Assessment and Monitoring of Bioremediation

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It was generally considered until the middle of 20th century that all the pollutants discharged into the environments could be readily degraded by microorganisms and their degradation products recycled. However, as various industries developed, the resulting pollutants have been increasingly diversified in species and toxicity and hence their treatment methods required more advanced technologies. This indicates that only use of the conventional physical and chemical treatment technologies cannot guarantee the ultimate and environment-friendly treatment of complex, toxic and recalcitrant contaminants any more (20). The environmental biotechnologies (particularly bioremediation), which began to be flourished in 1970's, have been frequently covered in the media. Especially the role and reliability of bioremediation technology demonstrated in treatment of the oil spill by Exxon Baldez in Alaska established a new horizon in publicity of the technology, in which a bioremediation technology has been boosted to a practical remediation technology for the environment (22, 24). This reflects that the environmental biotechnology is socially and politically acceptable on the basis of environmental protection law. In this review, the general concept and scope of bioremediation as an environmental biotechnology, and its monitoring and assessment will be discussed.

Scope and feature of bioremediation as an environmental biotechnology

Environmental biotechnology for environmental pollutants can be defined as the use of microorganisms (and/or other organisms) and their processes for socio-economic benefits in environmental protection and restoration (28). The technology generally utilizes microorganisms and other living organisms in nature as a vehicle to remove pollutants from the environment, and requires little input of extra energy and resources and is processed under ambient conditions, indicating these systems are believed to be low in cost compared with other methods for clean-up of environmental contamination. Bioremediation as one of the environmental biotechnologies, therefore, utilizes living organisms (microbes, plants, algae, etc.) to remediate and restore the polluted environment through biodegradation and stabilization of pollutants. From an industrial perspective the practice of bioremediation requires an interdisciplinary research and development in the fields of life sciences, environmental science and engineering, ecology and geology, etc. On the while, the principal purpose of environmental cleanup programs through bioremediation organized by the office of Research and Development, EPA is summarized as follows: to make acceptable to the user community and the general public improved and novel biological treatment systems whose aim is the reduction or elimination of the risk associated with hazardous waste and other forms of environmental pollution, and to accomplish this in both an effective and least expensive manner (18). The basic research sectors of environmental biotechnology for hazardous waste treatment and environmental remediation are listed in Table 1. As shown in Table 1, the fundamental research agenda for environmental biotechnology comprised of four elements: microbial strain development and improvement, the development of improved bioanalytical methods for measuring biotechnological processes, and the development and environmental and reactor systems analysis techniques leading to better process understanding, control, and optimization (28). In particular, the bioanalytical methods for measuring biotechnological processes can play an important role in bioremediation in that the methods make it possible to track and monitor population dynamics of

Table 1. Fundamental research agenda for environmental biotechnology of hazardous wastes (28)

Agenda	Description
Strain development	Source and selection
	Characterization
	Modification and improvement
	Model systems (mixed and pure)
	Collections and libraries
Process and system analytical tools	Quantitative analytical techniques (chemical/physical measurements)
	Bioanalytical Methods
	Molecular analysis methods
	Biomonitoring
	Reporter-signal analysis/structure and function
Environmental system analysis	Environmental fate and abiotic processes
	Population dynamics (organisms and genes)
	Environmental stability
	Microhabitats-niche invasions
	Organismal or genetic mobility
	Stress-induced effects
Reactor system analysis	Reactor design
	Transient outcomes and perturbations
	Dynamic analysis
	On-line analysis and control
	Kinetic parameters analysis

Table 2. Programs of natural and accelerated bioremediation study in U.S. Department of Energy (31)

