UNCERTAINTY AND RESEARCH NEEDS IN THE AREA OF THE BIOLOGICAL RESTORATION OF CONTAMINATED SEDIMENTS

FABIO FAVA & SPYROS N. AGATHOS
DICASM, Faculty of Engineering, University of Bologna, Bologna, viale Risorgimento 2, 40126 Italy
Unit of Bioengineering, Faculty of Bioengineering, Agronomy & Environment, Catholic University of Louvain, Croix du Sud 2, 1348 Louvain-la-Neuve, Belgium

1. Background: Microbial processes in sediments and their potential role in the contaminated sediments remediation

A large array of microbial processes are taking place in aerobic or subsurface anaerobic sediments, where they are responsible for the turnover of naturally occurring organic matter and the N, P, S geochemical cycles (Kafkewitz and Togna, 1998). Oxidizing conditions generally exist only at the top few centimeters of surface sediments, providing a limited zone where oxygen is available as electron acceptor for aerobic biotransformation of organic and inorganic chemical compounds. Beneath the aerobic zone, sediments are typically dominated by a single electron acceptor process such as sulfate-reduction in marine sediments or methanogenesis in freshwater sediments. Bacteria and eukaryotic organisms are coexisting in such matrices, by strictly interacting and cooperating through complex and often not fully elucidated mechanisms. The microbial population might be responsible for the biodegradation/biotransformation of several chlorinated priority pollutants, such polychlorinated-biphenyls, -dibenzodioxins, -dibenzofurans, -phenols and -benzenes, as well as some hydrocarbons, in particular under anaerobic conditions. Under sulfidogenic conditions, it might be also responsible for the precipitation/immobilization of some toxic heavy metals (Lloyd and Lovley, 2001). These microbial processes might be in turn responsible for a significant and cost effective decontamination/detoxification of polluted sediments (Bedard and Quensen, 1995; Lloyd and Lovley, 2001; Haggblom et al., 2003). This potential becomes of special relevance when they are actively taking place

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in situ, where they might contribute to a significant mitigation of contamination (Natural Attenuation, NA) (Apitz et al., 2004). NA often results in a significant reduction of the area (volume of contaminated sediment) to be dredged or managed through suitable *in situ* physical-chemical treatments.

However, many among such observations are based on preliminary and/or incomplete/inadequate experimental evidence (Bedard and Quensen, 1995; Lloyd and Lovley, 2001). In fact, relatively little is known yet about the actual relevance of microbially mediated degradation/detoxification processes, such as those mentioned above, *in situ*, and in particular in the large number of marine contaminated habitats (Bedard and Quensen, 1995; Haggblom et al., 2003), often impacted by marked advective processes (Apitz et al., 2004). The few data coming from *in situ* monitoring (Monitored Natural Attenuation, MNA) generally indicate that microbiologically mediated biodegradation processes are slow, partial and very often constrained spatially and/or temporally (Apitz et al., 2004). Almost nothing is currently known about possible strategies/approaches suitable to efficiently and safely stimulate such processes *in situ*. These and other gaps of information on several other basic issues related to biological removal of pollutants from sediments (issues that are listed and discussed below) and on their actual relevance *in situ*, have dramatically reduced and are still adversely affecting the opportunities and perspectives of biological approaches in the management of the huge amounts of contaminated sediments.

2. Uncertainties in the area of biological treatment of contaminated sediments

2.1. UNCERTAINTIES ON BASIC ASPECTS OF MICROBIAL TRANSFORMATION OF POLLUTANTS IN SEDIMENTS

The biotransformation potential of microorganisms occurring in contaminated sediments is largely controlled by the pollutant bioavailability (Bedard and Quensen, 1995; Alexander, 1999; Haggblom et al., 2003). The value of this parameter is generally very low in sediments, in particular for highly hydrophobic pollutants, because of the generally very high content of sorbing or otherwise sequestering materials within the matrices. Microbes are also important determinants for this parameter, as they significantly contribute to the sediment redox potential, pH, precipitation and dissolution of minerals that compose the surface of sediments. Microbial degradation processes are probably able to remove the pollutants *per se* (assuming a relative abundance of competent microorganisms) and

the fraction of each pollutant loosely bound to the matrix (Alexander, 1999; Apitz et al., 2004).

Thus, a first point of uncertainty is whether we have to spend time and financial resources aiming at enhancing/speeding up the release of tightly bound contaminants out of sediments and if we can ever have an effective and biocompatible strategy to do so. Another uncertainty is related to the actual toxicity of the non-bioavailable pollutant fraction that, due to this feature, is remaining in the sediments. A further discussion of bioavailability and of its possible implications on sediment toxicity and biological restoration is available at the following web-site: http://www.sediments.org./sedmgt.pdf.

