Systems biology approach to bioremediation

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Bioremediation has historically been approached as a 'black box' in terms of our fundamental understanding. Thus it succeeds and fails, seldom without a complete understanding of why. Systems biology is an integrated research approach to study complex biological systems, by investigating interactions and networks at the molecular, cellular, community, and ecosystem level. The knowledge of these interactions within individual components is fundamental to understanding the dynamics of the ecosystem under investigation. Understanding and modeling functional microbial community structure and stress responses in environments at all levels have tremendous implications for our fundamental understanding of hydrobiogeochemical processes and the potential for making bioremediation breakthroughs and illuminating the 'black box'.

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Introduction

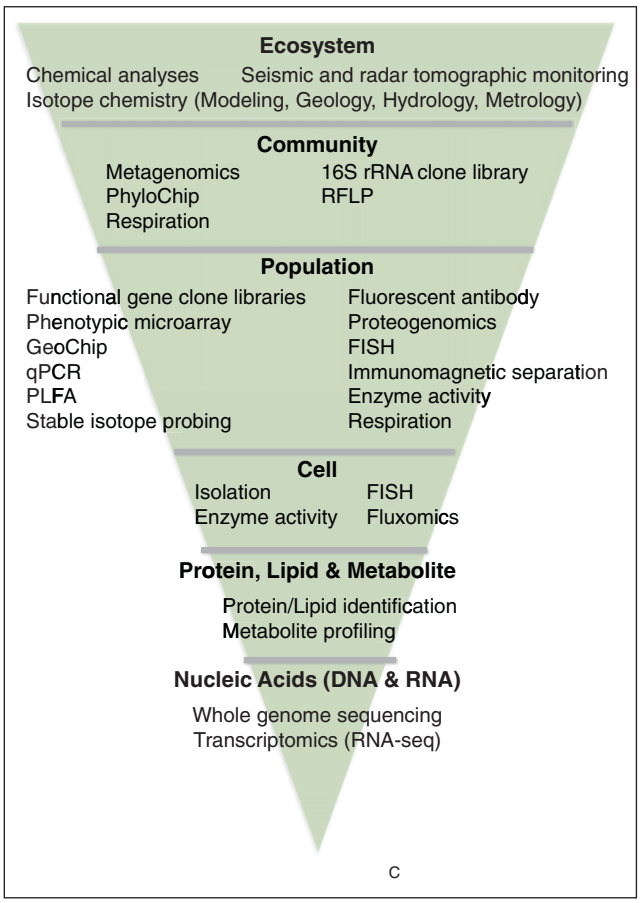
Bioremediation, a process mediated by microorganisms, is a sustainable way to degrade and detoxify environmental contaminants. Though bioremediation has been used to varying degrees for more than 60 years, for example petroleum land farming, it historically has been implemented as a very 'black box' engineering solution where amendments are added and the pollutants are degraded. This approach is often successful but all too often the results are less than desirable, that is, no degradation of the contaminant or even production of more toxic daughter products. The key to successful bioremediation is to harness the naturally occurring catabolic capability of microbes to catalyze transformations of environmental pollutants. Simulated experiments using defined microbial consortia in the laboratory is a great starting point in providing crucial initial indication (within certain constraints) of the process. However, unlike bench-scale simulations, in situ bioremediation in reality is a complex phenomenon involving more than one contaminant and mediated by different strains of microbes involving different metabolic pathways, across geochemical gradients, geophysical and hydrological complexities.

Systems biology approach

Recently, modern tools of genomics, transcriptomics, proteomics, metabolomics, phenomics, and lipidomics have been applied to investigate systems biology of microbial communities in a myriad of environments (Figure 1). Systems biology is an integrated research approach to study complex biological systems, by investigating interactions and networks at the molecular, cellular, community, and ecosystem levels. Amalgamation of the results from the various 'omics' tools has provided crucial insights into the survival, metabolism and interaction of microbes in their native environments including groundwater and marine systems [1–4], extreme milieus [5], deep-sea sediments and vents [6,7], and animal microbiomes [8]. A systems biology approach is being adopted to unravel key processes to understand, optimize, predict and evaluate microbial function and survival strategies in the ecosystem of interest. However, successful application of this approach requires over coming several challenges, including the high cost associated with sample processing equipment, materials and reagents, large amount of samples required, the need for skilled personnel to process the samples, massive amount of data generated, and the time consuming nature in integration and synthesis of the data. Currently, few bioremediation projects utilize the systems biology approach due to limitations in funding, expertise, and resources. This review will describe a compilation of research projects that would constitute a perfect study employing a systems biology approach for remediation of radionuclides, metals, hydrocarbons and chlorinated solvents, and suggest directions for future development.

