In situ groundwater and sediment bioremediation:
barriers and perspectives

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1. **Introduction**

Due to more than two hundred years of industrialisation and to the use and presence of dangerous substances in many production processes, Europe is facing the problem of contamination of soil, subsoils and groundwater. Soil and aquifer contamination has grown as a societal problem over the past two decades and has become a key issue for the political sphere, the business and scientific communities, and is catching the public interest. Contaminated sites generally result from the manufacturing, storage, use, and disposal of hazardous materials. It is now widely recognized that polluted sites pose threats to human health and the environment.

Recently adopted environment policy initiatives on waste, water, air, climate change, chemicals, flooding, biodiversity and environmental liability are now enforcing a strong array for protection of environmental resources against future risks. In particular, the Directive on environmental liability (Directive 2004/35/EC) creates a harmonized framework for the liability regime to be applied across the EU when contamination creates a significant risk for human health and possible damage of environmental resources. However, it does not apply to historical contamination or to damage prior to its entry into force, that still represents most frequent cases of site remediation.

As for previous soil contamination, the Commission adopted the Communication “Towards a Thematic Strategy on soil protection” in 2002 and a Proposal for a Directive of the European Parliament and of the Council establishing a framework for the protection of soil is presently under discussion. In this frame, it has been estimated that 3.5 million sites may be potentially contaminated, with 0.5 million sites being really contaminated and needing remediation. Though difficult to estimate, several studies demonstrate significant annual costs of soil contamination in the ranges of €2.4 – 17.3 billions, not including the damage to the ecological functions of soil as these were not possible to quantify.

According to the Proposal, Member States will perform a preliminary survey and subsequent site investigations to determine which sites are contaminated or not, taken from a pre-established screening list. The costs for preliminary survey, to be carried out within five years after transposition of the Directive, are estimated at about €51 million per year for EU25, followed by on-site investigations (up to €240 million yearly for the EU25 over 25 years), to finally conclude if there is indeed a significant risk to the human health or environment. If so, then the site will be classified as contaminated site and introduced in the inventory, to be monitored and remediated according to a National Remediation Programme. Such a tremendous effort will be also combined to other actions dealing with other soil threats: Erosion, Organic matter decline, Compaction, Salinisation, Landslides, Sealing, and Biodiversity decline.

In the perspective of such an enormous effort, new approaches to site remediation should be developed and implemented in order to increase the “sustainability” of remediation, both in the environmental and economic sense. Indeed, a recent survey (EURODEMO, 2006) confirmed that the Dig and Dump (D&D) and Pump & Treat
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(P&T) remain the most used approaches to soil and groundwater remediation, respectively.
On the contrary, we should move forward from such waste- and energy-intensive approaches, toward sustainable remediation approaches, in order:

- to recover natural functions and potential uses of environmental resources to be remediated (e.g., quantitative and qualitative preservation of groundwater resources);
- to minimise extraction of water and production of wastes to be disposed of;
- to favour the continued economic use of the site during remediation.

2. Inventory of contaminated sites at National Countries in Europe
A single comprehensive source of remediation activity information does not exist in Europe. So the desired information had to be compiled from different sources taking account of separate contexts. It is clear that the data derived from the differing sources is not mutually comparable. In addition, the information is incomplete, as from most sources only a portion of the existing remediation projects or technology applications is offered. So the country-wise display of projects and technologies is unrepresentative. Overall, the numbers presented in the text below cannot claim to be complete, representative, or comparable. The material contained in the next section is based on different kinds of information sources (EURODEMO http://www.eurodemo.info; Commission of the European Communities. 2005. Thematic Strategy on the sustainable use of natural resources. COM (2005) 670 final, Brussels).

2.1 Site remediation in Italy
In Italy, there are presently 57 sites whose remediation is considered to be of national interest, based on their environmental relevance (about 700,000 hectares, i.e. 2% of the country; National Programme for Site Remediation). Any remediation action at these sites has to be formally approved by the Ministry of Environment under and a national financial support of about 0.75 billions € was made available mainly for emergency containment of contamination. Many of sites from the National Programme are “megasites”, including 7 sites that are larger than 10000 ha and 30 sites larger than 100 ha). These sites include all main industrial fields and so a wide range of contaminants is present in soil, subsoil and groundwater. Many sites are in coastal areas, including harbors and lagoons; hence, the related pollution also extend to shallow sediments to be remediated too (a very rough estimate could range around 100 million tons).
Moreover, it has been estimated that about 15,000 contaminated sites of minor relevance will have to be remediated or at least monitored, with a cost of about 25-30 billions € in next 15 years.
When a groundwater is contaminated, the Italian national rule requires to take emergency safety actions, to avoid the spreading of the contaminated plume and the deterioration of nearly located sensible receptors. Due to their rapid viability,
emergency actions are usually achieved by passive (cut off walls and drainage trenches) or dynamic barriers (hydraulic barriers) and their realization brings with it the need of “pump-and-treat” (P&T). Moreover, the large use of P&T systems is due to their ease of use being based on consolidated approaches which require the adduction to a treatment plant and a final control of a localized effluent. A recent study from ENEA and “Sapienza” Università di Roma at 17 National Sites in Italy (about 1/3 of total) estimated an investment cost of 604 M€ for P&T systems, based on either hydraulic containment or impermeable walls and drains to catch the groundwater flow. 41% of the investment cost was for new and ad hoc designed “groundwater” treatment plants which average unit investment cost was around 50.000 €/(mc/h). The estimated overall flow rate was 45 Mm³/y (about 500.000 inhabitant equivalents) with an average operation cost of 2.4 €/m³. The high operation cost was mostly due to the most frequent use of physical-chemical treatment and very low threshold levels, even though reinjection of treated groundwater was seldom preferred. The time frame of P&T systems was usually undefined.

2.2 Site remediation in Austria
The information source used in Fig. 1 is the Register of remediated contaminated sites (http://www.umweltbundesamt.at/umweltschutz/altlasten/altlasteninfo/), a register operated by the Umweltbundesamt in Austria, which contains around 70 remediation activities undertaken with financial support from the Contaminated Sites Remediation Act (ALSAG), a funding programme from the Lebensministerium in Austria.

![Technology applications in Austria](image)

Fig. 1: number of remediation technology applications in Austria

It can be seen that Dig & Dump (D&D) is a dominant remediation component in the ALSAG execution. All other methods have few applications in comparison. Regarding innovative treatment, Austria does not have significant experiences, but one PRB application is generally regarded as very successful (see e.g. http://www.rtdf.org/public/permbarr/prbsumms/profile.cfm?mid=88).
2.3 Site remediation in Germany

The information sources used in Fig. 2 was the German Referenzkatalog Altlasten / Schadens-fallsanierung (RefAS) ([http://www.xfaweb.baden-wuerttemberg.de/alfaweb/index.html](http://www.xfaweb.baden-wuerttemberg.de/alfaweb/index.html)). The RefAS catalogue contains around 1000 remediation projects with different technology applications and this catalogue was published in 1995 in order to enable remediation planners to use experience from projects with comparable specifications.

The numerous remediation activities in Germany result to a great extent from the industrial past of the country and an early start of remediation activities. Here it can be seen that D&D, biological, and physical methods are mostly applied. Pump and Treat (P&T) and thermal methods have also been often applied. The overall high application numbers suggest that the treatment methods that have been used are rather well developed and used with confidence. Regarding thermal methods, some innovative in-situ applications have been reported. Additionally, some Permeable Reactive Barriers (PRB) projects exist in Germany ([http://www.rubin-online.de](http://www.rubin-online.de), [http://safira.ufz.de](http://safira.ufz.de)), where experience has been gained.

