Monitoring and evaluating the efficacy of bioremediation - a conceptual framework

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Monitoring and Evaluating the Efficacy of Bioremediation - a Conceptual Framework

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Introduction

The primary goal of cleaning up a contaminated site is the reduction of actual and potential future risks posed by the pollutants to the natural and social environment at and around the site. While a number of physical, chemical and biological remediation technologies and combinations thereof exist, bioremediation has received much attention because of its potential to detoxify and mineralize a wide variety of pollutants to carbon dioxide and water at low cost. However, the general acceptance and credibility of bioremediation as an environmentally sound and economic treatment technology for hazardous waste requires the demonstration of its efficacy, reliability, predictability and overall efficiency (5).

The goal of this contribution is the development of a systematic monitoring concept for the demonstration of bioremediation efficacy.

Efficacy and Efficiency in Bioremediation

The term efficacy refers to the power to produce an effect. Bioremediation efficacy*, as defined here, requires the demonstration of the treatment related biological nature of pollutant disappearance during a bioremediation process. The following three criteria, describing different qualities of bioremediation efficacy are going to be used:

1. Treatment related, biological nature of pollutant disappearance
2. Detoxification of the pollutant
3. Mineralization of the pollutant

The decision which of the above mentioned criteria are used, depends on the treatment goals and efficacy requirements. The qualitative and quantitative demonstration of process efficacy is a prerequisite for the evaluation of the overall efficiency of a bioremediation process.
Efficiency refers to the optimization of the ratio of expenditures such as, e.g., time, money, energy use, waste production or new pollution to benefits such as, e.g., clean up levels achieved, re-establishment of the original structure and function of the site matrix, quality and suitability of the formerly contaminated area for reuse.

**Pollutant Fate Processes**

Ideally, bioremediation efficacy is quantitatively demonstrated based on a complete mass balance for the pollutant, taking all relevant transport and reaction processes at the contaminated site into account. Figure 1 presents a generalized model for possible pollutant fate processes at the macro- and micro scale. However, with increasing bioremediation process scale and during in situ treatments in particular, general treatment system complexity as well as spatial and temporal site heterogeneities can make the establishment of complete mass balances very difficult. Under these circumstances the treatment efficacy may be demonstrated using more qualitative, ecophysiological characteristics of a pollutant biodegradation process. In general, the main reasons for the difficulties to obtain quantitative information include data and model uncertainties with their sources stochasticity, error and ignorance (17).

**Figure 1**: Generalized model for pollutant fate processes at a contaminated site comprising five different phases: Aqueous phase, gas phase, solid phase (including suspended solids), non aqueous phase liquid (NAPL), Biomass (pdB: pollutant degrading bacterium; npdB: non pollutant degrading bacterium).

The Monitoring Design

In order to reduce the uncertainty source "ignorance", a conceptual framework for the monitoring of bioremediation efficacy and efficiency was developed as illustrated in Figure 2. The first step in this procedure involves a precise goal definition, including the formulation of efficacy and efficiency criteria. Subsequently, the system under consideration needs to be defined and described in a model, based on information obtained from earlier investigations such as the site characterization as well as characteristics of the treatment scale, method and location. For this purpose it can also be helpful to use a generalized bioremediation process template (Figure 1) that is reduced or extended according to the treatment process under investigation. The monitoring strategy should be designed around the treatment system model. The resulting monitoring design should take the requirements set by the criteria for the process efficacy and efficiency into account.

Figure 2: Conceptual procedure for the monitoring of bioremediation efficacy and efficiency.
The complete monitoring design of a bioremediation process includes:

1. Monitoring parameter and method selection
2. Control strategy
3. Sampling strategy

For the successful assessment of bioremediation efficacy it is crucial to combine these approaches in a logical manner along a cascade of critical questions concerning treatment and bioremediation efficacy.

After the data acquisition step, the cascade of relevant questions may be answered qualitatively or quantitatively, as required. Subsequently, the efficacy and efficiency of the treatment process are evaluated according to the previously defined criteria before a decision is to be made. Selected monitoring parameters and the establishment of controls were analyzed and are subsequently discussed while the topic of spatial and temporal sampling strategies is not included in this contribution (for references see, e.g., (11) and (15)).

