# Using the stress response to monitor process control: pathways to more effective bioremediation

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Environmental contamination with a variety of pollutants has prompted the development of effective bioremediation strategies. But how can these processes be best monitored and controlled? One avenue under investigation is the development of stress response systems as tools for effective and general process control. Although the microbial stress response has been the subject of intensive laboratory investigation, the environmental reflection of the laboratory response to specific stresses has been little explored. However, it is only within an environmental context, in which microorganisms are constantly exposed to multiple changing environmental stresses, that there will be full understanding of microbial adaptive resiliency. Knowledge of the stress response in the environment will facilitate the control of bioremediation and other processes mediated by complex microbial communities.

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## Introduction

The contamination of large areas of the environment with organic and inorganic pollutants has promoted research for the development of effective bioremediation strategies. There is often no alternative to the bioremediation option, as the physical removal of soil, sediments and subsurface materials that can extend many hundreds of meters in area and depth is not feasible. The tremendous variety of pollutant types, including compounds such as halogenated organics that are recalcitrant to microbial degradation and others that cannot be degraded such as heavy metals and radionuclides, impose site-specific requirements. These specific requirements often prevent the general application of information developed at one site from being used to design treatment strategies for other systems and pollutant types. As a result, there has been an increasing emphasis placed on site monitoring — to ensure that specific microbial processes are operative or alternatively that intrinsic processes are sufficient to either control (e.g. minimize migration) or ultimately mitigate the contamination [1]. Existing monitoring tools attempt to develop clear links between pollutant transformation and microbial activity. Ideally, a stoichiometric balance would be achieved — the disappearance of the pollutant directly correlated with the appearance of a metabolite, such as $CO_2$ from pollutant mineralization or chloride ion release via microbial dehalogenation. As complete stoichiometric balance is virtually impossible in the open environment, having an unknown subsurface composition that is sampled at relatively few sites, monitoring has also included the use of stable isotopic measures to provide additional evidence of microbial attribution. Stable isotope probes (SIP) and compound-specific isotope analysis (CSIA) have been used to determine rates of substrate transformation and to link processes with specific populations. For example, SIP has been used to determine the rate of electron donor fractionation into microbial metabolites like $CO_2$ and $CH_4$ [2]. The contribution of specific populations has been determined by tracing the incorporation of specific organic components (e.g. $^{13}$C-labeled lactate) into diagnostic phospholipid fatty acids (PLFA) and nucleic acids [3•,4]. CSIA has been used recently to determine the rates of utilization of several electron acceptors (e.g. oxygen [5••], hydrogen [3•], sulfate [6], nitrate [5••] and carbon dioxide [2]) and to monitor the dehalorespiration of chlorinated solvents [7]. These techniques have been used both *in situ* and in bioreactors to provide more direct evidence of metabolic state, kinetics and stress status [8••]. Less specific measures include a demonstration of increased microbial abundance and activity in areas of pollutant disappearance or documentation that microorganisms possessing the desired metabolic characteristics are present at the bioremediation site and increase in numbers with increasing rates of pollutant loss.

These monitoring tools when combined (e.g. SIP and CSIA are now being used concomitantly with PLFA, nucleic acid, and protein analysis) offer a measure of process rates and some attribution to participating microbial populations. However, they are primarily retrospective measures that provide only a very blunt instrument for effective process control. We suggest that more

