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A STUDY ON INTRINSIC BIOREMEDIATION

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Abstract: Biological activity is utilized to neutralize harmful pollutants and transform them into innocuous products. Intrinsic bioremediation involves degradation of organic pollutants utilizing insitu micro-organisms via a natural process known as natural attenuation. The process has proven to degrade contaminants in ground water, soil and aquifer matrix.

Potential intrinsic bioremediation of tricholoroehylene (TCE) is possible using cholorobenzene (CB) as a primary substrate under aerobic and methanogenic conditions. Degradation of tricholoroethylene (TCE) is dependent on degradation of primary substrate cholorobenzene (CB). Microbial enumeration is performed to identify the occurrence of intrinsic bioremediation. The presence of daughter compounds is an indicator of successful remediation.

Studies performed to identify key factors used as indicators for intrinsic bioremediation and factors that control the biodegradation of dissolved BTEX compounds in ground water. Indigenous organisms exposed to xenobiotic compound capacity to degrade through genetic evolution or mutation. One of the most basic experiments of genetic modification is that the bacteria is tagged with a suitable gene construct which can be easily detected in extracts done from monitored site, with or without cultivation prior to detection of the microbes. Rhiosphere provides high energy conditions for the bacteria which successfully allows the expression and functioning of the bacterial degradative enzymes.

Biodegradation involves respiration process that bring about denitrification, iron reduction, sulfate reduction, methanogenesis and aerobic respiration. All this factors are major contributors to the effective intrinsic bioremediation or natural attenuation of BTEX compounds that are benzene, toluene, ethylbenzene and xylene.

Keywords: Intrinsic, Bioremediation, Indigenous organisms.

1. Introduction

Bioremediation is a biological process that utilizes biological activity to neutralize the harmful pollutants or to transform them into less harmful products. The main principle of bioremediation is to use a particular type of micro-organism to help improve the conditions of the degraded or contaminated site.

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Intrinsic bioremediation involves degradation of organic pollutants utilizing insitu micro-organisms. BTEX compounds are benzene, toluene, ethyl benzene and xylene result in ground water pollution. It is important to investigate the loss of contaminants in the field scale in order to verify the presence of intrinsic biodegradation.

Biogeochemical indicators, microbiological evidence are indicators for contaminant degradation however it is difficult to distinguish contaminant mass loss due to physical processes or sorption[1].

It is essential that the environment factors must favour the biological degradation. The conversion of the pollutants is a natural process also known as natural attenuation. Aerobic and anaerobic bio degradation are the major factors for intrinsic bioremediation. The basic concept of intrinsic bioremediation is utilizing naturally occurring micro-organisms in order to degrade contaminants that are released into the environment subsequently at the same time minimizing health risks.

The activities of intrinsic bioremediation is monitoring of site, evaluation of site, to ensure genetic potential or capacity of intrinsic microflora[2]. Intrinsic bioremediation conducts transformation of pollutants into innocuous products and eco-friendly.

Before considering the biological degradation at the contaminated site it is significant to simulate the behaviour of the micro-organisms used to degrade the organic pollutants this is essential for welfare of human health and environment. This study would be possible through laboratory experiments and molecular genetics.This pilot model would help forecast and evaluate the benefits of biodegradation on a contaminated site. This would help decide if exogenous organism would be required.

Genetic or natural modification techniques are performed on the organisms to ensure effective degradation of the contaminants. This mechanism can degrade the contaminants in the ground water, soil and aquifer matrix by stimulating and maintaining the microbial activity, pH, salanity, temperature, moisture content and also addition of nutrients, electron acceptors and carbon source[3].

2. Contamination of ground water at Robins Air Force Base, Georgia, USA

Contamination of ground water due to halogenated organic compounds such as tricholoroethylene (TCE) and cholorobenzene (CB). Two objectives were carried out (1) investigate the occurrence of intrinsic bioremediation at the study site, (2) evaluate the potential of intrinsic bioremediation of TCE using CB as the primary substrate under aerobic and methanogenic conductions[4].

Basic moniter wells were constructed at four different zones. The field investigations involved analysing the geochemical parameters of the samples at zones. The analysis of the sample was conducted according to EPA methods.

Microbial enumeration is done to identify the occurrence of intrinsic bioremediation. Microbial enumeration is accomplished to identify the number of iron reducers, sulfate reducers and methanogens in the ground water sample. MPN method and total plate count are the methods used for enumeration.

