Field applications of genetically engineered microorganisms for bioremediation processes

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Genetically engineered microorganisms (GEMs) have shown potential for bioremediation applications in soil, groundwater, and activated sludge environments, exhibiting enhanced degradative capabilities encompassing a wide range of chemical contaminants. However, the vast majority of studies pertaining to genetically engineered microbial bioremediation are supported by laboratory-based experimental data. In general, relatively few examples of GEM applications in environmental ecosystems exist. Unfortunately, the only manner in which to fully address the competence of GEMs in bioremediation efforts is through long-term field release studies. It is therefore essential that field studies be performed to acquire the requisite information for determining the overall effectiveness and risks associated with GEM introduction into natural ecosystems.

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Abbreviations
GEM genetically engineered microorganism
PAH polycyclic aromatic hydrocarbon
PMN premanufacture notice

Introduction
Application of genetically engineered microorganisms (GEMs) for use in bioremediation has seen little development over the past decade. Although one moderately large-scale, controlled field research test has been completed, the future use of engineered organisms remains cloudy. This apparent lack of direction or motivation for use of engineered organisms in bioremediation has a number of underlying root causes. Miller [1] has argued that the US Environmental Protection Agency’s risk-based regulatory approach continues to stifle both research and applications for engineered products in bioremediation. To an extent that appears true, but the problem is further confounded by other issues over the perceived need for engineered organisms in bioremediation and cost competitiveness with other technical solutions. These problems are true not only for engineered microorganisms but also for applications of “natural” organisms for use in areas such as trichloroethylene bioremediation [2].

Many companies engaged in remediation operate on very thin profit margins and few have a primary focus in bioremediation. Thus, there has been little in the way of a driving force to commercially exploit genetic engineering in bioremediation. Nonetheless, there continues to be significant research development in the genetics and molecular biology of biodegradative systems in bioremediation. One might argue that leadership in this area has shifted outside the US. This is somewhat of an anomaly given that many of the Organization for Economic Cooperation and Development (OECD) countries have a higher public sensitivity to genetic engineering methodology than does the US.

Regardless of this impression, there are several hundred genetic systems that have and can be exploited for developments useful in bioremediation [3,4]. Timmis and Pieper [5] have recently defined a number of opportunities for improving degradative performance using genetic engineering strategies. For example, rate-limiting steps in known metabolic pathways can be genetically manipulated to yield increased degradation rates, or completely new metabolic pathways can be incorporated into bacterial strains for the degradation of previously recalcitrant compounds. In addition, other strategies using engineered microorganisms for process monitoring and control, toxicity and stress response assessment, and endpoint analysis in bioremediation have also been summarized [5].

The University of Tennessee in collaboration with Oak Ridge National Laboratory has achieved the first, and so far only, field release of a GEM for bioremediation purposes [6••,7••]. The GEM involved was a Pseudomonas fluorescens strain designated HK44, released into a contained soil environment. The original parental strain from which strain HK44 was derived was isolated from a manufactured gas plant facility heavily contaminated with polycyclic aromatic hydrocarbons (PAHs). The naphthalene catabolic plasmid pUTK21 was introduced into this strain to form \( P. \) fluorescens HK44 [8••]. Additionally, strain HK44 contains a transposon-based bioluminescence-producing \( lux \) gene fused within a promoter for the naphthalene catabolic genes [9]. Therefore, exposure of strain HK44 to naphthalene (or the intermediate metabolite salicylate) results in increased catabolic gene expression, naphthalene degradation, and a coincident bioluminescent response. The purpose of such an engineering scheme was to develop a GEM capable of sensing an environmental contaminant and responding to it through an easily detectable signal, such as bioluminescence. In this manner, strain HK44 serves as a reporter for naphthalene bioavailability and biodegradation and, through bioluminescence signaling, can be used as an online tool for \( in \) situ monitoring of bioremediation processes. The utilization of this engineered strain in a field release study will be discussed in this review in order to assess the benefits and obstacles associated with the use of GEMs in bioremediation applications.
Risk assessment for the release of a recombinant microorganism

