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# Scientific Swift in Bioremediation: An Overview

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<http://dx.doi.org/10.5772/56409>

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## 1. Introduction

A pure environment gives a quality of life on earth. In ancient times, it was believed that people on earth had an unlimited abundance of land and resources; today, however, the resources in the world show a greater or lesser degree of our carelessness and negligence in using them. In many parts of globe, the problems associated with contaminated sites are now growing up. The actual cause of this scenario is result from past industrial activities when awareness of the health and environmental effects connected with the production, use, and disposal of hazardous substances were less well recognized than today. It became a global complication when the estimated number of contaminated sites became significant. There are several traditional methods which have been applied to overcome this inconvenience. From the list of ideas which have been applied the best ones are to completely demolish the pollutants if possible, or at least to transform them to innocuous substances. Bioremediation is an option that utilizes microbes to remove many contaminants from the environment by a diversity of enzymatic processes. However, it will not always be suitable as the range of contaminants on which it is effective is limited, the time scales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate. we attempted to assist by providing information how the bioremediation is linked with cutting edge sciences like genomics, transcriptomics, proteomics, interactomics and bioinformatics.

Some new techniques in molecular biology particularly genetic engineering, transcriptomics, proteomics and interactomics offer remarkable promise as tools to study the mechanisms involved in regulation of mineralization pathways. The strategies need to be refined in which transcriptomics and proteomics data are combined together in order to understand the mineralization process in a meaningful way. These techniques show great promise in their ability to predict organisms' metabolism in contaminated environments and to predict the microbial assisted attenuation of contaminants to accelerate bioremediation. Bioinformatics technology has been developed to identify and analyse various components of cells

such as gene and protein functions, interactions, metabolic and regulatory pathways. Bioinformatics analysis will facilitate and quicken the analysis of cellular process to understand the cellular mechanism to treat and control microbial cells as factories. The next decade will belong to understanding molecular mechanism and cellular manipulation using the integration of bioinformatics.

Bioremediation is an option that utilizes microbes to remove many contaminants from the environment by a diversity of enzymatic processes. The major positive shades of bioremediation are comparatively low-cost and techniques based on low-technology (Robb et al, 1995) which generally have a high public acceptance and can often be carried out on site. However, it will not always be suitable as the range of contaminants on which it is effective is limited, the time scales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate. Varying degrees of success bioremediation has been used at a number of sites worldwide (Ajay et al, 2009). Here, we attempted to assist by providing information how the bioremediation is linked with cutting edge sciences like genomics, transcriptomics, proteomics, interactomics and bioinformatics (Fleming et al. 1993., Schena et al. 1998., Sikkema et al. 1995., Kuhner et al. 2005., Ellis et al. 2000).

## 2. Genetic analysis of genes involved

Examining the presence and expression of the key genes involved in bioremediation can yield more information on microbial processes than analysis of 16S rRNA sequences (Rogers and McClure. 2003). In general, there is a positive correlation between the relative abundance of the genes involved in bioremediation and the potential for contaminant degradation (Rogers and McClure. 2003., Schneegurt and Kulpa. 1998).

However, the genes for bioremediation can be present but not expressed. Therefore, there has been an increased emphasis on quantifying the levels of mRNA for key bioremediation genes. Often, increased mRNA concentrations can be, at least qualitatively, associated with higher rates of contaminant degradation (Schneegurt and Kulpa. 1998). For example, the concentrations of mRNA for *nahA* a gene involved in aerobic degradation of naphthalene were positively correlated with rates of naphthalene degradation in hydrocarbon-contaminated soil (Fleming et al. 1993). The reduction of soluble ionic mercury, Hg(II), to volatile Hg(0), is one mechanism for removing mercury from water; the concentration of mRNA for *merA* a gene involved in Hg(II) reduction was highest in mercury-contaminated waters with the highest rates of Hg (II) reduction (Nazaret et al. 1994). However, the concentration of *merA* was not always proportional to the rate of Hg (II) reduction (Nazaret et al. 1994., Jeffrey et al. 1996), illustrating that factors other than gene transcription can control the rates of bioremediation processes.

Highly sensitive methods that can detect mRNA for key bioremediation genes in single cells are now available (Bakermans and Madsen. 2002). This technique, coupled with 16S rRNA probing of the same environmental samples, could provide data on which phylogenetic groups of organisms are expressing the genes of interest. Analysis of the mRNA concentrations for genes other than those directly involved in bioremediation might yield additional insights into

the factors that control the rate and extent of bioremediation. Sub-optimal nutrient levels, pH, salinity and other environmental factors can limit the growth and metabolism of organisms that are involved in bioremediation in contaminated environments. Ecological studies of phyto-plankton use molecular techniques to evaluate the stress response of photosynthetic microorganisms in the environment (Palenik and Wood. 1998). In a similar manner, evaluation of the metabolic state of bioremediating microorganisms through analysis of the mRNA concentrations for key genes that are involved in responding to stress could help to identify modifications to contaminated environments that might promote bioremediation.