To use a systems biology approach to bioremediation projects they must involve the characterization of microbial community composition, cellular and molecular activity and are complicated by the presence of toxic chemicals that alters the normal behavior of the microbial community. In addition, the ultimate objective of bioremediation projects is the elimination or detoxification of toxic compounds, which requires an understanding of all possible influence from environmental variables and cell–cell interactions. The selection process for which methods to use in a systems biology study includes consideration for cost, time frame, personnel, and the objectives of the project. If the

Figure 1



Systems biology from molecules to ecosystems. In general terms, an ecosystem consists of communities, populations, cells, protein, RNA, and DNA. The approaches use geochemcial, ecological, genomic, proteomic, metabolomic, and computational techniques. Analyze DNA, RNA, and protein at the cellular levels to understand impacts on the cell in terms of how bioremediation functions, and analyze communities, and populations to understand impacts on structure/function relationships and finally interactomes at the ecosystem level in terms of bioremediation practices.

focus is to elucidate microbial community composition, DNA based '-omics' tools such as 16S rRNA clone library, PhyloChip or sequencing should be used. If the interest is to understand cellular pathways and identify functional genes involved in microbially mediated reactions, tools that identify RNA, and proteins such as GeoChip, RNA-seq, and various mass spectrometry methods should be used. If the intention is to characterize small molecules produced by the microbes, matrix-assisted laser desorption/ionization (MALDI), desorption electrospray ionization (DESI), nuclear magnetic resonance (NMR) spectroscopy can be used. Concomitant monitoring of limiting nutrients, electron donors, electron acceptors, and hydrology is also crucial for a systems biology conceptual model to be useful.

To gain an understanding of complex *in situ* bioremediation processes, monitoring techniques that inventory and monitor terminal electron acceptors and electron donors, enzyme probes that measure functional activity in the environment, functional genomic microarrays, phylogenetic microarrays, metabolomics, proteomics, and quantitative PCR can provide unprecedented insights into the key microbial reactions employed (Figure 1). In general terms, an ecosystem consists of communities, populations, cells, protein, RNA, and DNA. We can analyze DNA, RNA, and protein at the cellular levels to understand the impacts on the cells, and analyze community and populations to understand effect of bioremediation on structure/function relationships (Figure 1). In some cases, a change in redox state is the simplest tool to bring about detoxification of hazardous metals and organic compounds. This is particularly true for metals and radionuclides like U(VI), Cr(VI), and Tc(VII). While these cannot be degraded, they can be biotransformed decreasing their bioavailability, mobility and thus toxicity [9–11]. Microbes can directly mediate such immobilization and detoxification by changing the valence states, utilizing them as electron acceptors [12,13] when appropriate electron donors are present. Measurement of enzyme activity during bioremediation is a reliable, inexpensive tool for measuring microbial respiration and metabolism. Dehydrogenase enzyme based assays such as INT (iodo nitro tetrazolium) [14] and TTC (triphenyl tetrazolium chloride) assays [15] have been used successfully in monitoring microbial respiration in the bioremediation of explosives [16], metals, PAHs [17], and oil [18]. Other enzymatic or DNA/RNA based probes from key microbial metabolic pathways can be effectively used as a tool in tracking bioremediation processes as used previously during degradation of trichloroethene (TCE) [19] and of petroleum hydrocarbons [20,21]. Proteogenomic analysis during U(VI) reduction field studies have been able to identify and track *Geobacter*-specific biomarker peptide citrate synthase [22] during the process. Using qPCR as a technique for detection of phylogenetic and catabolic genes as indication of microbially mediated remediation is a popular and successful approach for monitoring detoxification in metal as well as hydrocarbon contaminated sites. Examples include monitoring *Anaeromyxobacter* strains involved in reduction of U(VI) [23] and of *Dehalococcoides* spp. in bioremediation of chlorinated solvents [24]. In order to identify and track the entire microbial community during bioremediation processes, metagenomic analysis including 16S rRNA-based clone libraries has been broadly used for metals [25,26] as well as for hydrocarbons and chlorinated solvents [27•,28,29] among others. Collectively, these techniques for metagenomics have reiterated that the microbial diversity existing in most environments is greater than expected [30]. Recently, high throughput microarrays like the PhyloChip and the GeoChip have been extensively used in metal and organics bioremediation studies