In the majority of contaminated sediments there are occurring very complex mixtures of organic pollutants, often also associated to heavy metals, which can frequently act as inhibitors of microbial activities and/or sources of highly toxic and bioaccumulable biotransformation byproducts (e.g., methylmercury generated from the microbial methylation of mercury in surface sediments) (Lloyd and Lovley, 2001; Apitz et al., 2004). Further, the metabolism of several halogenated pollutants and high-molecular-weight hydrocarbons results in the accumulation of a number of potentially toxic, recalcitrant and often more water soluble (and therefore mobile) intermediates (Bedard and Quensen, 1995; Adriaens and Vogel, 1995; Haggblom et al., 2003). The uncertainties here are: a) how and how much does the coexistence of similar or different pollutants affect the biological fate of each single pollutant in terms of biotransformation rate and extent, breakdown mechanism and accumulation of transformation intermediates; and b) how and by how much do daughter products resulting from pollutant biotransformation contribute to the final sediment toxicity. There is a need to look not just at the disappearance of priority pollutants but also at the net changes in toxic effects in sediments before and after biological treatment. In addition, a very few studies have been aimed at understanding the interactions among microorganisms directly and indirectly involved in pollutant biodegradation in real sediments (Abraham et al., 2002).

Finally, new mixtures of chemicals have entered recently the large market of personal care products. Pharmaceuticals, hormones and a number of new endocrine disruptors are detected at growing concentrations (from ng to mg/l) in several aquatic systems (Lee et al., 2003; Pedersen et al., 2005). The uncertainties here are on a) our ability to detect and characterize them and their biotransformation products in sediments in a reliable manner through the currently available analytical procedures and b) on their potential biodegradability and degradation pathways in aerobic and anaerobic sediments.

2.2. UNCERTAINTIES ON THE APPLICATION/EXPLOITING OF MICROBIAL CAPACITIES IN THE RESTORATION OF CONTAMINATED SEDIMENTS, IN PARTICULAR UNDER IN SITU CONDITIONS

The majority of information available on the biotransformation of pollutants in sediments and on the microorganisms potentially responsible for it has come from studies performed in laboratory-scale microcosms very often developed with sediments spiked with well defined target pollutants and suspended in synthetic culture media (Bedard and Quensen, 1995; Adriaens and Vogel, 1995; Haggblom et al., 2003). Thus, little is known about the biological fate of several priority pollutants in real sediments under actual site geochemical conditions. In addition, a very few studies have been aimed at a) investigating the occurrence and the main features in marine sediments of several biodegradation processes already elucidated in freshwater sediments, which markedly differ in many geochemical and biological properties from the marine ones (Haggblom et al., 2003; Apitz et al., 2004), b) developing strategies/approaches to stimulate pollutant biodegradation processes in sediments and c) understanding the interactions between microorganisms directly and indirectly involved in the pollutant biodegradation and the eukaryotic organisms, such as benthic organisms, co-occurring in real sediments.

In any case, laboratory-scale evidence does not prove that biodegradation will provide sufficient natural attenuation at the site (Apitz et al., 2004). Indeed, the coexistence of several different known and unknown pollutants along with the occurrence of lower and variable temperatures, (bio)turbation phenomena, etc, in the actual site sediment might markedly affect the occurrence and the route of biotransformation processes under *in situ* conditions (Bedard and Quensen, 1995; Adriaens and Vogel, 1995; Apitz et al., 2004). A lack of integrated metabolic knowledge within the *in situ* biogeochemical context might further contribute to making bioremediation processes unpredictable. Finally, tidal events, creeks, streams and the other features that move the sediments through transport may further adversely affect the onset and predictability of pollutant biodegradation *in situ*. Therefore, the main uncertainties are focused on a) how and to what extent the information currently available from laboratory experiments can be extrapolated and used for an effective prediction of microbial process potential and features *in situ*; b) how the spatial and temporal interactions between the large variety of native microorganisms with distinct metabolisms (reductive dechlorinators, methanogens, sulfate reducers, methanotrophic organisms) directly or indirectly involved in pollutant biodegradation can be managed and optimized in an *ex-situ* or an *in situ* treatment.

3. Needs in the area of biological treatment of contaminated sediments

Too much laboratory-scale research has been performed on spiked sediments suspended in artificial mineral media (Bedard and Quensen, 1995; Haggblom et al., 2003). We need studies performed on real contaminated sediments suspended in their own real water under laboratory conditions that closely mimic those occurring *in situ* or those under which the sediments are subjected to *ex-situ* treatment (Apitz et al., 2004). This might allow us to collect information of some relevance for predicting, when combined to lines of biogeochemical evidences (Apitz et al., 2004), the actual potential of biological processes in the final *in situ* restoration of contaminated sediments.

