) studied the effect of TCE adsorption on granular activated carbon (GAC) and possibility of biological degradation and to determine the adsorption capacity of bio-regenerated GAC. 82.5% of the total TCE (26.2 mg) adsorbed in the GAC was degraded successfully. They concluded that the adsorption capacity of the bio regenerated GAC was very low and was not feasible to be recycled for TCE adsorption without thermal regeneration.

Dolasa and Ergas (2000) used a hollow fiber membrane bioreactor to investigate the degradation of TCE by mixed culture using toluene as growth substrate. The membrane bioreactor consisted of a bundle of polypropylene fibers and air containing volatile organic compounds was subjected to pass through theses fibers. Batch culture experiments showed that no inhibition to the toluene degrading ability was observed at up to 15 mg per L toluene or up to 1.5 mg per L TCE and about 80% TCE degradation was

observed. Brar and Gupta (2000) used rotating biological contactor (RBC) at laboratory scale to treat synthetic wastewater containing 30 mg l^{-1} of TCE. They used mixed culture consisting of nitrifiers, heterotrophs and *Thiosphaera pantotropha* to achieve 99.89% removal of TCE under loading of 0.0039m^3 $m^{-2}d^{-1}$ and HRT 3.5 days. Guo *et al.* (2001) developed a fibrous bed reactor todegrade TCE by *Pseudomonas putida* F1 (P. putida F1) using toluene. They reported that competitive inhibition of toluene on TCE removal could be prevented in this bioreactor. 90% TCE was removed over 4 h when 95 mg toluene l^{-1} was presented simu^{l}taneously. Lee *et al.* (2003) developed a trickling biofilter system for continuous treatment of gas-phase trichloroethylene. The system consisted on two units. Unlike to the previous studies, they used first unit to degrade TCE and the second for the reactivation of inactive biofilm. Gas phase TCE was degraded continuously by *Burkholderia cepacia* G4 achieving a 100% removal of TCE with a maximum TCE elimination capacity of 17mg per L per day. Dursun and Tepe (2005) used Ca-alginate immobilized *Ralstonia eutropha* to degrdade TCE using phenol as substrate. They found that Phenol had a strong inhibitory effect and the results showed that intra-particle diffusion resistance was important for this system. Tartakovsky *et al.* (2005) used coupled anaerobic–aerobic granular biofilm reactor to degrade TCE. The experiment showed the degradation of TCE due to the co-existence of methanogenic and methanotrophic populations in a single reactor. Chen *et al.* (2007) encapsulated P. putida F1 strain into chitosan beads and successfully degraded TCE using phenol as co-substrate. They found that TCE degradation kinetics were government by diffusion kinetics. Kim *et al.* (2010) degraded TCE via polyethylene glycolpolymer encapsulation of P. putida and *Bacillus Sp* strain via toluene metabolism. The degradation by Bacillus Sp was reported first time and the specific transformation efficiency of TCE was found higher than the other species reported in the literature. Kumar *et al.* (2012) used *Burkholderia vietnamiensis* G4 (the pure cultures of naturally-occurring bacteria are) in engineered systems in the biodegradation of TCE. Hamed *et al.* (2013) used P. putida F1 (strain ATCC 700007) at different concentrations in both single- and two-phase systems using 2-undecanone as the second organic phase. Two-phase system not only prevented the inhibition effect of high concentrations of TCE on the microbial biomass but also decreased the biodegradation time of TCE.

5. Future work and conclusions

TCE is found in all mediums of environment including soil, air and water. Although at some polluted sites natural attenuation of TCE has been found to occur but generally the natural process is very slow. Biological engineered systems have been found to degrade TCE on much faster rates and higher concentrations. Different combination of materials, microorganism and reactor setup have been presented in this study which emphases on the importance of various inter disciplinary (e.g. physico-biological, chemical-biological) approaches to get sustainable and highly efficient TCE detoxification and degradation processes. The composite nanofibrous mats having high surface area to volume ratio and porosity and immobilized with the bimetallic nanoparticles are the promising future applications in remediation of various environmental contaminants like TCE.

The question emerges, however, as to what kind of engineered systems would be most useful for maximizing the degradation of TCE in a sustainable way and where it might be implemented. Cometabolism may not be a sustainable TCE degradation approach and so as the reductive dechlorination unless an electron donor is provided. After reviewing the literature, it can be concluded that cometabolic degradation of TCE at low concentrations may not be an issue but its biological treatment is limited by the intrinsic properties of different methods and bacteria due to their toxicity. It is recommended to add to the aqueous medium a second distinct organic phase for degrading fast and high concentration of TCE. By using biocatalysts containing water-immiscible organic solvents, it is possible to degrade high concentrations of TCE at fast rates especially when it is known that TCE is toxic for the microorganisms. These catalysts will help maintain a low concentration of TCE in the aqueous phase even though the overall concentration remains high. This second phase (i.e. organic) can be reused again since it does not mix with the aqueous phase which can be easily separated and discharged.

Currently, there is a pressing need to identify the appropriate microorganisms in any engineered system that can effectively provide a low-cost treatment option for TCE degradation. Indigenous populations of bacteria may be stimulated by various sources of nutrients that selectively enhance the biodegrading bacteria present in any engineered system. Analytical chemistry can reveal what nutrients might be limiting for biodegradation to occur at a maximal rate. It is equally important to point the way toward identification and characterization of the most suitable bacteria that thrive in the presence of available nutrients. The best microorganism for TCE degradation should have high TCE-degrading enzyme activity, high transformation capacity for TCE, and grow relatively rapidly. The effect of the substrate concentration and the cell concentration on the biodegradation of TCE should be investigated carefully to degrade its high concentrations at fast rates. The second phase (i.e. organic) should be selected in an appropriate way considering its insolubility, volatility, non-biodegradability by the selected microorganism and the cost of the overall engineered system. In conclusion, we feel that biodegradation offers the potential of cost effective treatment of TCE. It is necessary, however, that engineered systems effectively use the biodegradative metabolism that nature has evolved.

Acknowledgments

"The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project no. RGP-VPP-283."

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