2.4 Site remediation in United Kingdom

The information source used in Fig. 3 was a remediation status survey article undertaken in 2005 with the aim of ascertaining opinions and facts on remediation practices (Henstock, J. 2005. “CL:AIRE Contractor-Consultant Remediation Status Survey 2005“. In: Brownfield Briefing (Newzeye publication). Issue 4 Remediation Solutions, Issue No. IV, pg. 23, 2006). The number for the D&D applications is derived from the article which states that around 41% of undertaken remediation activities have a D&D component.
The CL:AIRE remediation survey reveals, that besides the conventional methods (D&D, P&T) used, there is a high amount of biological measures undertaken in the UK. Moreover, all considered treatment technologies have been applied recently to some extent. The given numbers suggest that the UK has a broad experience in remediation.

2.5 Other Information
A very interesting material could be found in The Netherlands, where 74 full-scale in-situ remediation projects have been documented in the course of a European project called Case Based Reasoning (see Leijnse et al. CBR – Case Based Reasoning: hidden soil knowledge unveiled. Learning from finished in-situ remediation projects. Stichting Kennisontwikkeling Kennisoverdracht Bodem, Final report projectnr. SV613, Gouda, 2004). This database was developed with the aim to design a tool with which new in-situ projects can be designed based on the experience from already performed projects. In this database, more than 60 in-situ bioremediation technology applications are reported which shows a high interest in this method in The Netherlands.

Another interesting source of information is the Eurodemo demonstration project database (EUGRIS. 2006. Eurodemo Remediation Demonstration Project list. Hosted by EUGRIS at: http://www.eugris.info/eurodemo192192/eurodemoMainProjectlist.asp). This public database was initiated by the EC funded project Eurodemo and it is geared to collect information on innovative demonstration projects in Europe with “demonstration” meaning “post-pilot, full scale implementation of a technology (…)”. It implies that the remediation approach is not yet commercial. The data in this database is supplied by volunteering reporters who can here promote remediation projects in which they are involved. In Fig. 4, 36 demonstration projects (out of around currently 50 pilot and demonstration projects) reported in the Eurodemo demonstration project database are displayed in order to show that innovative efforts are being undertaken in several European countries. As the database has just recently been published (May
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2006), only a few projects are contained herein. However, the reporting to this database shows that international experience exchange and knowledge transfer is desired by people involved in remediation.

![Demonstration projects in European countries](image)

**Fig. 4**: number of remediation projects in European countries

### 2.6 Conclusion

Figs. 1 to 3 show that a variety of methods is applied throughout the illustrated countries, but with a clear dominance of conventional methods. Regarding Germany and the United Kingdom, the relative amount of D&D, P&T, and biologic applications is very similar. But apart from that, the relative distribution of technology applications seems to be different throughout the countries, indicating that the individual countries have rather individual remediation experience. Nevertheless, innovation appears to be an important topic for several countries, as the Dutch information and the demonstration project information show.

Making national remediation efforts and especially innovative remediation efforts visible and accessible on a European level would support international experience exchange and transnational knowledge transfer. Thus, planned remediation activities and especially innovative applications – often connected with high learning costs – could be optimized with regard to improved technology performance and more sustainability with optimal cost-efficiency.

By better connecting European remediation efforts and thus minimizing the duplication of efforts, an overall increased effectiveness of remediation activities and a faster advancement of innovative technologies could be achieved to the benefit of all involved parties. Finally, the competitiveness of European technologies could be strengthened in a global market and a European State-of-the-Art in remediation could be approached (Commission of the European Communities. 2004. Stimulating technologies for...

3. Conventional vs. sustainable remediation of contaminated groundwater

3.1 Conventional plume management through P&T

In almost all sites, a groundwater contaminated plume is formed as a result of the contamination. In order to prevent potential targets (e.g., drinking water wells) from being impacted by the contamination a plume containment and or remediation system needs to be put in place. For several reasons this is most commonly realized by means of a “Pump & Treat” (P&T) approach, whereby the extracted groundwater is usually treated in physical-chemical units based on air stripping, activated carbon adsorption, etc…

P&T approach has several drawbacks:

- it is often associated with a high request of energy and treatment costs (pumping, adsorbents, regeneration); it is also noteworthy that presently used processes for treatment of contaminated groundwater are mainly an adaptation of processes previously developed for industrial wastewater. As an example, for chlorinated compounds, this is basically achieved through activated carbon adsorption (either directly on the water stream or on the stripped gas stream) which has a very high operating cost and requires post-treatment step to actually “destroy” the chlorinated compounds.

- Contaminants can be strongly sorbed onto solid phase or even present as a separate phase (Light or Dense Non Aqueous Phase Liquid, LNAPL or DNAPL, respectively), i.e removal rate is mostly controlled by the slow dissolution kinetics and the P&T system has to be maintained active for long time. It is also worth noting that the long required operational times are hardly considered during the design and cost analysis of the P&T systems.

- With respect to the final destiny of the treated water, reinjection is not usually the preferred alternative as well as reuse is not always feasible due to stringent regulatory issues. Most frequently, the “treated” groundwater is discharged into surface waters or into the sewage, for which less stringent discharge limits are typically permitted. In other words, P&T cannot preserve the groundwater as a quantitative resource potentially available for human uses.

A lot of attention has been also to in-situ treatments, where the remedial action is carried out directly below the ground, acting either on the contaminant plume or directly on the contamination source. These treatments offer potential advantages concerning with lower operating costs (due to lower request of energy), the absence of external effluents to be treated and discharged as well as lesser disturb to the original use of soil. Moreover, usually in-situ treatment allows an effective degradation of contaminants
instead of simple phase transfer (as usual in most P&T treatments like stripping and/or adsorption). During last years, most scientific research has been dedicated to development of reliable in-situ technologies, including biological, chemical and thermal treatment processes.

On the other hand, chemical or even biological in-situ treatments may cause the possible onset of secondary contamination (i.e. accumulation of intermediates as toxic or parent compounds) and present difficulties in reaching the requested Low Threshold Values (MCLs). Moreover, in-situ treatments require a deeper knowledge of local conditions because designing and monitoring standardized protocols and methodologies are not available so far. For this reasons, in-situ technologies suffer of a lack of “cultural” consensus from the administrative audience and local authorization is sometimes problematic.

3.2 Outside Europe – The US experience from a EPA survey of National Priority List

An US EPA inventory of groundwater remedial actions undertaken at 877 sites from the National Priority List (relative to the period 1982-2005) reveals that, also overseas, P&T is the most commonly adopted remediation approach. Notably, 83% of the considered sites include a P&T system along with other approaches (e.g., Monitored Natural Attenuation; In situ remediation), while in around 55% P&T is the only approach being implemented. It is also worth noting that, in spite of the fact that the survey covered a period of over 20 years, out of the 725 P&T projects, 521 (72%) are still operational, and only 73 (10%) have been completed and ultimately shut down.
In spite of that, however, a new trend in groundwater remediation has emerged in the US after 1997, showing a substantial increase in the application of in situ remediation approaches and a gradual, yet continued, decline of those based on P&T (Figure 6).
Figure 6. A) Trends of distribution of remediation technologies applied in the US (1982-2005) at 877 sites from the National Priority List. (B) Trend of in situ treatment

Among in situ technologies, in most recent years bioremediation and chemical treatment were overcoming air sparging as top in situ remedies. In particular, bioremediation is presently applied for most contaminants and in the period 1982-2005, it was the first or second choice among in situ treatments (see Figure 7)
3.3 Potentials and barriers for further implementation of in situ technologies

As above reported, in situ technologies are good candidates to replace D&D and P&T, everywhere possible, because they usually:

- do not require external treatment and water discharge
- require no or less pumping energy
- cause minimal disturbance of land use
- are often based on effective degradation of contaminants, not only on phase transfer
- can usually be more oriented toward the treatment of the source rather than the plume
can also be effective on separate phases (e.g. source treatment of chlorinated DNAPLs)

On the other hand, some possible drawbacks are:

- it may be difficult to reach very low threshold values (MCLs)
- “injection” of substances into groundwater is often required, with specific limitations
- caution about possible secondary contamination is necessary (e.g. toxic intermediates or side-products)

More in general,

- The implementation of in situ technologies is quite more site-specific and requires very good expertise on underlying processes as well (so called knowledge-intensive approach).
- Often no standardised design methodology is available and pilot-scale field tests are required for appropriate design.
- Because field tests often require specific permissions, a preliminary agreement has to be reached among managers, technician and public authorities
- Appropriate design of in-situ technologies is more time-consuming and expensive than usual approaches as well as overall remediation is often longer.