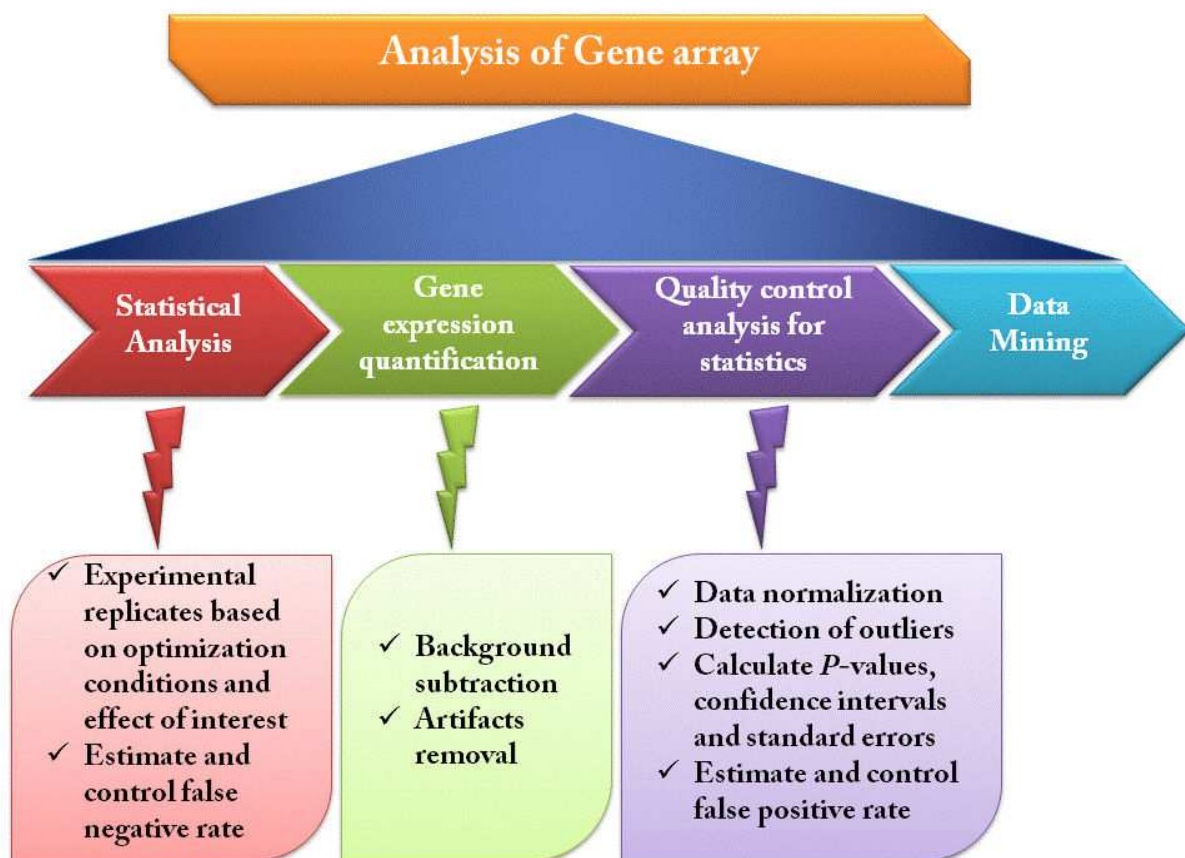
### **3. Role of transcriptomics**

The subset of genes transcribed in any given organism is called the transcriptome, which is a dynamic link between the genome, the proteome and the cellular phenotype. The regulation of gene expression is one of the key processes for adapting to changes in environmental conditions and thus for survival. Transcriptomics describes this process in a genome wide range. DNA microarrays are an extremely powerful platform in transcriptomics that enable determination of the mRNA expression level of practically every gene of an organism (Schna et al. 1998., Golyshin et al. 2003., Diaz. 2004) The most challenging issue in microarray experiments is elucidation of data (Dharmadi and Gonzalez. 2004). Often, hundreds of genes may be up- and/or down-regulated in a particular stress condition. In this context, several statistical issues become tremendously complex, including accounting for random and systematic errors and performing poor analysis.

### **4. Applications of DNA microarray**

Even with the complete genome sequences of microorganisms with the potential for bioremediation (Golyshin et al. 2003., Tiedje. 2002., Heidelberg et al. 2002., Seshadri et al. 2005., Rabus et al. 2005), studies are not accelerating in a rapid manner. With the completed genome sequences, it is possible to analyse the expression of all genes in each genome under various environmental conditions using whole-genome DNA microarrays (Gao et al. 2004., Muffler et al. 2002., Schut et al. 2003). Such genome-wide expression analysis provides important data for identifying regulatory circuits in these organisms (Lovley. 2003., Rabus et al. 2005., Muffler et al. 2002). In the past, DNA microarrays have been used to evaluate the physiology of pure environmental cultures (Schut et al. 2003) and to monitor the catabolic gene expression profile in mixed microbial communities (Dennis et al. 2003). More than 100 genes were found to be affected by oxygen-limiting conditions when a DNA microarray was used to study changes in mRNA expression levels in *Bacillus subtilis* grown under anaerobic conditions (Ye et al. 2000). Sensitivity may often be a part of the problem in PCR-based cDNA microarrays, since only genes from populations contributing to more than 5% of the community DNA can be detected. Several parameters were evaluated to validate the sensitivity of spotted oligonucleotide DNA