The degradation of tricholoroethylene (secondary substrate) is dependent on the degradation of primary substrate used in the microcosm study such as phenol, methanol and cholorobenzene. Three groups for aerobic conditions and three groups for anaerobic conditions were established to simulate the degradation of tricholoroethylene. The presence of the daughter compounds is the indicator for successful intrinsic bioremediation.

3. Geochemical Indicators of Intrinsic Bioremediation

An investigation was carried out at a gasoline – contaminated aquifer at Rocky Point to examine rate of intrinsic bioremediation and the possible indicators and factors responsible for its activity. The aquifer consists of benzene, toluene, ethylbenzene and xylene (BTEX) compounds and biodegradation was evident.

The objective of the study was to identify the key factors that are used as indicators of intrinsic bioremediation and to improve the factors that control the biodegradation of dissolved BTEX compounds in ground water[5].

The contaminated aquifier consists of a nonaqueous phase liquid (NAPL) hydrocarbon trapped in the soil matrix.

Samples were collected in three continuous soil cores to the depth of 14 ft. The BTEX concentration in each sample was identified by adding 1gm of soil sample to 1ml of water, sonicating for 5minutes, equilibrating at 80º C and eventually measuring the BTEX concentration via gas chromatography.

Dissolved BTEX and related parameters were monitored several times for months. The NAPL migrated to the sand unit where the water table is low. Contaminants were only observed in the sand aquifer. In order to determine the occurrence of bioremediation a graph was prepared where the individual BTEX compound was plotted versus the time on a logarithmic scale.

10 slopes were prepared out of which 7 slopes had a negative slope, which was determined by a linear regression which states that the decrease of concentration and 3 slopes showed

increase of concentration over a period of time[6]. The negative slope indicated 0.8% decrease in concentration.

On the basis of the results it was possible to determine the occurrence of intrinsic bioremediation by monitoring the geochemical parameters.

4. Intrinsic bioremediation techniques on contaminated soil

Intrinsic bioremediation has proved to be cost efficient, eco-friendly and reliable. The process requires relentless monitoring, analysis or introduction of nutrients, indigenous or exogenous bacteria, geochemical and physical properties of the soil. Isolating the indigenous bacteria and taking on account the physical parameters is essential to help forecast the effective mitigation of the contaminated site.

Indigenous organisms exposed to xenobiotic compound capacity to degrade the compound can occur through genetic evolution or mutation. Sometimes the organism acquire genes and degradation pathways from bacterial cells immigrating from elsewhere (McGowan et al., 1998) these bacterial cells or strains can also be artificially supplied by using genetic recombinant techniques also known as laboratory strain. Transfer of genetic material can occur by conjugation (1946 Joshua Lederberg & Edward Tatum), transduction or transformation.

One of the most basic experiments of genetic modification is that the bacteria is tagged with a suitable gene construct which can be easily detected in extracts done from monitored site, with or without cultivation prior to detection of the microbes (Jansson et al.,2000). The purpose of the gene tagged is not always degradation, they provide information on survival, spread and possibly metabolic activity (Ford etal., 1999).

Bioavailability is a crucial step in case of intrinsic bioremediation in this case biological materials are used to investigate the presence of various chemicals. In these biosensors a promoter that is the controlling element in the expression of a gene is linked with a gene for the investigation. The reporter genes commonly used are the bioluminescent (luc,lux) genes (Jansson et al., 2000), and gfp (Calfie et al., 1994), green fluorescent proteins, blue fluorosescent protein, redshifted gfp, (Tsien, 1998.). Pseudomonas fluorescens strains produce light upon exposure to mercury or arsenite in the presence of the luc substrate luciferin (Petanen, unpublished).

DNA shuffling is an invitro process that generate mutation and evolution artificially (Stemmer, 1994). It generates many variety of the same type of gene using homologus gene.

This technique has been used to improve the degradation of polychlorinated biphenyls and other aromatic compounds (Kumamaru et al., 1998).

Co-metabolism is a process of degradation of two compounds, in which the degradation of the secondary substrate depends on the primary substrate. Methanol, phenol, cholorobenzene, toluene have been used insitu to induce indigenous bacteria to degrade trichloroethylene and other hazardous cholorinated compounds (Hopkins and McCarthy, 1995).