As a result, in situ technologies still suffer of a lack of general consensus and regulatory permits are more difficult to obtain. In order to overcome these possible drawbacks, any contaminated site should be considered and dealt with as a “case study”, where best scientific and technical knowledge should be used. In general, this target needs to obtain the most accurate “up-stream” knowledge:

- to perform site-sensitive characterisation and to fit it to the processes under evaluation,
- to combine techniques (chemical, geophysical, microbiological, isotopic)
- to look for secondary sources (e.g. DNAPL) and to try to act as closest as possible, in order to minimise volumes under consideration
- to calibrate the process kinetics to the rate determining step (e.g. dissolution or desorption from separate phases) and to take advantage of downstream natural attenuation processes.

As for evaluation of “down-stream” impacts

- to try to preserve natural conditions (organic matter, soil texture, biological activity) as much as possible
- to evaluate all potential impacts (individuation of possible toxic intermediates, side- or end-products, ecotoxicology tests)
- to appropriately calibrate monitoring to remediation targets and potential impacts.

Of course, such a general approach needs to be detailed and tuned to different matrixes, contaminants and technologies under evaluation. As an example, some in situ technologies, such as soil vapour extraction, air sparging, and in-well stripping, are well
established and usually require simpler and less case-sensitive design. However, these technologies are actually based on in-situ phase transfer of contaminants and on-site treatment of gas phase, i.e. they suffer, to a more limited extent, of some drawbacks of P&T approach. Similarly, other emerging technologies, such as soil flushing and in-situ thermal treatment, are mainly based on further acceleration of in-situ phase transfer and on-site treatment. So, they require very good comprehension of physical-chemistry, geochemistry and hydrogeology aspects under strongly modified conditions. Finally, other techniques (such as chemical oxidation, electrochemical remediation, bioremediation) are based on induction and/or enhancement of in-situ degradation of contaminants and also require full comprehension of in-situ degradation mechanisms, including modifications that are caused on the environmental matrixes to be remediated.

In general, all techniques where chemical substances have to be added are considered with much caution because possible negative modifications of aquifer and soil natural conditions. On the other hand, it is obvious that the above reported techniques are quite different with respect to induced modifications because thermal or chemical techniques can in principle be supposed to cause stronger modifications than biological techniques. As a golden rule, techniques that simply accelerate transport and degradation mechanisms that are “naturally” occurring should be preferred (i.e. enhanced natural attenuation). Moreover, long actions should not necessarily considered as negative, if coherently tuned to down-stream environmental protection and to present or future site use.

From this short summary, it is evident that the concept of “sustainability” in the field of site remediation still requires to be detailed and comparative evaluation of techniques mainly remain an empirical exercise. Under FP7, several research actions have been taken in this context and include coordination and demonstration programmes on innovation on remediation technologies, as well as development of certification procedures for advancement of environmental technologies and expert systems (see below).

4. In situ bioremediation of contaminated groundwater and sediments

Bioremediation, i.e. the destruction, detoxification, or immobilization of harmful compounds by living microorganisms, has gained wide acceptance as a viable, environmentally friendly, and cost-effective alternative to physico-chemical cleanup options. The remarkable metabolic versatility of microorganisms makes bioremediation applicable to treat a large and ever-increasing number of environmental pollutants such as pesticides, industrial chemicals, jet fuel and gasoline, and metals. Even compounds that were once believed to be recalcitrant, such as chlorinated solvents, polychlorobphenyls (PCBs), methyl ter-butyl ether (MTBE), and other stable synthetic organics have been shown to be degraded by microorganisms.
The underlying principle for bioremediation is to enhance pollutant degradation/ transformation/ immobilization kinetics through stimulation of the growth and metabolism of microorganisms that are involved in the bioremediation processes. Hence, the key feature of in situ bioremediation systems is that the aquifer is used as the biological reactor for contaminant biodegradation, under natural or enhanced conditions.

Some of the successful approaches have been based on addition of nutrients or electron donors/ acceptors, on manipulation of environmental parameters such as pH, redox state, or temperature, or by increasing contaminant availability. In some other cases, pollutant degradation was increased through exogenous introduction of microorganisms harboring desired metabolic capabilities (bioaugmentation). In the following, a few examples are given for some relevant cases.

4.1 The case of chlorinated solvents

Chlorinated aliphatic solvents (chlorinated methanes, ethanes, ethenes) are a large family of compounds that are used in several industrial applications (chemical cleaning, dry cleaning, textile dyeing, solvent formulations etc…). Due to improper use and disposal practices, chlorinated aliphatic solvents are among the most common organic contaminants of soil and groundwater throughout Europe.

Chlorinated solvents are highly toxic and some are also carcinogenic, so their presence in groundwater is considered not to be acceptable (or at least potentially critical) even when at very low levels. A key feature of chlorinated aliphatic hydrocarbons is their tendency to occur as dense non-aqueous phase liquids (DNAPLs). DNAPL released to the subsurface can migrate through the soil and reach the groundwater table. Then, an aliquot of DNAPL (referred to as “free” or “mobile” DNAPL) move downward through the aquifer and eventually forms “pools” of free liquid phase on low permeability surfaces, such as clay layers. DNAPL movement is more often controlled by gravity than by hydraulic head in the groundwater. Therefore, a thorough understanding of site geology and hydrogeology is needed before likely locations and pathways of DNAPL movement can be identified. Both the residual and mobile DNAPL act as long-term sources of groundwater contamination, in that they slowly dissolve into the water path, often generating large contamination plumes. Clearly, a full comprehension of the environmental factors affecting the fate of chlorinated solvents in subsurface environments, as well as the role they play at the local scale, is necessary in order to eventually implement appropriate remediation actions.

In the aquifer, chlorinated aliphatic solvents can be transformed via several reactions either abiotic (chemical reactions) or biotic (biological reactions), the latter being usually predominant.

Because of the electronegative character of chlorine atoms, polychlorinated aliphatic compounds often behave as electron acceptors (oxidants) and are reductively dechlorinated in the reaction. The two main microbiologically-mediated reductive
dechlorination reactions are: 1) hydrogenolysis and 2) dichloroelimination. Hydrogenolysis, often simply known as reductive dechlorination (RD), involves the replacements of chlorine with hydrogen with a net input of 1 proton and 2 electrons. As an example, the reductive dechlorination of Perchloroethylene (PCE) to ethene proceeds through a series of hydrogenolysis reactions, where trichloroethene (TCE), cis-dichloroethene (cis-DCE), and vinyl chloride (VC) are typical intermediates. Dichloroelimination has been observed only on sp^3 hybridized vicinal carbon atoms (e.g. chloroethanes) carrying a halogen substituent each. The reaction results in the replacement of the chlorine substituents and the formation of a double bond between the two carbon atoms with a net input of 2 electrons. Chloroethanes also undergo dehydrochlorination, an abiotic reaction, that consists in the removal of a halogen from one carbon atom and concomitant removal of a hydrogen atom from an adjacent carbon. This reaction converts a chlorinated alkane into a lesser chlorinated alkene. Dehydrochlorination is not a reductive reaction and does not require the input of electrons. Clearly, elucidation of factors controlling the occurrence of these different reaction pathways under field conditions is crucial because of differing toxicity, mobility, and persistence of the intermediate daughter products.