microarrays and their applicability for bacterial functional genomics (Denef et al. 2003). Optimal parameters were found to be 50-C6- amino-modified 70 mers printed on CMT-GAPS II substrates at a 40 mM concentration combined with the use of tyramide signal amplification labelling. Based on most of the known genes and pathways involved in biodegradation and metal resistance, a comprehensive 50-mer-based oligonucleotide microarray was developed for effective monitoring of biodegrading populations (Rhee et al. 2004). This type of DNA microarray was effectively used to analyze naphthalene-amended enrichment, and soil microcosms demonstrated that microflora changed differentially depending on the incubation conditions (Cho and Tiedje. 2002 ).A global gene expression analysis revealed the co-regulation of several thusfar- unknown genes during the degradation of alkylbenzenes (Kuhner et al. 2005). Besides this, DNA microarrays have been used to determine bacterial species, in quantitative applications of stress gene analysis of microbial genomes and in genome-wide transcriptional profiles (Muffler et al. 2002., Greene and Voordouw. 2003).



**Figure 1.** Work flow of gene array analysis. Diagrammatic representation of DNA microarray data analysis and relative limitations under each category of data analysis during data mining.



## 5. Foot prints of proteomics

The terms 'proteomics' and 'proteome' were introduced in 1995 (Wasinger et al. 1995), which is a key postgenomic feature that emerged from the growth of large and complex genome sequencing datasets. Proteomic analysis is particularly vital because the observed phenotype is a direct result of the action of the proteins rather than the genome sequence. Traditionally, this technology is based on highly efficient methods of separation using two-dimensional polyacrylamide gel electrophoresis (2-DE) and modern tools of bioinformatics in conjunction with mass spectrometry (MS) (Hochstrasser. 1995). However, 2-DE has been considered to be a limited approach for very basic and hydrophobic membrane proteins in compartmental proteomics. In bioremediation, the proteome of the membrane proteins is of high interest, specifically in PAH biodegradation, where many alterations in any site specific bacterium affects cell-surface proteins and receptors (Sikkema et al. 1995). The improvements in 2-DE for use in compartmental proteomics have been made by introducing an alternative approach for multidimensional protein identification technology (MudPIT) (Paoletti et al. 2004). MS has revolutionized the environmental proteomics towards the analysis of small molecules to peptides and proteins that has pushed up the sensitivity in protein identification by several orders of magnitude followed by minimizing the process from many hours to a few minutes (Aebersold and Mann. 2003). The advancement in MS techniques coupled with database searching have played a crucial role in proteomics for protein identification.

Matrix associated laser desorption/ionization time-of-flight MS (MALDI-TOF-MS) is the most commonly used MS approach to identifying proteins of interest excised from 2-DE gels, by generation of peptid mass fingerprinting (Aebersold and Mann. 2003., Aitken and Learmonth. 2002., Landry et al. 2000). Surface-enhanced laser-desorption-ionization MS (SELDI-TOF-MS) is the combination of direct sample fractions on a chip integrated with MALDI-TOF-MS analysis (Merchant and Weinberger. 2000., Seibert et al. 2005). A variety of differentially expressed signature proteins were analysed using SELDITOF- MS in blue mussels (*Mytilus edulis*) exposed to PAHs and heavy metals (Knigge et al. 2004). The liquid chromatography MS (LC-MS) technique has begun to open a new analytical window for direct detection and identification of potential contaminants in water (Joo and Kim. 2005). In addition, the metabolites and degradation products have been taken into account to assess the fate of organic contaminants such as pesticides, surfactants, algal and cyanobacterial toxins, disinfection by-products or pharmaceuticals in the environment and during water treatment processes (Joo and Kim. 2005).

## 6. Interaction of interactomics

Genome-wide mRNA profiling is unable to provide any information about the activity, arrangement, or final destination of the gene products, the proteins. Various proteomic approaches, on the other hand, can successfully provide the straight answers. It is very rare that any protein molecule acts as a unique pillar during the physiological response in biore-

mediation process of any contaminant when cellular proteins and various other related cellular expressions are on crest (Muffler et al. 2002., Kuhner et al. 2005., Eyers et al. 2004., Segura et al. 2005). In general, cellular life is organized through a complex protein interaction network, with many proteins taking part in multicomponent protein aggregation. The detection of these aggregated proteins, i.e. 'interactomics', is usually based upon affinity tag/pull down/MS/MS approaches at a proteome level (Lee and Lee. 2004., Coulombe et al. 2004., Gingras et al. 2005). Studies on protein–protein interaction and supermolecular complex formation represent one of the main directions of functional proteomics and/or second generation proteomics.