Plants play an important role to improve soil contamination. Some plants are highly tolerant to alkaline conditions, prevent erosion, produce co-substrates inducing the degrading genes of the bacteria and also provide nutrients for the bacteria eventually increasing the population of the microflora subsequently enhancing the degradation of the contamination. Rhiosphere provides high energy conditions for the bacteria which successfully allows the expression and functioning of the bacterial degradative enzymes.The roots are colonized by bacteria (Suominen et al., 2000) or mycorrhiza i.e. symbiotic relationship with fungus and roots of the plant are densely colonized by bacterial film (Sarand et al., 1998). A Microcosm study Suillus Bovinus mycorrhiza performed rapid degradation on compound Meta-toluate at high initial concentrations (Sarand et al., 1999).

5. Mode of action

In the year 1986 a gasoline spill had occurred at automobile service station. The spill left untreated for eight years. On March 1994 a detailed study was carried out on fuel spill location on Patrick Air Force Base in Cocoa Beach, Florida. Samples were collected in order to forecast the influence of natural attenuation and also for bioavailability.

Intrinsic bioremediation is an approach that relies on natural attenuation to mitigate or remediate the pollutants or contaminants in soil or water. This process involves several processes such as sorption, volatilization, dilution, dispersion, advection, recharge and biodegradation that involves transforming the contaminants into innocuous by products. Microbial reactions and pathways that undergo in the degradation process are dechlorination in this process the chlorine atom is replaced with the hydrogen atom. Cleavage an organic compound is split or the terminal carbon is eliminated in the chain and redox reactions that involve oxidation donating electrons and reduction accepting electrons.

Biodegradation involves respiration process that bring about denitrification, iron reduction, sulfate reduction, methanogenesis and aerobic respiration. All this factors are major contributors to the effective intrinsic bioremediation or natural attenuation of BTEX compounds that are benzene, toluene, ethylbenzene and xylene.

The microorganisms tend to obtain energy for survival and reproduction from electron donors to electron acceptors. This process generally takes place through redox reaction in which oxidation of the electron donor takes place and reduction of the electron acceptor. The electron acceptors are generally the compounds that are in an oxidised form such as nitrate, sulfate, iron (III) and dissolved oxygen. The donors would be fuel hydrocarbons, organic compounds or BTEX compounds.

Depending on the availability of and types of electron acceptors along with the physical factors of the site such as nutrients, pH, temperature and moisture. Bioremediation can occur with aerobic respiration, iron reduction, sulfate reduction, methanogenesis as terminal electron accepting process (TEAP). Microorganisms generally utilize electron acceptor in preferred order while metabolizing fuel hydrocarbons (Bouwer 1992). For instance dissolved oxygen is the primary electron acceptor in case of aerobic respirations. Depending on the microbial competition, environment conditions and changes in the concentration of the electron acceptors will ultimately determine the terminal electron accepting process.

The pathways of biodegradation of compounds such as toluene, ethylbenzene and xylene biodegradation is identical to benzene degradation and involves the process of dehalogenation. Changes in the concentrations of the electron acceptors can help determine the TEAP.

6. Aerobic Pathways

Dissolved oxygen is the primary electron acceptor for aerobic biodegradation the first step towards aerobic biodegradation involves the enzyme deoxygenase causing hydroxylation of the ring (Rochkind et al., 1986). The resultant intermediate formed is cis-1, 2- dihydroxy-1.2-dihydroxybenzene losing two hydrogen compounds forming cathechol. Under aerobic process this common pathway is applicable for all BTEX compounds. (Bouwer 1988; Doong et al., 1995) indicated addition of electron acceptors and carbon sources boost the biotransformation of the aromatic hydrocarbons.

The degradation of TCE was accomplished by cometabolismi.e degradation of primary substrate serves as an energy producer, enzyme inducer and a carbon supplier that helps in order to degrade the secondary substrate. Pseudomonas cepacia G4, requires toluene, o-cresol, m-cresol or phenol for TCE degradation activity (Folsom et al., 1990,Krumme et al., 1993).

Degradation of cholorobenzene studied by Reineke and Knackmuss (1988) and Nishino et al.(1992). According to their study they justified that cholorobenzene is transformed into

cholorocathechol following the ortho-cleavage of the ring. The muconic acid is dechlorinated and the non-chlorinated compound formed is metabolized to form a 3- oxadipate which enters the tricarboxylic acid (TCA cycle). Bouwer (1989) discovered that addition of acetate, nitrogen and phosphate enhanced the biotransformation of the cholorobenzene.