Several bacteria have been isolated that can couple the reductive dechlorination of chlorinated aliphatic compounds to energy conservation and therefore are of great interest for bioremediation of chlorinated solvent sites (El Fantroussi et al., 1998 Biotechnol. Progr. 14: 167; Häggblom et al., 2003 Adv. Appl. Microbiol. 53: 61; Maphosa et al, 2010 Trends Biotechnol 28: 308). These microorganisms differ in their electron donor requirements, kinetics, end-points of dechlorination, and maximum concentration of chlorinated solvent tolerated. Several strains are quite restrictive in terms of electron donor requirements, such as Dehalobacter and Dehalococcoides that can only utilize H_2. On the other hand, other strains (Dehalospirillum, Desulfitobacterium) are quite versatile utilizing a broad spectrum of electron donors. Desulfomononas spp. are unique since they are the only strains that can utilize acetate as electron donor for PCE dechlorination. Remarkably, only members of the genus Dehalococcoides seem to be able to drive the dechlorination of chloroethenes to harmless ethene.

Presently, microbial dechlorination is the one of the most promising approach to in situ remediation of chlorinated solvents in contaminated groundwater. A survey of 93 sites worldwide (mostly USA, but also UK, The Netherlands, Japan) has been published by the Environmental Security Technology Certification Program, ESTCP (www.estcp.org), where enhanced in site anaerobic bioremediation has been applied or is presently ongoing (21 sites at full scale, 9 sites at pilot and full scale, 59 sites at pilot scale). In situ enhanced RD was accomplished by stimulating the activity of native dechlorinating populations through the addition of electron donors to provide the electrons required for RD. Several substrates were used as the electron donors, including single soluble compounds (14 lactate, 3 butyrate, 3 acetate, 6 others), soluble
or emulsified mixtures of substrates (15 molasses, 10 vegetable oils), slow-release soluble polymers (35 HRC®, a polylactate-based commercialized by Regenesis Inc.), organic solids (3 mulch, 1 chitin), and gas (3 molecular hydrogen). Of course, each type of substrate required the appropriate and different delivery systems.

Several protocols have been proposed to drive the evaluation processes and the preliminary design in the perspective of in situ enhanced bioremediation through RD. As an example, the RABITT protocol (Morse et al., 1997) is based on preliminary site assessment by using a ranking system, that includes contaminant, geochemical and hydrogeological data. Hydraulic conductivity is considered as the most important parameter to understand whether substrate addition can be appropriately managed.

Then, a treatability study has to be performed by using microcosms and/or field studies. A similar approach is also advised in “Principles and practice of enhanced anaerobic bioremediation of chlorinated solvents” (available on-line at www.estcp.org), where a preliminary screening system is based on “red flags” (occurrence of negative conditions that exclude effective application of RD).

More in general, a typical flow-sheet of evaluation procedure that should be adopted for application of RD is presented in Figure 8.

**Figure 8.** In situ RD evaluation flow-chart.

The available knowledge on the environmental factors affecting the RD process is not typically sufficient to predict the likelihood of success of an in situ bioremediation treatment. Ad hoc microcosms studies (possibly conducted under conditions that more closely resemble those occurring in situ) and field tests need to be performed. Microcosms are particularly useful:

- to verify the presence of native microorganisms able to dechlorinate and their possible end-products
• to individuate the type and optimal amount of electron donor and/or growth factors to add
• to evaluate the influence of competing metabolisms and their effects on substrate dosage
• to evaluate possible effects of co-contaminants and other substances in the groundwater (e.g. inhibition), including possible accumulation of toxic intermediates or side-products
• to verify the need and the effectiveness of bioaugmentation with specialized inocula.

Although more expensive and time-consuming, small-scale field tests should be also conducted along with microcosm studies to assess the potential for in situ bioremediation of chlorinated solvents at specific sites, particularly when the presence of a DNAPL is anticipated. As a pilot test evaluates a much larger, more representative volume of the aquifer, it make possible that microorganisms grow and become more active and widely distributed in the treatment zone, even if they were initially present in only a relatively small portion of the aquifer. In this respect, indicative information about the costs of both field tests and microcosms studies obtained from the application of the RABITT protocol at different sites have been recently published (available online at ESTCP website). This documented indicated a total cost in the range of 84-111 K$ or 124-154 K$ for a field test involving a 10 m or 60 m drilling, respectively. As for the microcosm studies, the document indicated a total cost in the range of 77-94 K$ (10 m depth of drilling) or 94-111 K$, (60 m depth of drilling). In order to be successful, these field tests need a research-quality approach including careful design, operation, and monitoring. Moreover, the design of field test is quite more variable than microcosm procedure, depending on remedial target, aquifer characteristics, and chosen substrate (either soluble or solid substrate). Dealing with the addition of soluble electron donors, a small-scale field test is usually based on a pilot-scale hydraulic system which is used to extract groundwater and re-inject it, after amendment in a mixing chamber. The design of the field test should be aimed at creating a hydraulically controlled reaction volume in the aquifer; controlling residence time in the reaction volume; obtaining good mixing of contaminated groundwater with substrates and good distribution of amended groundwater in the reaction volume, and carefully monitoring inside and outside the test area. The choice of the system arrangement should be strictly dependent on aquifer characteristics. Appropriate extraction flow rates (usually in the range of L/min) and field scale (usually 10-20 meters) have to be chosen in order to create the appropriate hydraulically-controlled reaction volume in the aquifer and to control residence time in the reaction volume (based on microcosm results and at least of 30 days). A conservative tracer test should be usually performed before adding substrates along with hydrodynamic modeling in order to confirm good control of the fluid dynamics of the system as well as good substrate distribution. Where the choice of an extraction-re-injection system is hindered by local constraints such as regulatory permits, internal
recirculation in a single well can be also performed. In general, field tests provide a greater level of confidence in estimating the in situ extent and rate of dechlorination, and provide more information for design purposes (e.g., injection well spacing, injection pressures and frequency, substrate loading requirements). In particular situations, field tests may preclude the need for laboratory studies. On the other hand, they are usually done with a single pre-chosen substrate that could have better optimized by a microcosm study. Moreover, field tests require a large array of caution procedures to avoid that malfunctioning will increase any environmental and health risks (sometimes, including physical containment of test area); thus specific permits are required and are often the rate determining step of the overall design procedure. These permits could be more easily obtained and some cautions avoided in the presence of clear and good microcosms results.

Given the high specificity of the microorganisms responsible for the RD processes, the application of molecular methods is increasingly being considered (in addition or alternative to microcosm studies and field tests) to qualitatively assess the “potential” for in situ bioremediation of chlorinated solvents. There is an increasing interest in many molecular techniques and combinations of these techniques are applied in scientific research for the identification and quantification of members of dechlorinating microbial communities. However, to date, PCR (Polymerase Chain Reaction), qPCR (quantitative PCR) and PLFA (Phospholipids fatty acids) analysis, are the only molecular tools on practice use at field level on a somewhat regular basis. Certainly there have been field applications of other techniques but mostly in the context of field research, not as a routinely adopted monitoring or assessment tool. Recently, in situ methodologies, such as FISH (Fluorescence In Situ Hybridization) and CARD-FISH (Catalysed Reporter Deposition-FISH), that give additional information on activity and on the actual biodiversity and structure of the microbial communities, have been shown to be reliable and easily applicable tools for the molecular monitoring of known dechlorinators present in both laboratory bacterial enrichments and contaminated sites.