A lot of work needs to be done under these laboratory-scale conditions in order to collect reliable information on a) the rate, extent and mechanism of biodegradation of aged priority pollutants occurring in a representative number of both freshwater and marine sediments, and b) how these parameters might change by enhancing the bioavailability of pollutants through the increase (*via* spiking) of the concentration of these same pollutants in the same sediments or through the addition of specific nutrients, electron donors/acceptors, specialized microorganisms, etc. The microcosms thus developed need to be monitored through an integrated chemical, molecular and ecotoxicological analytical methodology, able to provide holistic information on a) the fate of the parent pollutants and of the metabolites generated from their biotransformation and the potential role of bioavailability on such parameters, b) the basic microbial processes (nitrate-, sulfate-, Fe(III) or Mn (IV)-consumption and volatile fatty acids, CH_4 or H_2 production) in progress in the sediment and the structure and key catabolic potential of indigenous microbial community and c) the toxicity of the sediment throughout the whole treatment.

These data, in turn, can be valuable in predicting: i) the potential fate of common mixtures of pollutants in different types of sediments, ii) the main background products that might be expected from their biodegradation and their impacts on the final sediment toxicity, iii) the nutrients or inocula useful for the process and iv) the possible dynamics through which the main members of the indigenous microbial community interact temporally and are potentially involved in the pollutant removal.

The last group of findings might be of great relevance in order to develop sediment-specific biostimulation or bioaugmentation strategies. However, because microbial metabolism in sediments is still poorly understood and only little information on key catabolic genes involved in such processes is available yet, for this aim there is a need to develop better,

more robust monitoring tools, including molecular and other culture-independent approaches.

More knowledge on the interactions between indigenous microorganisms and benthic organisms might be also useful. The latter organisms can be responsible for an improved pollutant bioavailability, partial pollutant biodegradation and the establishment of geochemical conditions favorable for pollutant-degrading microorganisms. On the other hand, microorganisms might support benthic organisms by removing toxic pollutants, complementing their metabolism towards useful sediment substrates.

Laboratory scale investigations performed as described above can provide a body of reliable knowledge essential for a preliminary evaluation of the actual potential/relevance of an *in situ* natural recovery (NA/MNA) for a contaminated area. As stated above, such experimental evidence does not necessarily prove that biodegradation will provide sufficient natural decontamination of the site. However, it is a key line of evidence that, properly combined with other lines of microbial and biogeochemical evidence coming from the same site (Apitz et al., 2004), can permit the assessment of the role of biodegradation in the eventual natural recovery of the site.

Information obtained from microcosm studies performed with nutrients and inocula might be also useful in the preliminary design of a site-specific biostimulation/biaugmentation strategy. Here a lot of new knowledge is needed, in terms of commercially available nutrients, electron donors/acceptors, suitable inocula and the strategies/technologies through which to efficiently incorporate them into the contaminated sediment/water system.

The site conceptual modelling is also of great relevance for a reliable understanding/prediction of the susceptibility of a site to *in situ* natural recovery (Apitz et al., 2004). Here we need monitoring which not only provides qualitative descriptions of the *in situ* processes, but that also has the potential to supply parameter values for mathematical modelling of these processes. This is crucial in order to link conceptual understanding to performance models of biotreatment.

The hyporheic zone or interface between groundwater and surface water seems to play an important role in the natural attenuation of groundwater pollutants flowing from the sediment into surface water. This zone is a unique niche created by the sediment in which (bio)reductive processes can take place. For instance several chlorinated aliphatic hydrocarbons (PCE, TCE, etc) can be dehalogenated in this zone. Heavy metals such as Cd and Zn, can be precipitated as metal sulfides (formed by sulfate reducing bacteria, SRB). However some of these zones do have higher groundwater

influx rates than others leading to shorter residence times. If the residence time is shorter than the time needed to perform the dehalogenation process, incompletely dehalogenated compounds are produced and enter the surface water. The need here is to develop site specific strategies, which might also consist of injecting redox manipulating compounds, to enhance the reactivity in the hyporheic zone.

Ex-situ treatment is successfully applied to bioremediation of PAH- and mineral oil-contaminated sediments under aerobic conditions (landfarming or bioreactors). The residual pollutant concentration represents a key limiting factor in this treatment and it is generally responsible for prolonged treatment times (Harmsen, 2004). Concentration limit values and standard guidelines for sediment reuse should be developed and applied at the international level.

Here more information on the possibility of detoxifying sediments through a biologically or chemically mediated pollutant binding to the sediment inorganic/organic components or combining biodegradation and catalyst-enhanced chemical degradation would contribute considerably to the development of improved or innovative highly effective *ex-situ* remediation strategies. Furthermore, to accomplish the same aim, it would be very useful to select biogenic, biodegradable and non-toxic pollutant "solubilizing" agents able to intensify in a totally biocompatible way the bioavailability and therefore the biodegradation of aged hydrophobic pollutants during sediment conventional *ex-situ* bioremediation (via bioreactor systems) or the pollutant mobilization during sediment washing operations (Abraham et al., 2002). This choice should be modulated by a prior assessment of the local microbial community, since, at least in some cases, addition of exogenous "solubilizing" or surfactant agents may interfere with the natural tendency of specific bacteria (e.g. *Mycobacteria*) to directly adhere on target pollutants (e.g. PAHs). The laboratory-scale microcosms approach described above should be the preferential strategy of choice for performing such investigations.

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