**Selection of Monitoring Parameters and Methods**

After the bioremediation system model has been described, a set of monitoring parameters for the investigation of process efficacy, has to be selected. While the environmentally sound reduction of the pollutant and its toxicity is the ultimate goal of the treatment, measurement of the pollutant alone is not sufficient for the demonstration of efficacy according to the criteria defined earlier. Additional monitoring parameters have to be integrated in the monitoring design, depending on the ecophysiological characteristics of the pollutant degradation process, as illustrated in Figure 1. These biotransformation products, by-products, end products, biomass and its activities. Further, lumped parameters and toxicity as well as pollutant bioavailability can serve as important parameters. In Table 1, an overview for selected parameters including their utility as indicators for the efficacy criteria biodegradation/ biotransformation. Mineralization and detoxification is presented. The footnotes include some of the remaining uncertainties characteristic for these parameters which can be reduced using appropriate supplementary parameters and overall monitoring designs.
Table 1: Analysis of selected monitoring parameters for pollutant biodegradation. (Modified from Ref. 5).

<table>
<thead>
<tr>
<th>Monitoring parameter</th>
<th>Examples</th>
<th>Pollution type</th>
<th>Indication of biodegradation</th>
<th>Indication of biotransformation</th>
<th>Indication of mineralization</th>
<th>Indication of detoxification</th>
<th>Application scale</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollutant concentration</td>
<td>TCF, jet fuel, PCB Congener/34-34CB/236-34CB</td>
<td>all types</td>
<td>Yes(^a)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Full scale</td>
<td>(7)</td>
</tr>
<tr>
<td></td>
<td>PCB</td>
<td></td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Pilot scale</td>
<td>(3)</td>
</tr>
<tr>
<td></td>
<td>C18/phytane, C17/pristane</td>
<td>crude Oil</td>
<td>Yes(^b)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Full scale</td>
<td>(13)</td>
</tr>
<tr>
<td>Terminal electron acceptors</td>
<td>O(_2)</td>
<td>aerobically degrad. pollutants</td>
<td>Yes(^c)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Full scale</td>
<td>(6)</td>
</tr>
<tr>
<td></td>
<td>N(_0)</td>
<td>pollutants degrad. under denitrif. conditions</td>
<td>Yes(^d)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Full scale</td>
<td>(7)</td>
</tr>
<tr>
<td>Transformation products</td>
<td>tDCE epoxide chlorobenzoates</td>
<td>transDCE</td>
<td>Yes(^e)</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Pilot scale</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>CO(_2)</td>
<td>organic pollutants</td>
<td>Yes(^f)</td>
<td>No</td>
<td>Yes(^g)</td>
<td>Yes(^h)</td>
<td>Full scale</td>
<td>(6)</td>
</tr>
<tr>
<td></td>
<td>C(_1)</td>
<td>jet fuel</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Pilot scale</td>
<td>(6)</td>
</tr>
<tr>
<td></td>
<td>Cl(_-)</td>
<td>Chlorinated organics</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Full scale</td>
<td>(1)</td>
</tr>
<tr>
<td>Endproducts or byproducts</td>
<td>total heterotrophs</td>
<td>all pollutants</td>
<td>Yes(^i)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Full scale</td>
<td>(1)</td>
</tr>
<tr>
<td></td>
<td>Microtox</td>
<td>all pollutants</td>
<td>No</td>
<td>Yes(^j)</td>
<td>No</td>
<td>Yes</td>
<td>Bench scale</td>
<td>(19)</td>
</tr>
<tr>
<td>Biomass Toxicity</td>
<td>DOC</td>
<td>organic pollutants</td>
<td>Yes(^k)</td>
<td>Yes(^l)</td>
<td>No</td>
<td>No</td>
<td>Full scale</td>
<td>(8)</td>
</tr>
<tr>
<td>Lumped parameters</td>
<td>TPH</td>
<td>diesel fuel, jet fuel</td>
<td>Yes(^m)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Full scale</td>
<td>(1)</td>
</tr>
</tbody>
</table>