While use of these tools is still quite limited, it is evident that their application will grow, especially as evidence for the value added increases and as protocols and methodology become more standardized and automated. However, because the lack of an elective molecular tool, the use of multiple molecular approaches is recommended to get multiple lines of evidence especially for the analysis of site samples. An overview of the main available techniques utilized for the molecular identification of dechlorinating microbial communities, the related obtainable information and their current applications, is reported in Table 1.
Table 1 Main molecular tools for microbiological characterization of dechlorinating microbial communities.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Obtainable information</th>
<th>Current application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct and nested PCR (16S rRNA gene)</td>
<td>Qualitative: presence/absence of 16S rRNA genes of interest.</td>
<td>F*</td>
</tr>
<tr>
<td>qPCR (16S rRNA gene; mRNA; functional genes)</td>
<td>Quantitative: presence of specific organisms of interest.</td>
<td>F</td>
</tr>
<tr>
<td>Clone library (16S rRNA and functional genes)</td>
<td>It provides data on the gene biodiversity. Very useful for FISH probe design.</td>
<td>R§</td>
</tr>
<tr>
<td>DGGE</td>
<td>Qualitative: presence/absence of 16S rRNA and/or functional genes of interest.</td>
<td>F, its application is declining</td>
</tr>
<tr>
<td>FISH</td>
<td>Quantitative: information on activity, spatial distribution and the whole biodiversity of the sample. Monitoring of individual groups of organisms and for total biomass determination; it can be quantitative.</td>
<td>R</td>
</tr>
<tr>
<td>PFLA</td>
<td></td>
<td>F</td>
</tr>
</tbody>
</table>

*F: field application  §R: research application

4.2 Contaminated Sediments

4.2.1 Introduction
Contaminated sediments pose some of the most difficult site remediation issues today. Contaminated sediments are typically the ultimate repository for contaminants in the environment as a result of runoff and deposition. As such they pose long-term sources of contaminants back to the environment after land contamination issues are identified and controlled. The environmental security of both European and partner countries is at risk due to the pervasive nature of sediment contamination of rivers, lakes and harbors.

Contaminated sediments typically reside in spatially variable and dynamic systems subject to seasonal flow variations and episodic storm events. The volume of sediments that must be managed at particular sites often exceeds one million cubic meters, dwarfing many contaminated soil sites. These sediments are also associated with equally daunting volumes of water and efforts to remove the contamination typically entails even more water. The risks associated with these sediments depend on the processes controlling both contaminant release from the sediments and the transfer to benthic, aquatic, and land-based organisms. Observations of impairments in ecological or human health can indicate potential pollution problems; however, linking
these adverse effects to contaminated sediments requires an understanding of the processes leading to exposure and uptake. In addition, the selection of cost-effective and environmentally protective remedial alternatives is dependent upon the ability to predict the risks during implementation and into the future. Due to the volume and complexity of contaminated sediment sites, few economic and effective solutions are available.

Many of the potential technologies for contaminated sediment management were initially developed to manage contaminated soils. Unfortunately, many of these technologies are difficult either to apply or impose potentially unacceptable risks when applied to contaminated sediments. Identifying, comparing, and selecting remedial options for contaminated sediment is complicated also by the multiple technologies often involved. For example, ex situ treatment or sediment disposal typically introduces a complete train of technologies, including removing material by dredging, temporarily storing or pretreating to reduce water content or volume, treating or disposing of final dredged material, and managing any residually contaminated materials. Large contaminated sediment sites generally require applying different options at different areas on-site, each containing multiple technologies. Therefore, identifying sediment management and remediation options must recognize the entire train of technologies that constitute each option and the interaction of these technologies with the natural sediment processes leading to exposure and risk. Risk reduction has been generally accepted as the metric by which various options are judged and selected. Use of this metric, however, places a premium on understanding the natural processes that link sediment contaminants to exposure and risk. Evaluating management or remedial options requires defining remedial action goals and objectives and developing a valid conceptual model of the sediment system to be remediated, an exceedingly difficult proposition in complex contaminated sediment sites.

Many of the problems with managing contaminated sediments have been recognized only in past few years as large contaminated sediments sites such as the Hudson River in the United States have come under scrutiny. In the United States, the US Army Corps of Engineers, the US Environmental Protection Agency and University consortia such as the Hazardous Substance Research Center/South and Southwest have focused attention and resources on improving the understanding of contaminated sediment processes and their management. In Europe, a loose organization of scientists and engineers, SEDNET, have led the way to organizing contaminated sediment information.

Some key topics in this sector are:

a) understanding of the key sediment issues and concerns limiting their management in Europe;

b) the tools, including the biological ones, available for sediment site characterization and analysis;
c) understanding how the pollutant fate, in situ biodegradation and transport processes influencing contaminants and the resulting exposure to human health and the environment

d) sustainable technologies for the in situ remediation of sediments and technical deficiencies in both assessment and remediation that limit our ability to effectively respond to contaminated sediment problems with in situ sustainable approaches.

4.2.2 Assessment
For the assessment of contaminated sediment, there is not one ‘best’ method available. Each specific management question requires a tailor-made solution. Chemical analysis can be used to determine concentrations of selected hazardous chemicals and then it can be checked if the concentrations exceed pre-defined standards or guideline values. Using bioassays can test the toxic effects of sediment on organisms. Through a field inventory the long-term impact on sediment biota can be investigated. These assessment methods (chemical, bioassay, field) are complementary by giving a unique answer that cannot be given by any of the individual methods by themselves. But each method also has its own unique drawbacks and uncertainties (Salomons and Brils, 2004. SedNet booklet “Contaminated Sediments in European River Basins”).

4.2.3 Management
Contaminated sediments are mostly managed in Europe and USA through dredging and dump site accumulation. Sediment and dredged-material management challenges and problems relate to quality and quantity issues. Quality issues relate to contamination, legislation, perception, risk-assessment, source control and destinations of dredged material. Quantity issues mainly relate to erosion, sedimentation, flooding, the effects of damming and the resulting morphological changes downstream. Contamination can inflict severely the management of dredged sediments. The costs for the removal of excess sediment increases when it is too contaminated for unrestricted relocation. Port managers are concerned that they have to bear the extra costs for managing contamination which is derived from contributions along the river basin. The ‘polluter pays’ principle is far from being applied. The problem is left for the problem owner and there is no link to those that have caused it. Besides complicating dredging activities per se, contaminated sediment may pose ecological risks or risks to water quality. The relation between sediment quality and risks is complex and site specific, requiring assessment methods based on bioavailable contaminant fractions and bioassays rather than results based on the traditional total contaminant concentrations. However, if sediment quality impairs the chemical or ecological status, remediation measures may be needed. An integrated approach for sediment management is presently lacking in Europe (Salomons and Brils, 2004. SedNet booklet “Contaminated Sediments in European River Basins”).
Costly end-of-pipe solutions may be unavoidable for the management of contaminated sediment and dredged-material. Depots for contaminated dredged material can be an option in this situation, but they are expensive, often lack public acceptance and are subject to complex legislation. Alternatives include treatment for beneficial use and controlled (confined) disposal. Treatment and re-use is politically encouraged, but is currently applied only at a small scale because of the higher costs compared to disposal and the lack of product markets. However, in some cases treatment and beneficial use may be a competitive alternative for confined disposal. Confined disposal will remain the first choice solution for the time being. For the realization of new confined disposal sites (both upland and sub-aquatic), public involvement and support are needed. In many cases the procedures are very time consuming (10-15 years) and/or the lack of public acceptance can complicate matters and their implementation (Salomons and Brils, 2004. SedNet booklet “Contaminated Sediments in European River Basins”). In recent years, natural attenuation has received increasing attention and it is generally accepted that microorganisms are the principal mediators of the natural attenuation of many pollutants. However, the complexity of environmental systems such as sediments requires a multifaceted approach to understand microbial processes and their potential. This is even more so under in situ conditions, where the activity of pollutant degrading microorganisms is generally slow, partial and constrained spatially and/or temporally.