Specific research topics	Description
Biotransformation and biodegradation	Degradation mechanisms of complex contaminant mixtures
Community dynamics and microbial ecology	Interactions between biotic and abiotic components of ecosystems to understand their influence on the degradation, persistence and toxity of mixed contaminants
Biomolecular science and engineering	Fundamental research in molecular and structural biology to enhance understanding of bioremediation; to improve the efficacy of bioremedial organisms
Biogeochemical kinetics	Dynamic interactions among in situ geochemical, geological, hydrological and microbiological processes
Assessment	Measuring and validating the biological and geochemical processes of bioremediation
Acceleration	Interdisciplinary on flow and transport of nutrients and microorganisms, focused on acceleration and optimization of bioremediation
System integration, prediction and optimization	Development of conceptual and quantitative methods to describe community dynamics, biotransformation, biodegradation and biogeochemical dynamics processes in complex geological systems

the specific degradative microorganisms and their activities during the remediation of environmental pollutants. The monitoring techniques include detection and quantification of specific microbial populations, their genes and their degradation activities in the pristine or polluted environment. A few examples of the state-of-the-art techniques are: nucleic acid analysis and gene probe technology serving in rapid and specific measurement of the biodegradative communities and their related communities (26), techniques allowing the rapid extraction of DNA and RNA from the environment in order to directly quantify the frequency and activity of degradative genes without cultivation and enumeration of individual microbial populations (27), bioluminescent reporter technology for the on-line measurement of biodegradative activity which is a good example of genetic engineering to develop an in situ sensor technology for the presence of specific chemicals, their bioavailability, and their degradation (16). For the reliable, effective and predictable application of the environmental biotechnologies in pollution control, there will be major works to be done to identify and quantify specific biotic and abiotic processes affecting chemical transformation, the dynamics, and dominant variables useful in developing system

control strategies (28).

New bioanalytical technologies as well as the accompanying physicochemical analytical techniques will, therefore, become important in that they will permit timely and efficient analysis of the specific biological mechanisms and activities involved in waste treatment and bioremediation systems. In this sense, the biological monitoring technologies can build a bridge between biotechnology and bioremediation practices.

Evaluation on the research and development of bioremediation as an environmental remediation technology was performed several times since 1991 (6). Most of fundamental and field application researches on bioremediation have been accomplished according to the Natural and Accelerated Bioremediation Research program managed by U.S. Department of Energy (Table 2) (31). The long term goal of this program is to provide the scientific understanding needed to harness natural processes and to develop methods to accelerate these processes for the bioremediation of contaminated soils, sediments, and groundwater at DOE facilities.

Monitoring of pollutants during biodegradation

Prudent pollutant management strategy requires that we monitor potential contaminated or contaminated sites in order to take remedial action before or after human or environmental exposure occurs. Design of monitoring systems for polluted sites poses several tasks: where to place monitoring wells, what species to monitor for to serve as remediation indicators, what methods to employ for detection of these indicator parameters, and finally how to evaluate the monitoring data.

The goal of the pollutant monitoring is to determine the identity of chemicals present and their concentrations in order to set biodegradation possibilities based on established chemical, physiological, biochemical knowledges. The potential target chemicals found in the polluted environments are generally classified by 10 groups: monocyclic aromatic hydrocarbons; phenolic compounds; polyaromatic hydrocarbons; halogenated aliphatics; halogenated aromatics and cyclics; pesticides; alkanes and aliphatics; nitrogen and sulfur containing heterocyclic compounds; nitroaromatics; inorganics and organometals. The set-up of reliable quantitative analytical strategy for the target chemicals will be the first requirement of the pollutant monitoring in the bioremediation. Here sampling strategy and techniques based on statistical design will be important because sampling procedures need to allow trends indicative of on-site bioremediation process. Moreover, these monitoring processes and data should contribute to the determination of mass balance and the evaluation of the hydrogeological fate of the target chemicals. For an example, brief biodegradation pathways and the representative degradative indicators of the fre-

Table 3. The typical halogenated hydrocarbons and their representative degradation products, and their biodegradation indicators