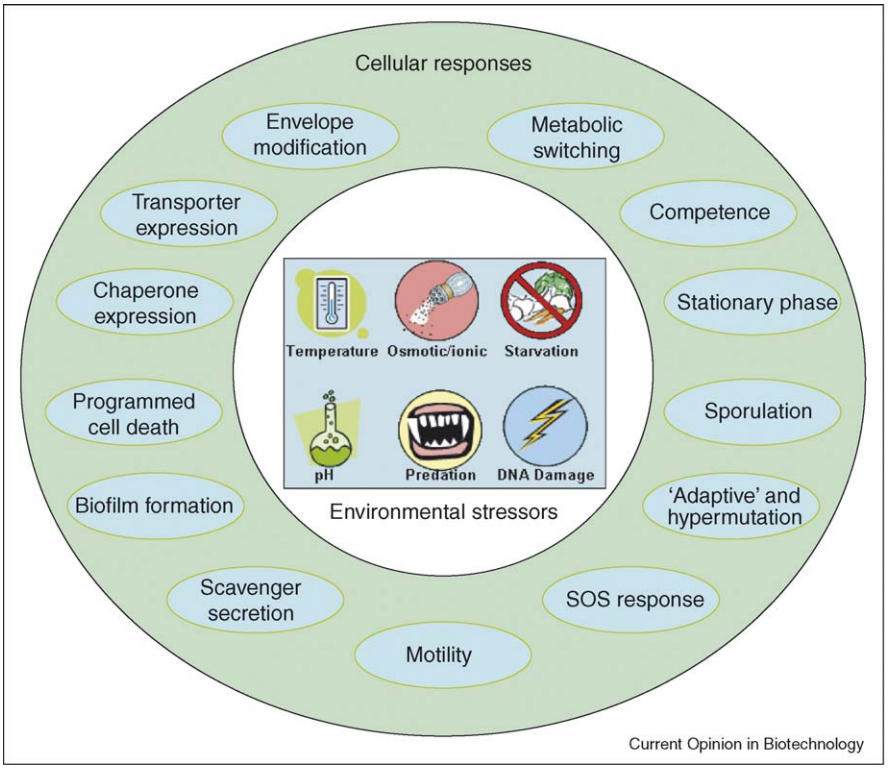
effective process control will derive from the development of monitoring tools that more directly determine the physiological status of organisms participating in pollutant transformation. This information can then be used for the more effective optimization of site conditions as needed to promote rapid and desired microbial transformations. Thus, this review primarily concerns the development of microbial stress response systems as an emerging tool for the more effective and general process control of bioremediation and other processes mediated by complex microbial communities. This tool would build directly upon existing monitoring technologies, as knowledge of the microbial populations active in a desired transformation would provide a framework for using the stress response for more effective process control.

## What is stress?

Microorganisms have relatively few behavioral options for coping with constantly changing environmental conditions (Figure 1). Although motility and chemotaxis are important for optimizing the local physical/chemical environment, they do not provide protection from rapid system-wide changes. As running away is generally not an option, the microbial alternative is rapid adaptation, often involving one or more systems of the stress response. Although microorganisms in the open environment are often stressed, and simultaneously exposed to multiple stressors, study of the microbial stress response has been primarily restricted to laboratory systems, considering the response of well-characterized 'model' organisms to very specific types of stress. This somewhat reductionist characterization of stress has resulted in a rather unsatisfying set of definitions of microbial stress, which generally includes: any deviation from optimal growth conditions that results in reduced growth rate; an environmental situation that results in damage of cellular components in the absence of a cellular response; and any situation that stimulates the expression of known stress-response genes.

The laundry list of better-studied stressors and microbial response systems includes starvation, heat shock, cold shock, envelope stress, oxidative stress, oxygen deprivation, osmotic challenges, acid stress, sodium stress, and SOS response to DNA damage. Stress is also relative — temperatures and pH that are stressful to one species could be optimal for the growth of another. Thus, we suggest the need for a more environmentally relevant definition of stress, one that incorporates the concept of microbial niche and the adaptive landscape. The most generally accepted definition of niche is that proposed by

**Figure 1**




Multiple response pathways to environmental stress. Environmental stressors can cause a large number of cellular responses that include physiological, biochemical, and behavioural responses that help avoid stressors, provide protection and repair stress damage. (Figure kindly supplied by AP Arkin.)

Hutchinson [9] — 'An N-dimensional hypervolume of environmental conditions within which the organism can maintain a population'. There are two categories of environmental conditions: physical/chemical (e.g. temperature, salinity, flow and pressure) and resources (e.g. nutrients, energy sources and space). The adaptive landscape for any one species includes three elements: the range of conditions in which the species functions competitively and can maintain a population; an adaptive range in which the population can function but is no longer competitive; and an adaptive limit at which individuals cannot persist. For example, organisms transiently exposed to an adaptive limit of temperature elicit the heat shock stress response that serves for their recovery; however, they cannot persist at the elevated temperature. It is also evident that the adaptive limit for any one stressor can change when the organism is exposed simultaneously to multiple stressors.