Specific uncertainties:
- Only if controls were conducted to exclude losses due to abiotic processes.
- Pristane and phytane are not completely recalcitrant. (Biodegradation has been reported)
- Only if consumption is linked to pollutant degradation.
- Only if abiotic production can be excluded.
- Only if origin from pollutant is demonstrated.
- Detoxification can only be demonstrated when complete massbalances were obtained after stoichiometric and quantitative pollutant conversion.
- Only if correlation between the increase in number of the organisms and pollutant degradation has been demonstrated.
- Only if controls were conducted to demonstrate biological basis of toxicity reduction or increase.
- Only in combination with other monitoring parameters and if increase due to biotic processes is demonstrated.
- For single pollutants the reduction of DOC can be an indicator of toxicity reduction. For pollutant mixtures it cannot be used without additional standardization with toxicity assays or detailed analytical characterization and quantification.

While the selection of a "good" parameter set is very important, its practical utility is mostly dependent on the quality of data and information provided by the monitoring method used. In Table 2 some bioanalytical methods are compiled with respect to their utility as indicators for population size, population composition, specific catabolic activity and pollutant bioavailability.
Table 2: Analysis of selected bioanalytical monitoring methods for microbial population size, specific population composition, specific catabolic activity and pollutant bioavailability. (Modified from Ref. 5).

<table>
<thead>
<tr>
<th>Bioanalytical monitoring method</th>
<th>Indication of application status</th>
<th>Application status</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct microscopic counts (AODC)</td>
<td>Yes, No</td>
<td>No, No</td>
<td>Numerous environmental applications (9)</td>
</tr>
<tr>
<td>Plate counts: total heterotrophs</td>
<td>Yes, No</td>
<td>No, No</td>
<td>Full scale (1)</td>
</tr>
<tr>
<td>Selective plate counts</td>
<td>Yes, No</td>
<td>Yes, No</td>
<td>Full scale</td>
</tr>
<tr>
<td>Monoclonal antibodies with AODC</td>
<td>Yes, No</td>
<td>Yes, No</td>
<td>Numerous environmental applications (9)</td>
</tr>
<tr>
<td>Colony hybridization</td>
<td>Yes, Yes</td>
<td>No, No</td>
<td>Demonstrated in contaminated environmental matrices (14)</td>
</tr>
<tr>
<td>Direct DNA extraction</td>
<td>Yes, Yes</td>
<td>No, No</td>
<td>Demonstrated in contaminated environmental matrices (14)</td>
</tr>
<tr>
<td>Direct RNA extraction</td>
<td>No, Yes</td>
<td>Yes, Yes</td>
<td>Demonstrated in contaminated environmental matrices (2)</td>
</tr>
<tr>
<td>Bloluminescent reporter bacteria</td>
<td>No, No</td>
<td>No, Yes</td>
<td>Demonstrated in contaminated environmental matrices (4)</td>
</tr>
<tr>
<td>Respirometry</td>
<td>No, No</td>
<td>Yes, Yes</td>
<td>Bench scale (18) Field scale (6)</td>
</tr>
<tr>
<td>Radio-respirometry</td>
<td>No, No</td>
<td>Yes, Yes</td>
<td>Biotreatability studies (14)</td>
</tr>
</tbody>
</table>

Controls

The application of controls in combination with specific monitoring parameters allows to discriminate between general aspects such as abiotic and biotic processes or between treatment related and non treatment related pollutant disappearance. The basis for the control is the selection of an appropriate reference condition to which the actual test condition is compared to. Ideally, the two should be identical for all but one critical variable which is to be investigated. Table 3 provides a general and systematic protocol for the combination of reference and test conditions in order to obtain specific information on processes causing pollutant disappearance. Different types of control strategies may be used: The application of replicate experiments or plots provides a useful approach even at the full scale level as has been exemplified during the Exxon Valdez oil spill (13). If the establishment of replicate plots becomes technically difficult or economically unrealistic, dynamic control studies involving the sequential application of different treatment regimes and/or the use of conservative tracers can provide analogous information (16).