Pollutant	Metabolites		Biodegradation indicators	
	Aerobic	Anaerobic	Indicators	Reference
Perchloroethylene (PCE)	Not known	TCE*, DCE, VC, CO_2, and CH_4	anaerobic mineralization	33
Trichloroethylene (TCE)	TCE-epoxide*; dichloroacetic acid*; formate*; glyoxylate*; CO; CO_2; HCl	DCE*, VC, CO_2, and CH_4	aerobic mineralization sulfate as a electron acceptor	19 21
Vinyl chloride (VC)	CO_2; HCl	ethylene; ethane; CO_2	aerobic mineralization	14
1,1,1-trichloroethane (TCA)	2,2,2-trichloroethanol*	1,1-dichloroethane; chloroethane; ethanol;	aerobic transformation sulfate as a electron acceptor	24 17
1,1,2-trichloroethane (TCA)	acyl chloride*; chloroacetic acid*	Not known	aerobic transformation	5
Carbon tetrachloride	Not known	chlorofrom; methylene chloride; CO_2	anaerobic mineralization (sulfate reducers and methanogens)	9
Chloroform	phosgene*	chlorofrom; methylene chloride; CO_2	Aerobic transformation	2

*To be used for specific monitoring of the fate of parent chemical.

quent polluting chemicals such as halogenated aliphatics are described to obtain idea on monitoring parameters (Table 3). An efficient way to measure and monitor these pollutants has been made possible by using a suite of sensitive, broad spectrum analytical methods chosen for their cost-effectiveness, and ability to detect a wide variety of potential contaminants. These methods include purge and trap gas chromatography using a mass spectrometer detector with liquid/liquid extraction followed by gas chromatography using a mass spectrometer detector for the non-purgeable gas chromatographable organics, combined with plasma spectroscopy for metals.

The representative chemicals of this group are perchloroethylene (PCE), trichloroethylene (TCE), vinylchloride (VC), 1,1,1- and 1,1,2-trichloroethanes (TCA), carbon tetrachloride (CT), and chloroform. PCE and TCE have been used widely as a degreasing agent in industry and dry cleaning. They are common ground water pollutant. PCE, not shown to be biotransformed under aerobic conditions, can be degraded by reductive dehalogenation under anaerobic conditions, generating the metabolites, TCE, dichloroethylene (DCE) isomers, VC, and eventually ethane or CO_2 which can be aerobically degraded. The accumulation of VC in the anaerobic degradation process is not desirable because VC is potential human carcinogen. TCE is microbially transformed under both aerobic and anaerobic conditions. Its eventual metabolic products are CO_2 and Cl^-. It can be also anaerobically dehalogenated.

VC is a highly volatile chlorinated ethylene that is used in the synthesis of plastics (e.g. PVC). It can be mineralized aerobically, generating CO_2.

1,1,1-TCA and 1,1,2-TCA have been used as industrial solvents which ended up with common ground water pollutants. Both compounds can be biotransformed under aerobic and anaerobic conditions. Their eventual aerobic product is CO_2 and anaeroic produces are

Table 4. Pollutant treatability in bioremediation (7)

Pollutant Class	Bioremediation Status*	Evidence of Future Success	Limitations
Hydrocarbons and Derivatives			
Gasoline, fuel oil	Established	-	Forms non-aqueous-phase liquid
Polycyclic aromatic hydrocarbons	Emerging	Aerobically biodegradable under a narrow range of conditions	Sorbs strongly to subsurface solids
Creosote	Emerging	Readily biodegradable under aerobic conditions	Sorbs strongly to subsurface solids; Forms non-aqueous-phase liquid
Halogenated Aliphatics			
Highly chlorinated	Emerging	Cometabolized by anaerobic microbes; cometabolized by aerobes in special cases	Forms non-aqueous-phase liquid
Less chlorinated	Emerging	Aerobically biodegradable under a narrow range of conditions; cometabolized by anaerobic microbes	Forms non-aqueous-phase liquid
Halogenated Aromatics			
Highly chlorinated	Emerging	Aerobically biodegradable under a narrow range of conditions; cometabolized by anaerobic microbes	Sorbs strongly to subsurface solids; forms non-aqueous-phase liquid--solid or liquid
Less chlorinated	Emerging	Readily biodegradable under aerobic conditions	Forms non-aqueous-phase liquid--solid or liquid
Polychlorinated Biphenyls			
Highly chlorinated	Emerging	Cometabolized by anaerobic microbes	Sorbs strongly to subsurface solids
Less chlorinated	Emerging	Aerobically biodegradable under a narrow range of conditions	Sorbs strongly to subsurface solids
Nitroaromatics	Emerging	Aerobically biodegradable; converted to innocuous volatile organic acids under aerobic conditions	
Metals	Possible	solubility and reactivity can be changed by a variety of microbial processes	Availability highly variable--controlled by solution and solid chemistry