7. Anaerobic Pathways

Depletion of dissolved oxygen results in the establishment of anaerobic conditions in the environment. Regardless of the absence of dissolved oxygen certain requirements must be made in order to ensure anaerobic degradation such as pH, carbon sources, nutrients, electron acceptors, temperature and salanity. In presence of oxygen the enzymatic mechanisms of the anaerobic bacteria is unsuccessful. According to Bossert and Compeau study in the year 1995 aromatic hydrocarbons can be biodegraded under anaerobic conditions.

A detailed study of the metabolic pathway under methanogenic conditions was studied by Grbic-Galic and Vogel (1987) and Evans et al (1998). Under anaerobicenvironments PCE is degraded into their daughter compounds such as cis-1,2-dicholoroethylene (cis-1,2,DCE), chloroethylene (VC) and ethylene the ultimate biotransformation of this compound is ethane (Holliger et al., 1993, Freedman, 1990).

Denitrification takes place under anaerobic conditions once all the dissolved oxygen is absent in the site, nitrate is used as an electron acceptor in order to degrade the BTEX compounds. Once the nitrate is depleted iron reduction takes place iron (III) is utilized as an electron acceptor. Iron (III) is converted to soluble form that is ferrous iron or iron (II). Once the dissolved oxygen, nitrate and iron (III) is depleted the sulfate reducing bacteria can act upon the BTEX compounds. The increase level of methane observed at site is a good indicator for methane fermentation. BTEX acts as a substrate for the methanogensis.

References

[1] Bedient, Philip B. 1991. “MEMBER ASSOCIATIONS.” Research Journal of the Water Pollution Control Federation 63: 97.

[2] Chen, Ku-Fan, Chih-Ming Kao, Chiu-Wen Chen, Rao Y. Surampalli, and Mu-Sheng Lee. 2010. “Control of Petroleum-Hydrocarbon Contaminated Groundwater by Intrinsic and Enhanced Bioremediation.” Journal of Environmental Sciences 22 (6): 864–71. doi:10.1016/S1001-0742(09)60190-X.

[3] Kao, C.M., and Jason Prosser. 1999. “Intrinsic Bioremediation of Trichloroethylene and Chlorobenzene: Field and Laboratory Studies.” Journal of Hazardous Materials 69 (1): 67–79.

[4] Mills, Marc A., James S. Bonner, Thomas J. McDonald, Cheryl A. Page, and Robin L. Autenrieth. 2003. “Intrinsic Bioremediation of a Petroleum-Impacted Wetland.” Marine Pollution Bulletin 46 (7): 887–99. doi:10.1016/S0025-326X(02)00367-3.

[5] Romantschuk, M., I. Sarand, T. Petänen, R. Peltola, M. Jonsson-Vihanne, T. Koivula, K. Yrjälä, and K. Haahtela. 2000. “Means to Improve the Effect of in Situ Bioremediation of Contaminated Soil: An Overview of Novel Approaches.” Environmental Pollution 107 (2): 179–85.

[6] Saunders, F. Michael, Sheau-Yun Chiang, and J. Wynn. 1999. Sediment-Based Intrinsic Bioremediation at Robins Air Force Base. Technical Report Presented to Robins Air Force Base (WR-ALC/EMQ). https://smartech.gatech.edu/bitstream/handle/1853/9610/e-20-w92_6910.pdf.

[7] Solano-Serena, Remy Marchal, Denis Blanchet, Jean-Paul Vandescasteele, “Intrinsic Capacities of soil microflorae for gasoline degradation”, 1998, pg319-326

[8] Wiedermeier, T.H.; Wilson, J.T,; Kampbell. D.H; Miller, R,N.; Hansen, J.E, Technical protocol for implementing intrinsic bioremediation with long-term monitoring for natural attenuation of fuel contamination dissolved in ground water, US, Air Force Center for Environmental Excellence: San Antonto, TX,1995

[9] T.H. Wiedermeier, Ross N. Miller and John T. Wilson and Donald H. Kampbell ‘Significance of Anerobic Processes for the Intrinsic Bioremediation of Fuel Hydrocarbons’

[10] Richard Schaffner, ‘Bioremediation offers an effective, ethical and economical alternative to standard clean-ups, Journal (American Water Works Association), Vol.96, No.1, January 2004, pg22-23.

[11] F. Michael Saunders, Sheau-Yun Chiang and Jennifer C. Wynn, ‘Sediment-Based Imtrinsic Bioremediation at Robins Air Force Base’, School of Civil and Environmental Engineering, Georgia Institute of Technology.