Accurate field release studies is the securing of the required governmental permission, which is often a difficult and lengthy endeavour. Although necessary to ensure environmental and public health safety, the process often leads to an overall aversion to GEM implementation in environmental systems, with researchers concentrating rather on the optimization and commercial development of naturally occurring (intrinsinc) microbial degradation [10•]. Also, during the approval process the GEM might undergo significant refinement, and genetic restructuring while in the hands of researchers, making the originally proposed release microorganism somewhat obsolete. This unfortunately prevents the integration of state-of-the-art engineered microbes into field release studies.

The US Environmental Protection Agency’s Office of Pollution Prevention and Toxics regulates on a broad basis the production and application of GEMs through its Toxic Substances Control Act (TSCA) [11,12]. This risk assessment process used by TSCA involves 10 official forms reviewing potential health and environmental effects to be detailed within a premanufacture notice (PMN). The University of Tennessee submitted a PMN (#P95-1601) in July of 1995 signifying its intent to conduct field research tests with P. fluorescens HK44 for applications in bioremediation [13]. Rather than outright approval of the field test, in March of 1996 the University received a consent order sanctioning the release under guidelines outlined in the PMN research design. This was because of species classification ambiguities, possibility of transfer of introduced foreign genetic elements, and will therefore be unable to compete under real-world conditions [21,22].

In the case of P. fluorescens HK44, no such extra energy burden was observed and these microbes survived for the duration of the field release study [6••,7••]. Although numerous other studies have similarly shown GEM survival to be non-problematic, an equal number have not, begetting the conclusion that GEM survival in the environment is inherently unpredictable [21,23]. Considering the vast array of environmental factors influencing GEMs, both biotic (competition and predation) and abiotic (temperature, pH, moisture and adsorption), it is understandable that deriving a component modeling scheme for GEM survival will be a daunting task.

Bioprocess monitoring and control

One of the most common criticisms of bioremediation in general is the inability to document the efficacy of a potentially lengthy clean-up process without the use of expensive chemical analysis methods such as gas chromatography/mass spectrometry. It is often stated that bioremediation is the most economically effective treatment technology available, costing at least one-third less than conventional incineration or landfill methods [24,25•]. When considering the added expense of continuous monitoring, however, bioremediation may become a much less cost-effective alternative.

To improve bioremediation monitoring and for online process control, the P. fluorescens HK44 GEM released in the lysimeter study was engineered to bioluminesce when physiologically active during a biodegradation process. As strain HK44 degrades naphthalene it concomitantly produces a bioluminescent signal that is detectable using fiber optics and photon counting modules, and thus functions as a continuous online monitoring tool for bioremediation process manipulations. Bioluminescence was successfully detected from HK44 cells residing directly within the PAH-contaminated lysimeter soil matrix as well as from HK44 cells contained within biometer devices that specifically monitored for volatile PAHs [6••,20].

One means of bypassing structural containment of GEMs is to design GEMs that are susceptible to biological containment. These GEMs would only be permitted to exist only under the selective environmental conditions for which they were designed (i.e. only in the presence of a specific environmental contaminant) or would contain self-destruction mechanisms (suicide genes or vectors) that could be induced when necessary to eradicate the GEM population [19]. Such strategies cannot, at present, ensure total GEM elimination after environmental introduction, and may represent more of a genetic engineering risk, due to, for example, gene transfer potentials, than the original bioremediative modifications themselves [20].

Survival in a harsh world

It is typically assumed that GEMs will exhibit a decreased level of fitness due to the extra energy demands imposed by introduced foreign genetic elements, and will therefore be unable to compete under real-world conditions [21,22]. In the case of P. fluorescens HK44, no such extra energy burden was observed and these microbes survived for the duration of the field release study [6••,7••]. Although numerous other studies have similarly shown GEM survival to be non-problematic, an equal number have not, begetting the conclusion that GEM survival in the environment is inherently unpredictable [21,23]. Considering the vast array of environmental factors influencing GEMs, both biotic (competition and predation) and abiotic (temperature, pH, moisture and adsorption), it is understandable that deriving a component modeling scheme for GEM survival will be a daunting task.
utilized for monitoring plume dynamics within a groundwater aquifer contaminated with a mock jet fuel at Columbus Air Force Base, Mississippi [27•].