*Established: successful bioremediation at commercial level; emerging: limited success at field sites; possible: future potential.

VC or glyoxilic acid.

Carbon tetrachloride is also used as an industrial solvent and common ground water contaminant. No evidence of aerobic transformation was shown while the reductive dechlorination is the dominant degradation process under anaerobic conditions. CO_2 is the final product degradation under denitrifying conditions.

Chloroform is a common industrial solvent and causes tumors in mice. The possible degradation products in aerobic conditions are CO_2 and HCl. Table 3 shows the representative halogenated hydrocarbons, and their degradation products and biodegradation indicators.

Pollutant treatability, and parameters for pollutant assessment and monitoring

One of the critical factors determining bioremediation success is the pollutant treatability in the site. The treatability is primarily dependent on biodegradation capability of the relevant microbes at the site and/or microbes to be augmented and its accompanying engineering implementations. The Table 4 provides an overview of classes of pollutant and their treatability for bioremediation. The table delivers a broad perspective on how chemical and microbiological properties affect prospects for bioremediation, that is, current status of pollutant treatability technology.

Pollutant assessment and monitoring in *in situ* bioremediation

Good pollutant assessment and monitoring strategy is one of the most critical parameters allowing a successful evaluation of in situ bioremediation. The general pro-

Table 5. Assessment and monitoring parameters for pollutants useful for evaluation of biodegradation during in situ bioremediation procedure (15)

Assessment and monitoring	Parameter examples	Types of pollution	Application status
Ratio of biodegradable to recalcitrant reference pollutant	Congener/3,4-3',4' CB	PCB	Limited pilot-scale field demonstration (10, 25)
	congener/2,3,6-3',4 CB		
	C18/pristane	Crude oil	Field demonstration (23)
	C17/phytane	Crude oil	Field demonstration (22)
	Hopanes	Crude oil	Proposed demonstration (1)
Reaction intermediates and transformation products	*trans*-DCE epoxide	*trans*-DCE	Limited pilot-scale field demonstration (30)
	Chlorobenzoates	PCB	Limited pilot-scale field demonstration (25)
Reaction end-products or by-products	CO_2	Organic pollutants	Proposed demonstration
	$^{13}CO_2/^{12}CO_2$	Jet fuel and other fossil fuels	Proposed limited field demonstration
	CH_4	Organic pollutants, degradable under methanogenic conditions	Bench-scale studies (12, 13)
	Cl^-	Chlorinated organic pollutants	Field demonstration
Loss of pollutant	TCE, gasoline, creosote, PCB, etc.	All pollutants	Field demonstration (4, 29)
Lumped parameters	COD	All organic pollutants	Field demonstration
	TPH (total petroleum hydrocarbon)	gasoline, diesel fuel, jet fuel	Field demonstration
Terminal electron acceptor	O_2	Aerobically degradable pollutants	Field demonstration (30)
	NO_3	Pollutants degradable under denitrifying conditions	Field demonstration (11)

cedure of in situ bioremediation procedure include 5 phases: Phase I, Preliminary site investigation; Phase II, Site assessment and characterization; Phase III, Pilot scale study; Phase IV, Full scale implementation, and Phase V, Closure (7).