The growing demands of genomics and proteomics for the analysis of gene and protein function from a global bioremediation perspective are enhancing the need for microarray-based assays enormously. In the past, protein microarray technology has been successfully implicated for the identification, quantification and functional analysis of protein in basic and applied proteome research (Labaer and Ramachandran. 2005). Other than the DNA chip, a large variety of protein-microarraybased approaches have already been verified that this technology is capable of filling the gap between transcriptomics and proteomics (Liu and Zhu. 2005). However, in bioremediation, microarray-based protein–protein interaction studies still need to make progress to understand the chemotaxis phenomenon of any site specific bacterium towards the environmental contaminant.

## 7. Revolution of genomics

A drastic innovation in the study of pure cultures has been brought by the application of genomics to bioremediation. (Nierman & Nelson, 2002). Next generation genome sequencing techniques play a vital role in advancing the understanding of physiological and genomic features of microorganisms relevant to bioremediation. Complete, or nearly complete, genome sequences are now available for several organisms that are important in bioremediation (Table. 1). The notions of researches have been changed after the application of bioremediation to the advanced sciences like genomics which gave different answers. For example, molecular analyses have indicated that *Geobacter* species are important in the bioremediation of organic and metal contaminants in subsurface environments. The sequencing of several genomes of microorganisms of the genus *Geobacter*, as well as closely related organisms, has significantly altered the concept of how *Geobacter* species function in contaminated subsurface environments. For instance, before the sequencing of the *Geobacter* genomes, *Geobacter* species were thought to be non-motile, but genes encoding flagella were subsequently discovered in the *Geobacter* genomes (Childers et al. 2002) Further investigations revealed that *Geobacter metallireducens* specifically produces flagella only when the organism is growing on insoluble Fe(III) or Mn(IV) oxides. Genes for chemotaxis were also evident in the *Geobacter* genomes, and experimental investigations have revealed that *G. metallireducens* has a novel chemotaxis to Fe(II), which could help guide it to Fe(III) oxides under anaerobic conditions. Pili genes are present and are also specifically expressed during growth on insoluble oxides (Childers et al.

Microorganism	Relevance to bioremediation	Web site for genome documentation
<i>Dehalococcoides ethanogenes</i>	Reductive dechlorination of chlorinated solvents to ethylene. The 16S rRNA gene sequence of <i>D. ethanogenes</i> is closely related to sequences that are enriched in subsurface environments in which chlorinated solvents are being degraded	<a href="http://www.tigr.org">http://www.tigr.org</a>
<i>Geobacter sulfurreducens</i> <i>Geobacter metallireducens</i>	Anaerobic oxidation of aromatic hydrocarbons and reductive precipitation of uranium. 16S rRNA gene sequences closely related to known <i>Geobacter</i> species predominate during anaerobic in situ bioremediation of aromatic hydrocarbons and uranium.	<a href="http://www.jgi.doe.gov">http://www.jgi.doe.gov</a> <a href="http://www.tigr.org">http://www.tigr.org</a>
<i>Rhodopseudomonas palustris</i>	Main organism for elucidating pathways of anaerobic metabolism of aromatic compounds, and regulation of this metabolism.	<a href="http://www.jgi.doe.gov">http://www.jgi.doe.gov</a>
<i>Pseudomonas putida</i>	Metabolically versatile microorganism capable of aerobically degrading a wide variety of organic contaminants. Excellent organism for genetic engineering of bioremediation capabilities.	<a href="http://www.tigr.org">http://www.tigr.org</a>
<i>Dechloromonas aromatica</i>	Representative of ubiquitous genus of perchlorate-reducing microorganisms and capable of the anaerobic oxidation of benzene coupled to nitrate reduction.	<a href="http://www.jgi.doe.gov">http://www.jgi.doe.gov</a>
<i>Desulfitobacterium hafniense</i>	Reductive dechlorination of chlorinated solvents and phenols. <i>Desulfitobacterium</i> species are widespread in a variety of environments.	<a href="http://www.jgi.doe.gov">http://www.jgi.doe.gov</a>
<i>Desulfovibrio vulgaris</i>	Shown to reductively precipitate uranium and chromium. An actual role in contaminated environments is yet to be demonstrated.	<a href="http://www.tigr.org">http://www.tigr.org</a>
<i>Shewanella oneidensis</i>	A closely related <i>Shewanella</i> species was found to reduce U(vi) to U(iv) in culture, but <i>Shewanella</i> species have not been shown to be important in metal reduction in any sedimentary environments.	<a href="http://www.tigr.org">http://www.tigr.org</a>
<i>Deinococcus radiodurans</i>	Highly resistant to radiation and so might be genetically engineered for bioremediation of highly radioactive environments.	<a href="http://www.tigr.org">http://www.tigr.org</a>