Clearly, the number of controls conducted should be reduced to a minimum with increasing process scale. However, the general principles presented in Table 3 can be adopted, irrespective of process scale. This is illustrated in Table 4 based on practical examples of control designs reported in the literature.
Table 3: Generalized protocol for the design of controls and relevant information obtained from combinations of different reference and test conditions: Discrimination between abiotic and biotic, natural, and treatment related pollutant disappearance; effects of treatment and pollutant on ecosystems. (Modified from Ref. 5)

<table>
<thead>
<tr>
<th>Test condition</th>
<th>Non-contaminated</th>
<th>Contaminated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biotic</td>
<td>Abiotic</td>
</tr>
<tr>
<td>Condition alone</td>
<td>no treatment</td>
<td>no treatment</td>
</tr>
<tr>
<td></td>
<td>with treatment</td>
<td>with treatment</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

- Table 4: Controls at different process scales: Selected examples reported in the literature that illustrate the utility of Table 3.

<table>
<thead>
<tr>
<th>Combination of reference and test condition</th>
<th>Process scale</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>Bench scale</td>
<td>(18); (13)</td>
</tr>
<tr>
<td>A6</td>
<td>Bench scale</td>
<td>(19)</td>
</tr>
<tr>
<td>B6</td>
<td>Bench scale</td>
<td>(18)</td>
</tr>
<tr>
<td>D4</td>
<td>Bench scale</td>
<td>(12)</td>
</tr>
<tr>
<td>D6</td>
<td>Bench scale, pilot scale</td>
<td>(18); (12); (16)</td>
</tr>
<tr>
<td>E6</td>
<td>Bench-, pilot- and full scale</td>
<td>Q 8); (19); (4); (13)</td>
</tr>
</tbody>
</table>

a This combination is important for the discrimination between pollutant and treatment related effects and between mmu-sl and treatment related effect on the monitoring parameters O₂ consumption or CO₂ production.
Uncertainty Reduction and Monitoring Design

Figure 3 illustrates the general contribution of the three monitoring design elements, monitoring parameter, control and statistical sampling to the reduction of uncertainty, with respect to the demonstration of treatment and bioremediation efficacy. Uncertainty is expressed as the information quality provided for each efficacy criteria. It is obvious that mere selection of monitoring parameters or its combination with a statistical sampling strategy does not provide sufficient information for all the efficacy criteria to be answered. In contrast, a combination of monitoring parameters with specific controls can provide a qualitative demonstration of bioremediation efficacy. However, for the quantitative demonstration of efficacy, it is a prerequisite to combine monitoring parameters, controls and statistical sampling.

The diagram presented in Figure 3 may be used to evaluate different bioremediation processes or monitoring designs for their utility to demonstrate treatment and bioremediation efficacy.

Figure 3: Monitoring design and uncertainty reduction for the demonstration of bioremediation efficacy: General contribution of the design elements, monitoring parameter, control and statistical sampling. Uncertainty is expressed as information quality.
Discussion and Conclusion

A conceptual framework for the systematic establishment of a monitoring design along a cascade of critical questions for treatment and bioremediation efficacy was developed. Generally applicable elements of the monitoring design include the selection of monitoring parameters, controls and statistical sampling. For the successful development of a design strategy, the ecophysiological characteristics of the biodegradation process as well as the site characteristics and the treatment method have to be taken into account.

The analysis of selected monitoring parameters and methods with respect to their utility as indicators for process efficacy has illustrated a significant methodological and technical framework. Its logical combination with the systematic protocol for controls proposed in this study allows the qualitative demonstration of process efficacy.

It is obvious that a rigorous monitoring design requires significant economic efforts which increase with process scale. Generally, the implementation of a monitoring design is less complex and laborious for homogeneous, contained, ex situ treatment processes than for heterogeneous in situ treatments. However, the systematic monitoring of selected aspects of an in situ bioremediation process still provides qualitative information which may contribute to the reduction of remaining uncertainties when only partial mass balances could be established.

While the demonstration of bioremediation efficacy has been a widely neglected topic, the economic aspects of treatment efficiency have received significantly more attention. However, if the environmental dimension of treatment efficiency is also to be considered, the demonstration of treatment efficacy must not be neglected. Clearly, a balance between effort, feasibility and economy has to be found, but the economics should not be the only driving force. Rather the process efficacy should also be demonstrated according to sound, scientific criteria in order to ensure the credibility of bioremediation as an environmentally sound, competitive technology. Therefore it can be concluded that a minor systematic effort during the planning and development phases of a bioremediation process can prevent major expenses of post-treatment proof of bioremediation efficacy.
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