In Phase I, a historical assessment of the site usage and a literature search of the similar sites will be performed (e.g. species and amount of pollutants used, potential hydrogeological and degradative fates of the pollutants and their by-product, and toxicity of the target chemicals). In Phase II, hydrogeological and hydrogeochemical characterization of the site, and the relevant microbiological and pollutant information will be collected. Therefore, Phases I and II are important steps enabling the ultimate decision of bioremediation strategy: engineered bioremediation (bioremediation intervention) or intrinsic bioremediation (natural attenuation). In Phase III of engineered bioremediation, pilot scale study will be performed to calibrate and select a final proponent for full-scale implementation. This level of study will not be necessary in intrinsic bioremediation. In Phase IV, full-scale implementation will be performed to maximize the biodegradation in the site. However, no additional implementation will not be considered except minimal monitoring of the site in the case of intrinsic bioremediation. In Phase V, in both cases of bioremediation strategy, pollutant concentration should be reduced to meet the regulatory level and confirmed by the regulation agency to close the site remediation project.

The successful assessment and monitoring of pollutants and their microbiological degradation is a critical task for a successful bioremediation. Moreover, the bio-

Table 6. Several emerging advanced environmental monitoring techniques and comparison with the traditional techniques (3, 8)

Monitoring Techniques	Mode of Operation	Advantages	Disadvantages
Traditional			
GC; GC-MS; HPLC	Chromatographic separation	High sensitivity and selectivity	Laborious sample preparation; less field deployable
Emerging			
Airborne laser fluorosensor	Measuring laser-induced fluorescence spectra of site and converting them into pollution profiles	Able to obtain extensive regional scale profiles of pollution	Ambiguities in data analysis due to abnormalities in spectra
Spectroscopic techniques			
Luminescence spectroscopy	Utilizing room temperature excitation spectra caused by laser	High sensitivity and selectivity; field deployable; little interference	Necessary to increase the spectrum of target chemicals
Ultraviolet-visible absorption and Colorimetry	Absorption of specific wave length by target chemicals	Simple sample preparation; field deployable	Less sensitivity and selectivity
Surface-enhanced Raman spectroscopy	Utilizing Raman scattering effect	Providing group frequencies; no interference with water	Complex instrumentation; fluorescence interference
Fibre optic chemical sensors (colorimetric; fluorometric; refractive index; fluoroimmunoassay)	Detecting optical changes caused by chemical reaction	Very high sensitivity and selectivity; field deployable	Extreme sensitivity assumes that the likely pollutant is known
Immunochemical assays	Antigen-antibody reaction	High sensitivity and selectivity; field deployable; rapid analysis	False negative or positve result
Biosensors			
Electrochemical	Converting biological changes into electrical signals; enzymes and antibodies	High accuracy	Electrical interference
Optical	Detecting spectral changes occurring when a substrate binds	Unaffected by electrical interference	Interference from light
Piezo	Detecting vibration frequency changes in proportional to pollutant concentration	Useful for monitoring individual exposure and indoor air quality; easy manufacturing	Less specific than electrochemical and optical biosensors

degradation capability of degraders will be evaluated by the pollutant transformation and removal rates which are based upon measurements of concentrations of pollutants and their metabolic products. Table 5 shows important assessment and monitoring parameters for pollutants during the bioremediation process.

Emerging analytical techniques for assessing and monitoring pollutants *in site*

Here are introduced several advanced emerging environmental monitoring techniques which include a remote sensing technique, spectroscopic techniques, and biosensor techniques. Their action mechanisms, advantages, and disadvantages are summarized in Table 6.