Another reason for using the stress response as a monitoring tool is that it provides a more complete understanding of the contribution of biotic interactions to the physiological state and stress response. Only in the laboratory do microorganisms function in isolation. An example of how a biotic interaction can change the spectrum of the stress response is provided by a metabolic interaction commonly observed in anaerobic communities that involves the interspecies transfer of hydrogen or formate. In the absence of sulfate, sulfate-reducing bacteria can remain a dominant population by using a hydrogen-consuming microorganism (such as a methanogen) as an alternative electron acceptor [10,11]. The methanogen serves to pull the reaction by removal of an end-product of substrate oxidation (hydrogen or formate). As many sulfate-reducing bacteria have the capacity to use hydrogen when respiring sulfate, these two alternative growth states alter the affect that increased hydrogen concentration has on cells when growing via sulfate respiration or syntrophically. In the first instance, hydrogen is a valuable substrate for growth; in the second, increased hydrogen inhibits growth. We anticipate that as stress response circuits are examined in environmentally relevant contexts, much of our current understanding of stress response will be modified. As many key environmental transformations, including the biodegradation of chlorinated hydrocarbon pollutants, are sustained by syntrophic interactions [12•], it is essential that the biological context of the stress response be better constrained.

Our ability to 'map' the stress response in relation to adaptive landscapes should also define the concept of microbial species, address environmental factors that determine microbial biogeography, further our understanding of pathogen survival in the environment, and serve to monitor any system — whether natural or engineered – for the physiological status of the resident microorganisms. Thus, our intention here is not to cover the many well-studied stress response mechanisms, but to identify a few areas of investigative overlap that should have value in biotechnological applications in which monitoring the stress response could contribute to process control.

## What are signature responses to specific stressors?

There are two general levels of possible interrogation of stress: the immediate regulatory and physiological response and subsequent changes in the transcriptome, proteome, metabolome and cellular architecture. Immediate changes in protein and mRNA structure are part of the first line of defense (e.g. [13–15]), but might not be easily monitored using available analytical methods. Alternative σ factors also play a critical role in the early adaptive response to transient and longer-term changes in environmental conditions. Many of these factors are conserved across wide phylogenetic groups and would offer general targets for assessing the stress response. However, the identity of the alternative σ factor regulons is needed to provide transcriptional and translational metrics of different stress response systems. To this end, the regulons for some better-studied organisms have now been characterized using a combination of similarity searches for conserved promoter sequences, characterization of changes in protein composition (e.g. using two-dimensional protein gel electrophoresis), microarray analysis, and more selective measurements of changes in transcription (e.g. reporter fusions; see [16•,17••,18]). The work by Rhodius *et al.* [17••] offers a nice perspective on the relationship between the lifestyle of the microorganisms and the complexity of the adaptive responsive mechanisms. For example, obligately intracellular *Mycoplasma* species contain only one housekeeping σ factor and no alternative factors, whereas common soil-dwelling organisms such as streptomyces that are exposed to constantly changing environmental conditions can have more than 60 alternative σ factors. Their analysis of the $\sigma^E$ regulon across nine Gram-negative genomes revealed a core response associated with the maintenance of outer membrane integrity, encoding for the synthesis and assembly of lipopolysaccharides and outer membrane proteins. However, the extended regulon for each organism included genes that appear to be niche specific; for example, encoding pathogenic-specific functions.

Research groups, including investigators supported by specific programs such as the US Department of Energy's Genomics: GTL program, are now developing the necessary bioinformatics tools to identify regulons controlled by specific σ factors (http:vimss.lbl.gov). The heat shock stress response, having relevance to food processing and sterilization, is one of the better-characterized systems. Our most recent studies show that heat shock stress analysis can be applied to sulfate reducers found at metal-contaminated sites, suggesting general stress

response pathways that could be relevant to other environmental stressors [19•]. Other possible signatures of the stress response include increased levels of expression of genes of lysogenic phage and transposons. For example, the global regulator H-NS has been reported to act directly on the transpososome to promote Tn10 transposition [20]. Continued advances in these areas should greatly enable the practical application of stress response data to systems level analysis of complex environmental systems, identifying both core regulons that are likely to have utility for general monitoring and the extended niche-specific regulon that in part defines individual species.