Recent developments in molecular biology and genomics are offering tools to explore microbial processes at a level that encompasses the genetic characteristics of the local microbial players, culturable or not, as well as their organization into complex communities and their interactions both with each other and with the target chemicals. It is now possible to study microbes directly in their environments at the population level as well as at the single cell level and to link biology to geochemistry. Integrative knowledge from culture independent studies based on functional characters and assessment of the diversity and quantity of catabolic genes in response to pollution, will allow a deeper understanding of and a rational intervention in environmental processes. Moreover, the use of genomic libraries to retrieve genes from natural bacterial communities without cultivation will allow a breakthrough in accessing new microbial capabilities.

In this chapter, the main features, advantages and limitations of these innovative approaches to the biomonitoring and analysis of intrinsic bioremediation potential of polluted environments and sediments are critically reviewed. Then, the potential of the same strategies in the integrated chemical, physical and biological monitoring and characterization of polluted sediments subjected to natural decontamination is shown through the description of the main results of case studies performed on a) polychlorinated biphenyl (PCB)-contaminated marine sediments of the Porto Marghera area of Venice Lagoon (Italy) in which the occurrence of PCB-reductive dechlorination processes has been demonstrated for the first time in the literature, b) sediments contaminated by chlorinated aliphatic hydrocarbons (CAHs)
collected from different positions of the eutrophic river Zenne (Vilvoorde, Belgium), where they have been found to act as a natural biobarrier for the CAHs occurring in the groundwater that is passing through the sediment zone, hereby reducing the risk of surface water contamination, and c) other environmental contaminated systems subjected to ex-situ and in situ active bioremediation, where these processes are described on the basis of the experience accumulated in pilot and real-life systems (Fantroussi et al. 2006. In: REIBLE D. LANCZOS T., Assessment and Remediation of Contaminated Sediments, AMSTERDAM, Springer, (NATO Science Series - IV. Earth and Environmental Sciences - Vol. 73)

5. Present projects in the frame of FP7
In the frame of the current FP7 projects there are several project dealing with innovative technologies for the in situ bioremediation of contaminated areas. Among them there are surely AQUAREHAB, MINOTOAURUS, and ULIXES

AQUAREHAB, i.e., Development of rehabilitation technologies and approaches for multipressured degraded waters and the integration of their impact on river basin management, (web-site: http://aquarehab.vito.be/home/Pages/home.aspx) is an EU financed large scale research project (FP7) lead by VITO (Belgium) involving 19 project partners. In the framework of AQUAREHAB, different innovative rehabilitation technologies for soil, groundwater and surface water are being developed to cope with a number of priority contaminants (nitrates, pesticides, chlorinated compounds, aromatic compounds, mixed pollutions…) within heavily degraded water systems (Bastiaens & Sethi, 2011, Ecomondo, Rimini, 9-12 November 2011). Methods are being developed to determine the (long-term) impact of the innovative rehabilitation technologies on the reduction of the influx of these priority pollutants towards receptors like drinking water wells and surface water. A connection between the innovative technologies and river basin management will be worked out, with focus on groundwater as well as surface water.

The innovative rehabilitation technologies that will be studied and enhanced in the project are:
- Activated riparian zones/wetlands (diffuse pollution);
- Biostimulation and/or bioaugmentation of pesticide-containing degraded water in open trenches with smart biomass containing materials;
- Bioreactive zones in aquifer and sediments (capping) to rehabilitate surface water degraded by influx of pollutants from the groundwater;
- Multifunctional permeable barriers (multibarriers) for mixed groundwater contamination plumes;
- In-situ reduction or oxidation of hazardous pollutants in groundwater/aquifer with injectable Fe-based particles.

Further aims of the project are:

- to develop methods (feasibility tests), tools (numerical models) and guidelines to design the mentioned rehabilitation technologies and to determine their (long-term) impact on local fluxes of pollutants;
- to develop a collaborative management tool ‘REACH-ER’ that can be used by stakeholders, decision makers and water managers to evaluate the ecological and economical effects of different remedial actions on river basins;
- the development of an approach to link the effects of the rehabilitation technologies with a river basin management tool REACH-ER;
- to evaluate and disseminate the generic rehabilitation guidelines, approaches and tools by applying them to other river basins with other pollutant conditions, climates, .. in collaboration with end-users.

During the first years the following 3 river basins were selected a study areas: The Scheldt River basin (Belgium/FRance), the Odense river basin (Denmark) and the Sechor-Beser river basin (Israel). One of the outcomes of the project will be a river basin management tool that integrates multiple measures with ecological and economic impact assessments of the whole water system. The project will aid in underpinning river basin management plans being developed in EU Member States, and will demonstrate cost effective technologies that can provide technical options for nationals and local water managers, planners and other stakeholders.

The recently EU funded project MINOTAURUS, i.e., Microorganism and enzyme Immobilization: NOvel Techniques and Approaches for Upgraded Remediation of Underground-, wastewater and Soil (web-site:www.minotaurus-project.eu/) is an EU medium scale research project (FP7) lead by the University of Applied Sciences Northwestern Switzerland (Switzerland) involving 15 project partners (Corvini et al 2011. 5th European Bioremediation Conference, Chania, Crete, 4-7 July 2011).

MINOTAURUS aims to deliver an innovative set of novel environmental biotechnologies, which are all based on the concept of immobilization of biocatalysts, in order to eliminate emerging as well as classic organic pollutants. The project addresses the elimination of compounds representative of several classes of pollutants and mixtures thereof as a wise and careful approach reflecting the real problem of contamination by organic pollutants. The proposed technologies apply to both engineered (ex-situ) and natural (in situ) systems for the bioremediation of groundwater, wastewater and soil.

Among these seven technologies proposed, five are reactor-based technologies (ex situ) and two are in-situ-based biotechnologies. Among the latter, there are: a) Intensified biodegradation of highly chlorinated CAH by microorganisms immobilized on polarized solid state electrodes (cathodes and anodes) in aquifer and b) intensified
biodegradation of PCBs and polybrominated flame retardant (BPA as a degradation product thereof) by naturally occurring microorganisms and exogenous ones immobilized on the roots of halophytes in wetlands systems depolluting soil/waste- and groundwater systems. To ensure the optimal development of the technologies, each bioremediation process will be monitored and assessed using a set of technology-tailored tools. The reliability of the analytical tools will be proofed through a set of ring trial proficiency tests for the chemical, biological, and ecotoxicological monitoring. The acquisition of reliable measurements will constitute a solid basis to develop and refine our biodegradation kinetics models, which will be the mean to improve the predictability of performances to be achieved with the investigated biotechnologies. To facilitate the transfer and upscaling of the technologies, on-site testing are performed for a selected number of the technologies. The direct implementation of the most promising technologies at five EU reference sites that are confronted with hazardous pollutants has been planned with support of the five SMEs participating in the project. The four sites are: Rho (aquifer, Italy), Heraklion (Greece, wetlands for soil/waste- and groundwater systems), Birsfelden (Switzerland, wastewater treatment plant (WWTP)), and Tel Aviv (Israel, WWTP of a hospital). The fifth site will be selected in Belgium in the course of the project and will be an aquifer contaminated with MTBE and BTEX. Thus, the principal outcome of MINOTAURUS is the set up of new enhanced and site-validated biotechnological processes based on the immobilization/retention of microorganisms and enzymes to intensify the biodegradation of xenobiotics in water (groundwater and wastewater) and in soil treatment applications. Throughout the on site validation, the new technologies will be comprehensively assessed and therefore adapted for the robust, reliable and predictable continuous bioremediation of a variety of European environmental matrices.