[31•,32,33,34•,35], in order to quickly characterize microbial community and function. PhyloChip, the 16S rRNA-phylogenetic microarray characterizes and monitors microbial community dynamics whereas GeoChip, the functional gene microarray tracks functional gene activity changes of microbes in the environment [34•,36•]. Microbial community proteomics and metabolomics have been a major breakthrough in providing deeper insight into the microbial cellular function and gene products interplaying in the environment [37•]. A novel application of Immunomagnetic separation for targeting and monitoring specific microorganisms during *in situ* bioremediation [38] holds promise to enable transcriptomics, proteomics, or metabolomics-based studies directly on cells collected from the field. Integration of all of these techniques using the latest advances in bioinformatics and modeling will enable break-through science in environmental biotechnology. We discuss a review of these techniques as used in field studies and lab simulations from sites contaminated with metals, radionuclides, hydrocarbons, and chlorinated solvents (Table 1).

Case studies

The U.S. Department of Energy (DOE) is responsible for the remediation and long-term stewardship of a significant number of plumes containing various contaminants including radionuclides and metals, at sites spread across the United States (http://www.em.doe.gov/Pages/siteslocations.aspx). Several groups of researchers have been involved since 2004 in active implementation of basic research to understand the systems biology of contaminated sites, and predicting feasible remediation technologies.

Radionuclide biotransformation

Groundwater and soil at the Area 3 FRC site in Oak Ridge is not only contaminated with Uranium (up to 200 μM), but poses an unique bioremediation problem due to its low pH (~3), high nitrate (200 mM), and high calcium concentrations along with presence of chlorinated organic solvents. Research at this site by various investigators exemplifies successful application of systems biology tools to reveal a deeper understanding of the microbiology at play in the subsurface. Previously, 16S clone library-based community analysis during an *in situ* biostimulation test at this site have identified *Desulfovibrio*, *Geobacter*, *Anaeromyxobacter*, *Desulfosporosinus*, *Acidovorax*, and *Geothrix* spp. present concomitant with U(VI) reduction [26]. Clone libraries of functional gene markers like *dsrAB*, *nirK*, *nirS*, *amoA*, and *pmoA* [39,40] showed high microbial diversity in functional genes. However, recent metagenomic analysis from well FW106 specifically using a random shotgun sequencing-based strategy revealed a highly enriched community dominated by denitrifying *β-Proteobacteria* and *γ-Proteobacteria* [2]. GeoChip analysis of several groundwater monitoring wells reported widespread diversity of *dsrAB* genes [34•,41], which showed that sulfate-reducing bacteria were key players in U(VI) reduction. During the U(VI) reoxidation phase as studied in a sediment column with samples from FRC, observed decrease in biomass, but increase in microbial activity [42]. Using the PhyloChip, the study showed no decline in *Geobacter* or *Geothrix* spp. during the reoxidation phase, but members of *Actinobacteria*, *Firmicutes*, *Acidobacteria*, and *Desulfovibrionaceae* exhibited increased abundance [42]. GeoChip analysis during the reoxidation phase from field samples showed a decline in *dsr* genes but reoxidation did not appear to effect microbial functional diversity [33] suggesting that the microbial community was able to recover and continue to reduce U(VI) in the post oxidation phase.

Metals bioimmobilization

The Hanford 100H area adjacent to the Columbia River in Washington is contaminated with Chromium (Cr) as a result of being a weapons production site. In 2004, Hydrogen Release Compound HRC^{tm} was injected in an effort to mediate sustained bioimmobilization of Cr(VI) *in situ* by stimulating indigenous microbial flora [43]. Hubbard *et al.* used time-lapse seismic and radar tomographic geophysical monitoring to determine spatiotemporal distribution of the injected HRC and biogeochemical transformations associated with Cr(VI) bioremediation post injection of HRC [44]. Direct cell counts revealed that while cell numbers reached 10^8 cells/ml [43], Cr(VI) levels decreased from 100 ppb to below background levels within a year. PhyloChip analysis showed enrichment of sulfate reducers along with nitrate reducers, iron reducers, and methanogenic populations during this time [43]. Targeted enrichments resulted in isolation of sulfate-reducing *Desulfovibrio vulgaris* like strain RCH1, nitrate reducing strain *Pseudomonas stutzeri* strain RCH2, and iron-reducing strain *Geobacter metallireducens* strain RCH3 [45], all capable of Cr(VI) reduction [45]. μFlowFISH (integrated fluorescence *in situ* hybridization and flow cytometry) analysis was able to detect and sort *Pseudomonads* similar to strain RCH2 directly from Hanford 100H field water samples collected in 2009 and 2010 [46].