**Table 1.** Genomes of microorganisms pertinent to bioremediation.

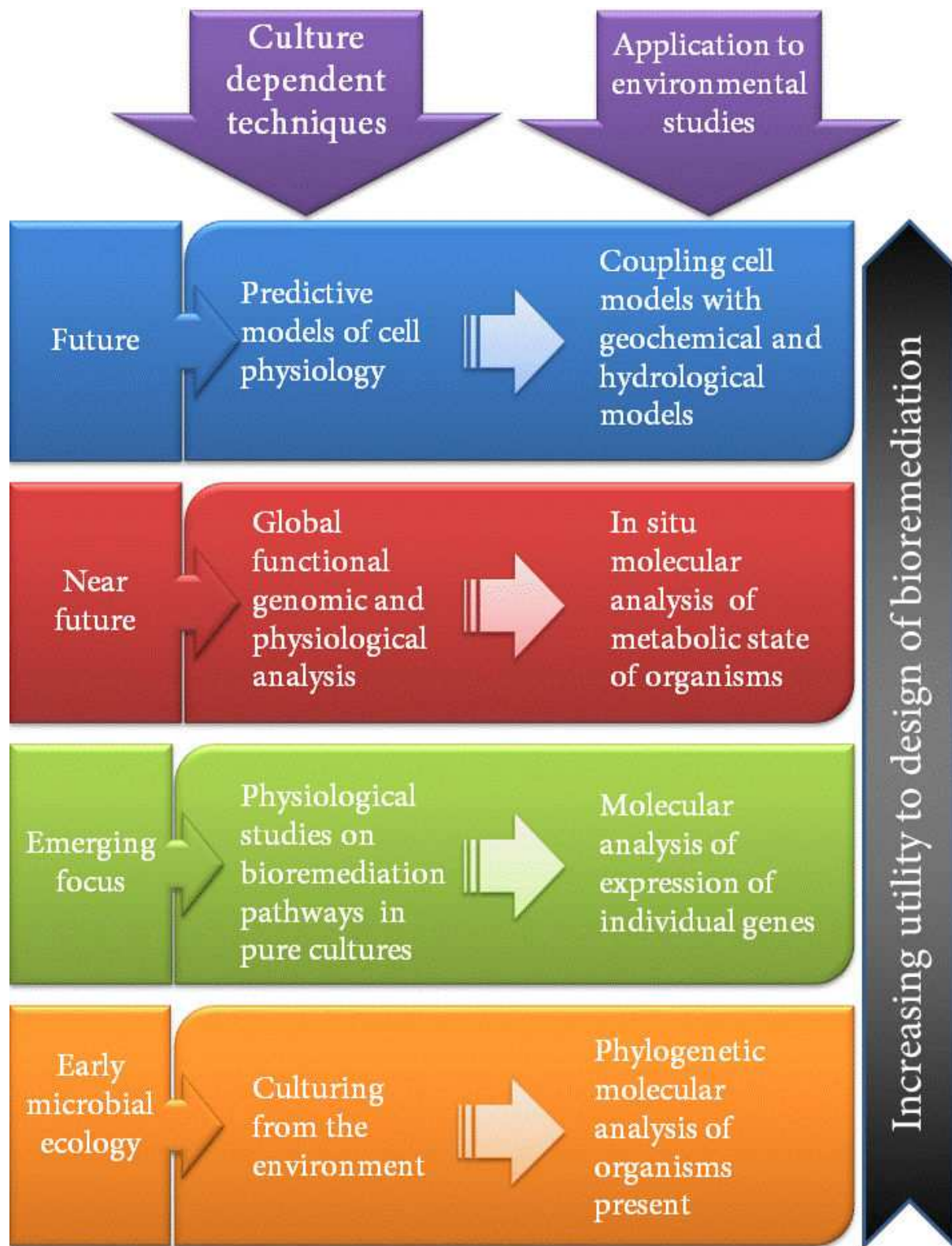


2002). Genetic studies have indicated that the role of the pili is to aid in attachment to Fe(III) oxides, as well as facilitating movement along sediment particles in search of Fe(III).

This energy-efficient mechanism for locating and reducing Fe(II) oxides in *Geobacter* species contrasts with the strategies for Fe(III) reduction in other well-studied organisms, such as *Shewanella* and *Geothrix* species. These other organisms release Fe(III) Chelators, which solubilize Fe(II) from Fe(III) oxides (Nevin and Lovley. 2002), and electron shuttling compounds, which accept electrons from the cell surface and then reduce Fe(III) oxides (Newman and Kolter. 2000., Nevin and Lovley. 2002). These strategies make it possible for *Shewanella* and *Geothrix* species to reduce Fe(III) without directly contacting the Fe(III) oxide. However, the synthesis of chelators and electron shuttles requires a significant amount of energy, and the lower metabolic energy requirements of the *Geobacter* approach is the probable explanation for the fact that *Geobacter* species consistently outcompete other Fe(III)-reducing microorganisms in several subsurface environments (Nevin and Lovley. 2002). Understanding this, and numerous other previously unsuspected physiological characteristics of *Geobacter* species, is important in guiding the manipulation of conditions in subsurface environments to optimize the ability of *Geobacter* species to remove organic and metal contaminants from polluted groundwater.