The recently EU funded project ULIXES “Unravelling and exploiting Mediterranean Sea microbial diversity and ecology for xenobiotics’ and pollutants’ clean up” (website: http://www.ulixes.unimi.it/) aims to unravel, categorize, catalogue, exploit and manage the microbial diversity available in the Mediterranean Sea for addressing bioremediation of polluted marine sites (Daffonchio et al 2011. 5th European Bioremediation Conference, Chania, Crete, 4-7 July 2011). It is an EU financed medium scale research project (FP7) lead by Università di Milano (Italy) involving 16 project partners. The idea behind ULIXES is that the multitude of diverse environmental niches of the Mediterranean Sea contains a huge range of microorganisms and their components, e.g. catabolic enzymes or products (e.g. biosurfactant) that can be exploited in pollutant- and site-tailored bioremediation approaches. ULIXES intends to provide the proof of concept that it is possible to establish and exploit for bioremediation site-specific collections of microbial strains, mixed microbial cultures, enzymes, biosurfactants and other microbial products. These biotechnological resources are mined by using approaches based on isolation of
culturable microorganisms as well as by extensively applying advanced novel ‘meta-
omics’ technologies. Three pollutant classes recognized worldwide as environmental
priorities are considered: petroleum hydrocarbons, chlorinated compounds and heavy
metals. Through the effort of twelve European and Southern Mediterranean partner
laboratories, it is intended to explore a large set of polluted environmental matrices from
sites located all over the Mediterranean Sea, including seashore sands, lagoon
sediments, deep sea sediments polluted by heavy oil hydrocarbons at oil tanker
shipwreck sites, hypersaline waters and sediments from polluted salty coastal lakes and
natural deep hypersaline anoxic submarine basins and mud volcanoes where natural
hydrocarbon seepages occur. The mined collections of microbial biotechnological
products are exploited for development of novel improved bioremediation processes
whose effectiveness is proved by ex situ and in situ field bioremediation trials.

6. How to overcome barriers and research “hot spots”

6.1 R&D Needs in the area of in situ biological remediation of contaminated sites.
Bioremediation has a great potential to increase the sustainability of remediation of
contaminated groundwater, both from the environmental and economic point of view.
However, its potential is partially unexploited because bioremediation still suffers of a
lack of general consensus and regulatory permits may be more difficult to obtain than
for conventional techniques, such as P&T. With particular reference to in situ
bioremediation, public concerns mostly deal with possible lack of effectiveness and
control, long term design and operation, and possible secondary effects, e.g. toxic
metabolites and pathogens.
Both basic and applied research is needed to overcome these barriers and to make
bioremediation more reliable, robust and acceptable to the public awareness as well as
more competitive from the economic point of view.
Research focus should be on any process steps, i.e. “up-stream” site characterisation,
bioprocess design, operation and control, “down stream” evaluation of effects.
As for the “upstream” step, full exploitation of bioremediation potential requires to
develop tools allowing for most accurate and quick characterisation of contaminated
sites as well as for more specific evaluation of relevant bioprocesses. First of all, this in
turn requires to further develop modern biolomolecular tools for analysis of natural or
modified biocenosis and to combine them with other techniques, such as chemical,
geophysical, isotopic analysis. This research topic should also include full exploitation
of largely unexplored biodiversity at contaminated sites as well as strong effort is
needed to combine species-specific genetic tools (to describe which microorganisms are
present) to metabolic-specific ones (to describe which metabolic activities are going
on), so to better describe bioremediation potential as function of groundwater
conditions. Increased use of biomolecular tools will also help to obtain a more specific estimate of concentration and activity of relevant microbial groups, as function of natural or engineered conditions, in order to obtain more general and robust modelling of relevant microbial processes.

As for relevant bioprocesses, research should focus on how to individuate secondary sources (e.g. DNAPL) and to better understand their interactions with microbial processes; this on one hand includes effects of microbial processes on contaminant dissolution and mobility and, on the other hand, on tolerance and competition among microbial processes under high contaminant concentration. The latter point is particularly important to better fit the kinetics of microbial processes to other physical-chemical processes, i.e. dissolution or desorption from separate phases, in the general frame of underground hydraulics, any of them possibly being the rate determining step of the overall attenuation process.

Moreover, innovative technologies are still under study and show high potential, especially when allowing to minimize the need for substrate or cosubstrate supply and/or make it more specific and reliable. As an example, bioelectrochemical processes do not require any addition of electron acceptors or donors, that are substituted from the appropriate electrochemical potential; the latter can be tuned at the desired value in well defined geometry and it appears in principle a quite more flexible and robust way to drive and control relevant microbial reactions. The combination of biological reaction with conductive or semiconductive nanoparticles should be also studied to enhance microbial activity in a highly specific way. Multipurpose reactive barriers also appears an interesting approach for long-term and slow-rate release of needed substrates, that can extend downgradient barrier activity towards sustainable enhanced attenuation. Interesting results have been obtained by combining zerovalent iron with microbial polymers, either in form of permeable trench of emulsified suspensions.

As for evaluation of “down-stream” impacts, it is truly necessary that ecotoxicology tests are further developed and specifically calibrated to possible toxic intermediates, side- or end-products. This will be particularly useful at prenormative level, in order to establish a specific regulation helping to plan, conduct and control field tests for checking innovative technologies.

Anyway, field tests require permission and implies that public authorities and end users are regularly involved, especially when planning research and testing of brand new bioprocesses. In this respect, many scientists underline that it is very difficult to move from lab-scale basic research of new processes and tools to their market exploitation, at least in the usual time frame of European Research Projects (3-4 years), especially when field tests are necessary in the between and require to obtain specific permission. In this respect, an innovative 2 step application/evaluation procedure could be experimented where the 2nd step, including field test, is described in greater detail once more basic research has been performed in the 1st step.
Besides of research efforts, full exploitation of innovative technologies, including bioremediation, would anyway require that groundwater remediation is put in the more general perspective of groundwater protection. Indeed, it is necessary to strengthen the importance of quantitative recovery of groundwater resources and so the added value of any in situ techniques with respect to P&T (unless groundwater is reinjected). Moreover, milder techniques should be preferred (e.g. bioremediation), that enhance natural attenuation by simply accelerating transport and natural degradation mechanisms and that in principle cause no or less modifications than stronger and quicker techniques. This approach would in turn require that slow remediation is not necessarily considered as negative, if coherently tuned to quality standards to be achieved for protection of downgradient water bodies as well as to present or future uses of the contaminated site. In this respect, when a contaminant plume is discovered, the possibility of ancillary measures for environmental and health protection (e.g. restricted admission, alternative supply of water) should be enforced to decrease the need for P&T emergency containment while at the same time planning stronger actions on primary or secondary contaminant sources. Correspondingly, the time frame for sustainable remediation of the source downgradient plume should be extended in order to take the maximal advantage of ongoing natural or enhanced attenuation.

The introduction of LCA assessment in comparing different approaches (so also including energy, chemicals, secondary effects, etc.) could likely demonstrate how bioremediation is more sustainable than other techniques, especially if this general perspective is adopted.

6.2 R&D Needs in the area of biological treatment of contaminated sediments

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. In Biological Treatment of Hazardous Wastes, Lewandowski and DeFilippi, eds., John Wiley & Sons, Inc, USA, Chapter 12 pp. 327). 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 Curr. Opin. Biotechnol. 12: 248). These microbial processes might be in turn responsible for a significant and cost effective decontamination/detoxification of polluted sediments (Lloyd and Lovley, 2001 Curr. Opin. Biotechnol. 12: 248; Haggblom et al., 2003. Adv. Appl. Microbiol. 53: 61). This potential becomes of special relevance when they are actively taking place in situ, where they might contribute to a significant mitigation of
contamination (Natural Attenuation, NA) (Apitz et al., 2004, http://www.spawar.navy.mil/sti/publications/pubs/tr/1918/tr1918cond.pdf). 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. In fact, relatively little is known yet about the actual relevance of microbiologically-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, in: Microbial Transformation and Degradation of Toxic Organic Chemicals, Young and Cerniglia, eds., Wiley-Liss, USA, Chapter 4, pp 127), 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, http://www.spawar.navy.mil/sti/publications/pubs/tr/1918/tr1918cond.pdf). 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 (Fava and Agathos, 2006 In: REIBLE D. LANCZOS T. Assessment and Remediation of Contaminated Sediments, AMSTERDAM, Springer, (NATO Science Series - IV. Earth and Environmental Sciences - Vol. 73)).