Hydrocarbon bioremediation

The dependence of petroleum-based energy source has fueled industrial growth and prosperity. However, it also brought dispersal of hydrocarbons into different environments. Fortunately, the organic nature of hydrocarbons enables microbes to metabolize these petroleum compounds as substrates. Notable reviews on a systems biology approach to bioremediation a Atlas and Hazen [47•], Harayama *et al.* [48], Zhou *et al.* [49], Fredrickson *et al.* [50], and de Lorenzo [51].

The MC252 oil spill in the Gulf of Mexico in 2010 was the largest in US history. Many environmental factors

Table 1

Fundamental systems biology parameters measured at the bioremediation sites

Site	Contaminant	Key parameters measured	References
Field Research Center, Oak Ridge, TN	Uranium (VI), nitrate	(a) 16S clone libraries	[26]
		(b) Metagenomics	[2]
		(c) PhyloChip	[42]
		(d) Functional gene clone libraries	[39,40]
		(e) GeoChip	[33]
Hanford 100H, Hanford, WA	Chromium (VI)	(a) Seismic and radar tomographic monitoring	[43]
		(b) Microbial cell counts	[44]
		(c) PhyloChip	[44]
		(d) Microbial isolation	[45]
		(e) FISH	[46]
Gulf of Mexico	Oil	(a) Dissolved oxygen	[52]
		(b) Enzyme activities	[53]
		(c) Microbial counts	[27•,53]
		(d) Hydrocarbon analyses	[54,27•,52,53]
		(e) 16S clone libraries	[27•]
		(f) PhyloChip	[27•]
		(g) GeoChip	[27•,56•]
		(h) PLFA	[27•,53]
		(i) Isotope chemistry	[27•,55]
Savannah River Site, SC	TCE, PCE	(a) Microbial cell counts	[62]
		(b) Fluorescent antibody	[62]
		(c) PLFA	[62]
		(d) Functional gene analysis	[62,66]
		(e) Isotope chemistry	[67]
Test Area North, ID	TCE	(a) Microbial cell counts	[68]
		(b) PLFA	[68]
		(c) Phenotypic microarray	[68]
		(d) DGGE	[68]
		(e) qPCR	[69]
		(f) RFLP	[69,70]
		(g) Functional gene analysis	[70]
		(h) PhyloChip	[31•]
		(i) Isotope chemistry	[63]

distinguished this spill from previous ones, including hydrocarbon composition, environmental variables, depth of the spill, and the availability of systems biology tools. Information on chemical analyses is crucial in support of a system's biology approach for oil bioremediation in the MC252 spill. While Camilli *et al.* [52] concluded that microbial respiration rates within the deep plume were extremely low based on dissolved oxygen concentration, measurement of microbial respiration rates, enzyme activity, phosphate concentration, and polar membrane lipid concentration in surface water affected by the oil spill. Edwards *et al.* concluded that enzyme activities and respiration rates were found to be higher inside the oil slick [53]. Valentine *et al.* [54] investigated the fate of methane, propane, and ethane gases of the deep hydrocarbon plume at depth greater than 799 m, and found that propane and ethane were degraded faster than methane. ^{13}C-labled substrates, as well as ^{13}C and ^{3}H tracers, were used to measure δ^{13}C-DIC. In another study, methane was found to be the most abundant hydrocarbon released during the MC252 spill, and that there was a rapid response of methanotrophic bacteria rapidly respiring the released methane [55]. PhyloChip, clone library, GeoChip, phospholipid fatty acid (PLFA), and isotope chemistry were used to compare microbial communities inside and outside the deep plume [27•]. The results identified *Oceanospirillales*, which were found to degrade hydrocarbons at 5°C inside the plume. The GeoChip demonstrated genes that were significantly correlated to concentration of oil contaminants, such as *phd*C1 (naphthalene degradation), and *alk*B (oxidation of alkanes), as well as a shift in C, N, P, S cycling processes in the deep plume samples [56•]. The involvement of federal agencies and pending lawsuits is the impetus for a concerted effort in collating all data collected resulting in a comprehensive database useful for researchers. By integrating chemical analyses with studies utilizing a systems biology approach, there was an unprecedented near real-time understanding of chemical and biological reactions involved in the hydrocarbon degradation. In order to gain a more comprehensive understanding of the microbiological processes, data from transcriptomics studies will provide information on whether the cultivatable dominant microbes are the *in situ* active ones, and

proteomics studies will identify enzymes central to hydrocarbon degradation.