The study of the physiology of other microorganisms with bioremediation potential, the genomes of which have been sequenced, is now accelerating in a similar manner. With the completed genome sequences, it is possible using whole-genome DNA microarrays to analyse the expression of all the genes in each genome under various environmental conditions. Using proteomic techniques, it is possible to identify which proteins are expressed (Nierman & Nelson, 2002). Such genome-wide expression analysis provides important data for identifying regulatory circuits in these organisms (Baldi and Hatfield. 2002). This is significant as the mechanisms that control the regulation of the catabolic and respiratory genes that are the most important in bioremediation are largely unknown. As genetic systems for these environmentally significant organisms become available, it is possible to elucidate the function of the many genes of previously unknown function and to decipher bioremediation pathways. For example, the availability of the *Geobacter* genomes and a genetic system for these organisms is leading to the elucidation of which of the more than 100 c-type cytochromes that are apparent in the genome are important in electron transfer to metals (Lloyd et al. 2003., Leang et al. 2003).

Treatability study is a process, in which samples of the contaminated environment are incubated in the laboratory and the rates of contaminant degradation or immobilization are documented (Rogers and McClure. 2003). Giving little insight into the microorganisms that are responsible for the bioremediation, such studies provide an estimate of the potential metabolic activity of the microbial community. When bioremediation processes are researched in more detail, attempts are generally made to isolate the organisms responsible (Rogers. et al. 2003). The isolation and characterization of pure cultures has been, and will continue to be, crucial for the development and interpretation of molecular analyses in microbial ecology (Fig. 1). The recovery of isolates that are representative of the microorganisms responsible for the bioremediation process can be invaluable because, as outlined below, studying these isolates provides the opportunity to investigate not only their biodegradation reactions, but also other



**Figure 2.** Evolution of increasingly sophisticated studies of pure cultures and their application to the study of microbial communities

aspects of their physiology that are likely to control their growth and activity in contaminated environments. However, before the application of molecular techniques to bioremediation, it was uncertain whether the isolated organisms were important in bioremediation *in situ*, or whether they were 'weeds' that grew rapidly in the laboratory but were not the primary organisms responsible for the reaction of interest in the environment.

## 8. The 16S rRNA approach

A significant advance in the field of microbial ecology was the finding that the sequences of highly conserved genes that are found in all microorganisms, most notably the 16S rRNA genes, could provide a phylogenetic characterization of the microorganisms that comprise microbial communities (Pace et al. 1986., Amann et al. 1995). This was a boon to the field of bioremediation because it meant that by analysing 16S rRNA sequences in contaminated environments, it was possible to determine definitively the phylogenetic placement of the microorganisms that are associated with bioremediation processes (Rogers and McClure. 2003., Watanabe and Baker. 2000)

One of the surprises from the application of the 16S rRNA approach to bioremediation has been the finding that, in some instances, microorganisms that predominate during bioremediation are closely related to organisms that can be cultured from subsurface environments (Lovley. 2001). This contrasts with the general dilemma in environmental microbiology that is, it can be difficult to recover the most environmentally relevant organisms in culture (Amann et al. 1995). For example, in polluted aquifers, in which microorganisms were oxidizing contaminants with the reduction of Fe (m) oxides, there was a significant enrichment in microorganisms with 16S rRNA sequences that were closely related to those of previously cultured *Geobacter* species (Rooney-varga et al. 1999., Snoeyenbos-West et al. 2000., Roling et al. 2001). Coupled with the fact that *Geobacter* species in pure culture are capable of oxidizing organic contaminants with the reduction of Fe(III) oxide (Lovley et al. 1989), this indicated that *Geobacter* species are important in contaminant degradation *in situ*. *Geobacter* species can also remove uranium from contaminated water by reducing soluble U(vi) to insoluble U(iv) (Lovley et al. 1991). 16S rRNA sequence analysis showed that, when acetate was added to uranium-contaminated groundwater to promote micro-bial reduction of U(vi), the number of *Geobacter* species increased by several orders of magnitude, accounting for as much as 85% of the microbial community in the groundwater (Anderson et al. In Press, Holmes et al. 2002) In aquifers in which the indigenous microbial community was degrading the solvent trichloroethene (TCE), 16S rRNA sequences that are ~99% identical to the 16S rRNA sequence of a pure culture of the TCE-degrader *Dehalococcoides ethanogenes*, were detected (Fennell et al. 2001., Richardson et al. 2002., Hendrickson et al. 2002). Marine sediments with high rates of anaerobic naphthalene degradation were found to be specifically enriched in microorganisms with 16S rRNA sequences closely related to NaphS2, an anaerobic naphthalene degrader that is available in pure culture (Hayes and Lovley. 2002). There was a close correspondence between the potential for aerobic degradation of the fuel oxygenate methyl tert-butyl ether (MTBE) in groundwater and the number of organisms with 16S rRNA sequences that had more

than 99% similarity to the MTBE-degrading organism, strain PM-1, which is available in pure culture (Hristova et al. 2003).

The primary limitation of the 16S rRNA technique is that knowledge of the phylogeny of the organisms associated with bioremediation does not necessarily predict important aspects of their physiology (Pace. 1997., Achenbach and Coates. 2000). For example, microorganisms with 16S rRNA sequences closely related to the TCE-degrader *D. ethanogenes* can differ in the chlorinated compounds that they can degrade (He et al. 2003., Bunge et al. 2003), and predicting which of these compounds an uncultured organism will degrade might not be apparent from analysis of its 16S rRNA sequence alone (Hendrickson et al. 2002). Predicting physiology from phylogeny is even more difficult if there are no closely related organisms available in pure culture.