The use of the hcs-based system affords several advantages for monitoring bioremediation processes: firstly, bioluminescence is easily detected and requires no substantial input of expensive or obscure survey devices; secondly, the production of bioluminescence by strain HK44 is completely self-contained, no exogenous addition of chemicals or co-factors is required; thirdly, bioluminescence can be monitored directly online, providing a continuous, near real-time profile of the bioremediation process; and finally, the use of intact microbes as chemical sensors allows for the monitoring of contaminant bioavailability rather than just contaminant presence. This is in contrast to analytical techniques that may determine contaminant presence in an environmental matrix, but without providing information as to the biological effect of the contaminant. Such data becomes extremely important when attempting to assess detrimental health effects of chemical pollutants on exposed populations, human or otherwise.

Bioremediation potential
Evaluating the overall success of an in situ bioremediation program is an often-difficult process, whether using genetically engineered or intrinsic microorganisms. These difficulties lie primarily in deducing to what extent the microbe itself actually contributed to the degradation process, recognizing that factors such as volatilization and chemical transformation are simultaneously occurring within the system. Specific to the use of GEMs, it can also be problematic distinguishing between GEM-specific degradation and biodegradation due to the indigenous microbial consortia. Another obstacle is the inability to statistically gauge bioremediation efficacy because of the highly heterogeneous distribution of the contaminants. Sample-to-sample chemical analyses can typically vary by up to 200%, making valid conclusions unconvincingly obscure [29•]. In P. fluorescens HK44 lysimeter release experiments, soil PAH concentrations were similarly heterogeneously dispersed, spatially ranging in some lysimeters from 0.04 to 192 ppm [6••]. Consequently, a precise evaluation of the effectiveness of P. fluorescens HK44 in contaminant remediation could not be adequately determined. Statistical models that can better incorporate chemical heterogeneity kinetics into their overall design are unquestionably required before valid bioremediation efficacy assessments can be obtained [29•].

Conclusions
The future potential for GEMs in bioremediation at the field scale may be limited to those problems that simply cannot be cost effectively managed by other treatment technologies. One example may be in situ source reduction of recalcitrant organics in the subsurface. Even here, effective delivery of the GEM may be limiting to the technology. Other opportunities do exist and may eventually use engineered microorganisms in contained reactor technology for bioremediation or waste treatment. Thinking broadly, these applications could extend to greenhouse gas control, carbon sequestration, or conversion of wastes to value-added products (sparking petroleum feedstocks and fossil carbon in the process). Regardless, there remains the need for a regulatory, safety, or costs benefit-driving force to make these potentials a reality.

Worldwide, it has been realized that pollution prevention is more economical and socially responsible than clean up, and inevitably the demand for waste site remediation technologies will abate. For pollution prevention to be successful, materials must be reused or recycled, and therein lies a new future for the bioremediation application of GEMs. Before such perceived advances can be realized, however, a fundamental knowledge database of GEM performance under complex environmental parameters must be attained. This can only be achieved through careful field studies and a comparative life cycle assessment that balances risk with biotechnological benefit.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
+ of special interest
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An overview of the field bioremediation experiments involving Pseudomonas fluorescens HK44. The function and design of the lysimeter facility are dis-cussed, stressing the engineering aspect of a field release study.


The author discusses the role of biotechnology in meeting the goals required for pollution prevention and environmental sustainability.


Husdal ML: Bioremediation: towards a credible technology. 


A computer program described in this paper, designated RT3D, represents perhaps the best modeling scheme yet available for predicting bioremediation potential.