Chlorinated solvents bioremediation

Chlorinated solvents, such as TCE and dichloroethene (DCE), are recalcitrant carcinogenic compounds that persist in the environment once released. Microbes, such as *Dehalococcoides*, are capable of using the chlorinated solvents as electron acceptors anaerobically and dechlorinating the compounds to ethene [31•,57]. Another biodegradation pathway is the aerobic co-metabolism of the chlorinated compounds to carbon dioxide and chloride by microbes such as methane-oxidizers with methane monooxygenases (MMOs) [31•]. Descriptions of techniques that monitor mass loss, geochemical fingerprints, isotope fractionation associated with biodegradation, microbial communities in biostimulation and natural attenuation studies, quantitative real-time PCR methods targeting reductive dehalogenase genes are included in several reviews [58,59,57].

Between 1955 and 1972, low-level radioactive isotopes, sewage and chlorinated solvents were injected into the aquifer through a 95 m deep well at Test Area North (TAN) in Idaho National Laboratory. The plume contained TCE concentrations ranging from 5 ppb to 300 ppm extending for more than 2 km. An enhanced *in situ* bioremediation pilot study started in 1999 to treat the chlorinated solvents contaminated groundwater by injecting the electron donor Lactate to stimulate *in situ* reductive dechlorination. A comparison of microbial communities in the core and groundwater samples was assessed by characterizing total biomass, PLFA analysis, culturing and community-level physiological profiling (CLPP) using Biolog GN microplates [60]. DGGE analysis indicated that wells with high concentrations of chlorinated solvents had different microbial communities from wells with minimal concentrations of the contaminants, and that attached and the free-living microbes had different functional and composition profile [60]. Additionally, qPCR of the *Dehalococcoides* sp. 16S rRNA genes provided the most convincing result in quantifying dechlorinating potential of a community compared to community analysis by terminal restriction fragment length polymorphism (T-RFLP), and RFLP analysis with clone sequencing [61]. Erwin *et al.* [62] demonstrated the presence of bacteria harboring MMOs and potential of TCE co-metabolism at TAN from a pristine area using PCR amplification to generate a function gene fragment library and sequencing. Stable carbon isotope ratios of groundwater samples taken in 2000 confirmed the complete conversion of TCE to ethene, and minimal biodegradation of t-DCE [63]. Using the PhyloChip for bacterial composition characterization, a decrease in reductive dechlorinating organisms and an increase in methane-oxidizing microbes capable of aerobic co-metabolism of TCE was observed [31•]. Further studies that would complement the investigation at the TAN site would be to employ a shotgun proteomics approach as reported by Werner *et al.* [64•]. Their method allowed for detection of peptides, such as *FdhA*, *TceA*, *PceA*, and *HupL* that could potentially be used as bioindicators of chlorinated ethene dehalorespiration.

Conclusion

The combination of the systems biology techniques as demonstrated in the case studies above allowed for enhanced understanding of complex bioremediation processes. Investigation of the MC252 spill is the most comprehensive bioremediation study using a systems biology approach to date as a result of available funding, resources, expertise, as well as, interests from the scientific communities and regulating agencies. Future projects can benefit from the experiences obtained from the MC252 spill investigation. However, while significant advances have been made in rapid generation and availability of 'omics'-based data in key microbial processes in the environment, a key bottleneck lies in the ability to quickly analyze the output using appropriate, user friendly, simplified bioinformatic tools to make meaningful conclusions. Currently, user-friendly bioinformatics pipelines available for analysis of sequencing and microarray data, include Qiime (qiime.sourceforge.net) [65] and PhyloTrac (www.phylotrac.org/), respectively. In order to fully utilize the data generated from the various 'omics' tools, better annotation of the genes, pathways, and metabolites are needed. A comprehensive database of all available genomics, proteomics, and metabolomics information from bioremediation research will provide a platform for scientist to exchange information including data obtained, and analysis methods and pipeline. This will require coordination from scientists to share data, and database managers to update, maintain, and provide quality control. Taken together, these tools will allow for accurate interpretation of the 'omics' data, leading to generation of judicious predictive models and strategies for successful implementation of bioremediation applications in the future.

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