## 9. Comparative analysis of Omics in bioremediation

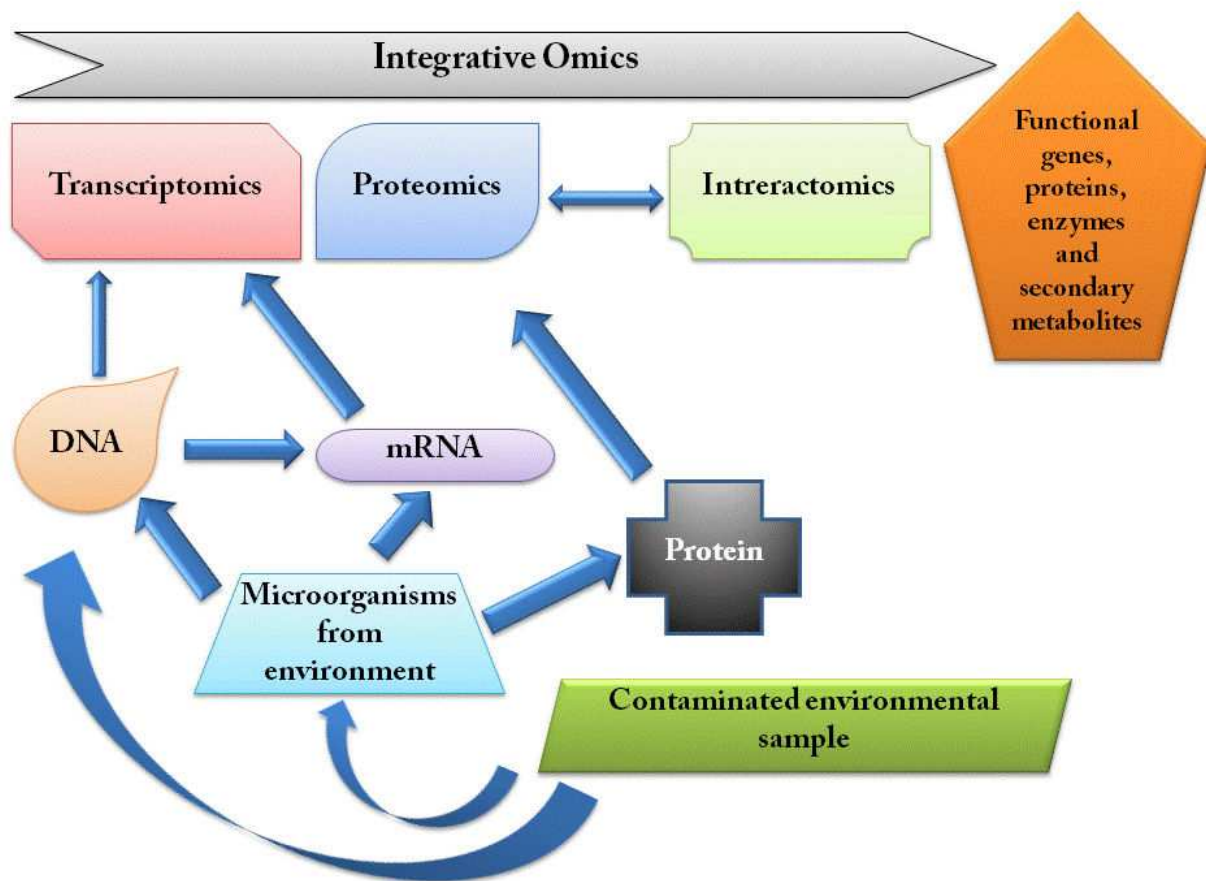
Based on an overall analysis of transcriptomics and proteomics, the comprehensive analysis of wholegenome sequencing is especially helpful to understand bioremediation-relevant microorganisms whose physiology has not yet been studied in detail. Global gene expression using DNA microarray technology, very much depends on the degree of coverage of the cellular mRNA and cellular proteins, whereas the coverage of the whole genome represents all the genes of an organism by definition. Cellular mRNA levels do not display as wide a dynamic range as the encoded proteins (Gygi et al. 1999). Thus, whole genome arrays are believed to provide a much more comprehensive overview of the actual gene expression pattern than proteomic studies.

According to global gene expression studies, both transcriptomics and proteomics support the view that the DNA array technologies record changes in gene expression more completely than the proteomics (Muffler et al. 2002., Kuhner et al. 2005., Eymann et al. 2002). Therefore, genomics data is deemed necessary to complement the proteomics approach (Hegde et al. 2003). However, proteomics would retain its central position in functional transcriptomics and/or genomics. The protein molecules, but not the mRNAs, are the key players in an on-site microbial mineralization reaction; the later are one of the highly unstable transmitters on the path from the genes to the ribosome, but each protein molecule represents the end product of gene expression (Kuhner et al. 2005). Complete protein profiling provides not only information on the individual organism, but also information on the fate and destination of protein molecules inside and outside the cell that can only be discovered via a joint transcriptomics, proteomics and interactomics approach (Figure 3).