## Environmental systems

Biostimulation through the addition of nutrient amendments to contaminated environments has recently started to focus on specific stressors that could affect biodegradation/biotransformation processes. Holmes *et al.* [21] monitored the *nifD* gene for nitrogen fixation during acetate stimulation of organic- and nitrogen-poor subsurface sediments. Although *nifD* expression decreased 100-fold after the addition of ammonium, it had no effect on rates of toluene degradation or Fe(III) reduction. Thermodynamic analysis of Cr(VI) exposure to sulfate reducers has also been shown to induce an inhibition of growth and energy production that is similar to oxidative stress responses [22]. This suggests that commonality in stress responses might provide strategies that can be used to maximize biodegradation/biotransformation processes *in situ* against specific contaminants without increasing biomass of the target organism. Bioaugmentation (the addition of living cells) for the biodegradation of carbon tetrachloride has also been shown to benefit not only from nutrient balance, but also from pH adjustments to avoid pH stress [23]. By adding a combination of alkali, acetate, phosphorus and carbon tetrachloride degrader in a biocurtain strategy, carbon tetrachloride biodegradation in the groundwater passing through the biocurtain could be sustained at 100%.

## Industrial systems: waste treatment and food processing

Trickle-bed bioreactor systems for treating industrial wastewater typically have problems with media clogging from excessive biomass that greatly reduces the overall efficiency of the system. Recent studies demonstrated that salt stress inhibited bacterial growth but not substrate degradation by benzene-toluene-xylene degraders, suggesting that limited stress can be used to control bioreactor efficiency [24]. Although short-term microbial adaptation to environmental stressors is protective at the cellular level, it could be disruptive in engineered bioreactors such as those used for wastewater treatment [25]. For example, *cis-trans* isomerization of cell membranes can decrease rates of active transport owing to a decrease in membrane fluidity. In turn, this alters linkages between cells and exopolymers (flocculation) and alters the transport of hydrolytic enzymes out of the cell, thereby affecting the biodegradation of extracellular material.

We also anticipate that there will be significant synergistic interaction in different areas of applied biotechnology that have a common interest in understanding the microbial stress response. An area of great potential overlap is in food processing [26]. As food-processing technology increasingly emphasizes the use of less destructive food preservation methods, the production and storage of minimally processed foods increases the likelihood of microbial contamination. In response, there is a significant research literature addressing the stress response of important food-borne pathogens such as *Listeria monocytogenes* [14]. The survival and growth of this microorganism at high osmolarity and at the low temperatures used for storage have received particular attention, examining different physiological roles of the alternative $\sigma$ factor, $\sigma^B$, in this and other Gram-positive bacteria. This $\sigma$ factor is involved in resistance to a variety of environmental stresses (including heat, high osmolarity, high ethanol concentrations, high and low pH, and oxidizing agents). The role of $\sigma^B$ has recently been reviewed by van Schaik and Abee [27], with an eye to using this knowledge to develop new food processing technology, possibly involving sequential preservation steps that do not activate stress response systems. Also, existing and emerging methods to monitor adverse physiological effects (stress) during the large-scale production of recombinant proteins could have more general application. For example, a surface plasmon resonance biosensor for monitoring profiles of the heat-shock protein DnaK was shown to provide a measure of the stress response associated with protein overproduction [28]. It is apparent that this kind of knowledge and associated technologies will have broad application in monitoring the physiological status of microbial populations, either to promote the growth of those that are favored or limiting the growth of those that are unwanted.

## Conclusions

The identification of general and species-specific stress response regulatory elements and regulons should serve to identify appropriate metrics for process monitoring. We anticipate that the rapidly developing bioinformatics tools will continue to make this an achievable objective in the near term; however, the remaining challenge is to develop appropriate analytical methods to selectively measure response. Here we anticipate that new advances in areas such as proteomics (e.g. using high-resolution mass spectroscopy) will be needed to effectively evaluate the stress response in open environmental systems. Amplification techniques suited to expression analysis might also have utility. Furthermore, RNA-based tools are being used to infer metabolic rates from both pure

cultures and environmental samples; for example, carbon dioxide fixation rates in marine systems have been monitored using mRNA levels from the ribulose-1,5-bisphosphate carboxylase/oxygenase (or RUBISCO) gene *rbcL* [29••]. Once specific markers are identified using these more labor-intensive, costly, and broader techniques, then we can use tools such as quantitative PCR, which is more applicable for routine, cost-effective use. In the near term, it is likely that use of the stress response for process control will initially find application in engineered systems, such as bioreactors designed for waste treatment. An early exploratory example of using the stress response as a monitoring tool is the immunochemical detection of GroEL (a highly conserved chaperone) in activated-sludge reactors [25,30].

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