Maximal and minimal requirements of pollutant assessment and monitoring during *in situ* remediation

Maximally and minimally required parameters for pollutant assessment and monitoring are suggested in Table 7. As shown in the table, engineered bioremediation requires all the parameters in phases III, IV, and V to be monitored for both cases of maximal and minimal requirements. In intrinsic bioremediation, however, parame-

Table 7. Maximal and minimal requirements for pollutant assessment and monitoring in in situ bioremediation strategy

Remediation stage	Maximal requirement	Minimal requirement
Phase I (Preliminary site investigation)	Conducting investigation of the polluted site history	Conducting investigation of the polluted site history
	Conducting literature searches relevant to the potential pollutants	Conducting literature searches relevant to the potential pollutants
	Conducting extensive data base searches concerning previous similar case studies	Conducting minimal data base searches concerning previous similar case studies
Phase II (Site assessment and characterization)	Extensive sampling design	Minimal sampling design in
	Assessment of surface and subsurface soil gases of volatile pollutants, CO_2, O_2, N_2, and CH_4	Assessment of the most critical target pollutants only
	Extensive assessment of pollutants in non-aqueous and liquid, and solid phase of surface and subsurface soil cores	Minimal assessment of pollutants in non-aqueous and liquid, and solid phase of surface and subsurface soil cores
	Extensive assessment of pollutants in soil pore water	Minimal assessment of pollutants in soil pore water
	Extensive assessment of pollutants in ground water	
Phase III (Pilot scale study)	Conducting extensive tracer studies to obtain hydrogeological information to delineate bioremediation feasible zone	Conducting minimal tracer studies to obtain hydrogeological information to delineate bioremediation feasible zone
	Redundant pollutant monitoring on all parameters from Phase II to optimize remediation conditions	Minimal pollutant monitoring on the most critical parameters from Phase IIto optimize remediation conditions
	Monitoring most species of important pollutants and their by-products	Monitoring only the most critical degradation parameters
Phase IV (Full-scale implementation)	Setting up of extensive monitoring wells and proper placement of previous monitoring wells	Setting up of minimal monitoring wells
	Conducting no redundant pollutant monitoring but complete tracking of the plume	Monitoring only the most critical degradation parameters
	Setting up of extensive degradation models	Setting up of minimal degradation models
	Conducting tracer studies to track ground water movement, if allowed (e.g. tritiated water or proper organic tracer)	
Phase V (Closure)	Finishing remediation project when the regulation requirements are met.	

Table 8. Microbiological techniques (or parameters) available for bioremediation assessment and monitoring

Microbiological assessment and monitoring			
Population		Activity	
Total biomass	*Community structure*	*Physiological status*	*Gene expression and activity*
Microscopic direct count	Plate count on selective media	Nucleotide incorporation	mRNA extraction
Plate count	Total DNA extraction	Amino acid biosynthesis	mRNA hybridization
MPN	Fatty acid profile	Alkaline phosphatase	Bioreporter gene expression
Turbidity	16S rRNA profile	Dehydrogenase	Catabolic enzyme activity
Total ATP measurement	ELISA	Lipase	
Total proteins	Monoclonal antibody	Phospholipds contents	
Tryptophan fluorescence			

ters in phases IV and V may be monitored but the focus is on only monitoring pollutants and their degradative products with minimal efforts.

Microbial assessment and monitoring techniques in *in situ* bioremediation

Microbial assessment and monitoring for *in situ* bioremediation includes two major categories: microbial population and microbial activity. When microorganisms degrade the pollutants in the field, they usually use the pollutants and their metabolites metabolically or cometabolically. Therefore, the observation of increase in the population of contaminant-degrading microorganisms with the loss of the contaminants in the site provides an indication of active biodegradation process. However, it is not always necessary to have increased microbial population as the evidence for biodegradation event. Active microbial metabolism can also indicate the process of bioremediation happening in the field. Table 8 lists the microbiological techniques (or parameters) available for bioremediation assessment and monitoring.

Conclusion

The remediation cost for the polluted area including land and aquatic environments in U.S. alone is estimated to be $1.7 trillion (7). The bioremediation technology becomes increasingly competitive with the traditional treatment technologies in cost and acceptability. The current bioremediation technology has been successful in *in situ* treatment of crude and fuel oil but the treatment efficacy is still unpredictable depending on target pollutants and environmental conditions. Development of the assessment and monitoring technologies for bioremediation will, therefore, contribute to a successful implementation of the remediation technology in the future.

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