## 10. Bioinformatics in bioremediation

MetaRouter is a system for maintaining heterogeneous information related to Biodegradation in a framework that allows its administration and mining (application of methods for extract-





**Figure 3.** Omic technologies using a systematic biology approach to track the insights of bioremediation. DNA is directly extracted from contaminant environmental sites and from organisms will end up on transcriptomics (DNA microarrays). Transcriptomics will expand towards proteomics followed by interactomics. Extraction of protein from pure culture using 2-DE and protein microarray platforms will allow us to explore the new molecules of interest during mineralization process.

ing new data). It is an application intended for laboratories working in this area which need to maintain public and private data, linked internally and with external databases, and to extract new information from it. The system has an open and modular architecture adaptable to different customers. This multiplatform program, implemented in PostgreSQL (standard language for relational databases) and using SRS as an indexing system (used to connect and query Molecular Biology databases), works using a client/server architecture that allows the program to run on the user station or on the company server, so it can be accessed from any place in a secure way just by having a web browser.

The University of Minnesota Biocatalysts/Biodegradation Database (<http://www.labmed.umn.edu/umbbd>) begins its fifth year having met its initial goals. It contains approximately 100 pathways for microbial catabolic metabolism of primarily xenobiotic organic compounds, including information on approximately 650 reactions, 600 compounds and 400 enzymes, and containing approximately 250 microorganism entries. It includes



information on most known microbial catabolic reaction types and the organic functional groups they transform. Having reached its first goals, it is ready to move beyond them. It is poised to grow in many different ways, including mirror sites; fold prediction for its sequenced enzymes; closer ties to genome and microbial strain databases; and the prediction of biodegradation pathways for compounds it does not contain ( Ellis et al. 2000).

## 11. Approaches of systems biology

The rise of genomic technologies and systems biology provide fresh approaches to currently untactable biological processes that are at the root of serious environmental problems. One formidable challenge in this respect is the biological fate of the nearly 8 operons, etc. implicated in this process. The biodegradation database of the University of Minnesota documented new chemical compounds (~40 000 predominant) which are common in modern Organic and Industrial Chemistry. A large number of microbial strains are able to grow on environmental pollutants (about 800 today). Bioremediation was studied from a molecular biology point of view, characterizing the chemical reactions, genes; University of Minnesota has made a pioneering effort in putting together nearly every aspect of our current knowledge on biodegradation pathways and in developing systems for dealing with that data e.g. to learn rules for predicting biodegradative features. Yet, most information available in the literature of microbial biodegradation of xenobiotics and recalcitrant chemicals deals with duos consisting of one pollutant versus one strain and thus, lacks essential aspects of the natural scenarios, like the interchange of genes between bacteria or their metabolic cooperation. This study of genomes and 'functionomes' from a community point of view (in contrast to organism point of view) is leading, for example, to the sequencing of 'genomes' of communities and ecosystems, instead of single organisms. These circumstances expose the need to qualify and to represent the information available in biodegradation databases in a fashion in which the entire known biodegradative potential of the microbial world can be crossed with the whole collection of compounds known to be partially or totally degraded through (mostly) bacterial action (Kitano 2002).

## 12. Conclusion

The application of omic sciences to the study of bioremediation is clearly in its infancy. There are many technical issues that will need to be addressed before some of the more novel approaches, such as environmental genome sequencing and arrays. To elucidate the function of most genes recovered from the environment, it will be necessary to recover the relevant organisms and study gene function in pure culture. Microorganisms closely related to those that predominate in some contaminated environments are already available in culture, and the careful replication of environmental conditions during isolation will probably yield more. Microorganisms that typically comprise about one-fourth of the marine microbial community, but the presence of which had only previously been detected from 16S rRNA sequences. This

search for previously uncultured organisms can be greatly accelerated with high-throughput culturing and screening strategies.

Some new techniques in molecular biology particularly genetic engineering, transcriptomics, proteomics and interactomics offer remarkable promise as tools to study the mechanisms involved in regulation of mineralization pathways. The applications of these techniques are still in their infancy, but the amount of data that is continuously being generated by today's genomics and proteomics technocrats needs to be organized in a stepwise manner within informative databases. The strategies need to be refined in which transcriptomics and proteomics data are combined together in order to understand the mineralization process in a meaningful way. These techniques show great promise in their ability to predict organisms' metabolism in contaminated environments and to predict the microbial assisted attenuation of contaminants to accelerate bioremediation. Bioinformatics technology has been developed to identify and analyse various components of cells such as gene and protein functions, interactions, metabolic and regulatory pathways. Bioinformatics analysis will facilitate and quicken the analysis of cellular process to understand the cellular mechanism to treat and control microbial cells as factories. The next decade will belong to understanding molecular mechanism and cellular manipulation using the integration of bioinformatics.

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