Too much laboratory-scale research has been performed on spiked sediments suspended in artificial mineral media (Haggblom et al., 2003. Adv. Appl. Microbiol. 53: 61; Fava and Agathos. 2006. In: REIBLE D. LANCZOS T., Assessment and Remediation of Contaminated Sediments, AMSTERDAM, Springer, (NATO Science Series - IV. Earth and Environmental Sciences - Vol. 73)). 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, http://www.spawar.navy.mil/sti/publications/pubs/tr/1918/tr1918cond.pdf) as this might allow to predict the actual potential of biological processes in the final in situ restoration of contaminated sediments. Under these laboratory-scale conditions can be obtained prominent information on a) the rate, extent and mechanism of biodegradation of aged priority pollutants in both freshwater and marine sediments, and b) how these parameters might change by enhancing the bioavailability of pollutants through the addition of specific nutrients, electron donors/acceptors, specialized microorganisms, etc. (Fava and Agathos, 2006. In: REIBLE D. LANCZOS T., Assessment and
Remediation of Contaminated Sediments, AMSTERDAM, Springer, (NATO Science Series - IV. Earth and Environmental Sciences - Vol. 73)). The biodegradation processes should be then 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, 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 under in situ conditions: i) the potential fate of detected pollutants, 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 stimulating 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 final pollutant removal. The last group of findings might be of great relevance in order to develop sediment-specific biostimulation or bioaugmentation strategies (Fava and Agathos, 2006 In: REIBLE D. LANCZOS T., Assessment and Remediation of Contaminated Sediments, AMSTERDAM, Springer, (NATO Science Series - IV. Earth and Environmental Sciences - Vol. 73)).

However, microbial metabolism in sediments is still poorly understood and only little information on key catabolic genes involved in such processes is available yet. Thus, 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 (Fava and Agathos, 2006. In: REIBLE D. LANCZOS T., Assessment and Remediation of Contaminated Sediments, AMSTERDAM, Springer, (NATO Science Series - IV. Earth and Environmental Sciences - Vol. 73)).

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 (Fava and Agathos, 2006 In: REIBLE D. LANCZOS T., Assessment and Remediation of Contaminated Sediments, AMSTERDAM, Springer, (NATO Science Series - IV. Earth and Environmental Sciences - Vol. 73)).
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 (Fava and Agathos, 2006 In: REIBLE D. LANCZOS T., Assessment and Remediation of Contaminated Sediments, AMSTERDAM, Springer, (NATO Science Series - IV. Earth and Environmental Sciences - Vol. 73).

When assessing research needs and opportunities for in situ bioremediation of the subsurface (soils, groundwater, sediments), it is becoming important to consider the biogeochemical activities that control elemental cycling. The various biogeochemical redox processes taking place in the subsurface have a decisive impact upon the behavior of both inorganic and organic contaminants, including their migration and degradation (Borch et al, 2010 Environ Sci Technol 44: 15-23). Understanding and controlling these redox processes can lead to novel, hybrid (biotic-abiotic) remediation strategies such as zero-valent Fe permeable reactive barriers for groundwater treatment to remove organochlorine compounds and reductively sequester heavy metals and radionuclides, often with the help of native microbial communities stimulated by amending groundwater with an organic electron donor such as ethanol or acetate.

Some of the emerging and future research needs in this context will be addressed with new analytical and computational tools for probing the complex determinants of these redox processes (spatial and chemical heterogeneity of subsurface environments, coupled redox kinetics, microbial community dynamics, electron transfer mechanisms, redox-induced mineral and organic transformations, etc.) (Borch et al., 2010 Environ Sci Technol 44: 15-23). Novel in situ electrode and gel probe techniques are starting to give access to direct and practically non-invasive field-scale observation of redox processes. Furthermore, progress in analytical instrumentation and sample preparation will continue to enhance our ability to detect redox-sensitive or trace quantities of pollutant species in the underground environment.

7. Conclusions
In situ bioremediation is a highly promising and cost-effective technology for the clean-up of soil and groundwater in contaminated sites. It can be quite promising also in the sustainable management of contaminated sediments. The wide metabolic diversity of
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microorganisms makes it applicable to an ever-increasing number of contaminants and contamination scenarios. On the other hand, in situ bioremediation is highly knowledge-intensive and its application requires a thoroughly understanding of the microbiology, ecology, hydrogeology, and geochemistry of contaminated soils and aquifers, and sediments, under both natural and enhanced conditions. Hence, its potential is partially unexploited because bioremediation still suffers of a lack of general consensus and public concerns are still present on possible lack of effectiveness and control, long term design and operation, and possible secondary effects, e.g. toxic metabolites and pathogens. Both basic, applied and prenormative research is needed to overcome these barriers and to make in situ bioremediation more reliable, robust and acceptable to the public awareness as well as more competitive from the economic point of view. From the short summary of research topics, it is evident how multidisciplinarity is really a key point because advanced research on microbes and microbial processes acting on xenobiotics and micropollutants has to be combined with many other scientific and technical fields. Bacterial diversity is still largely unexplored, especially under “extreme” conditions to which certainly belongs severe contamination from xenobiotics, and particularly for emerging pollutants. Moreover, several innovative technologies are emerging from recent studies on how microbial activities can be linked with electrochemistry and nanoparticles. Finally, research efforts should not be restricted to a deeper understanding of relevant microbial reactions only, but also of their interactions with the large array of other relevant phenomena, as function of really variable site-specific conditions. This need calls for further development of advanced biomolecular tools for site investigation as well as of advanced metabolic and kinetic modelling. This would allow quick evaluation of available metabolic activities, at least for preliminary assessment of technical feasibility of the chosen bioprocess, thus being also useful to substitute or at least minimize the need for time-consuming and high-cost field tests. At the same time, field test will probably remain unavoidable for detailed design of full scale remediation and in this respect the above reported tools will be anyway useful for better test design and more reliable operation, also including further implementation and intercalibration of ecotoxicological tests.

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Chair of the Experts Group and of the EFB section on Environmental Biotechnology), Spyros Agathos (University of Louvain, Belgium and Vice-Chair of the Experts Group and of the EFB section on Environmental Biotechnology), Valter Tandoi (CNR, Italy and Secretariat of the Experts Group and of the EFB section on Environmental Biotechnology), Mauro Majone (University of Rome La Sapienza, Italy and Delegate of SusChem Italy), Alessandro Sidoli (Italian Association of Biotech Industry – Assobiotech, Italy), Ludo Diels (VITO, Mol, Belgium), Willy Verstraete (Ghent University, Belgium), Nicolas Kalogerakis (Technical University of Crete, Greece), Daniel Mamais (National Technical University of Athens, Greece), Philippe Corvini (University of Applied Sciences NS Muttenz, Switzerland), Hans Peter Kohler (EAWAG, Dübendorf, Switzerland), Steven A. Banwart (University of Sheffield, United Kingdom), Piet Lens (UNESCO-IHE, Delft The Netherlands), Victor de Lorenzo (CSIC, Madrid, Spain), Katarina Demnerova (ICT, University of Prague, Czech Republic), Vladimir Brenner (AECOM, Czech Republic), Venko Beschkov (Bulgarian Academy of Sciences, Sofia, Bulgaria), Katarina Dercova (Slovak University of Technology, Slovak Republic), Katalin Belafi-Bako (University of Pannonia, Veszprem, Hungary), Maria Reis (University Nova de Lisboa, Portugal), Bruno Sommer Ferreira (APBIO, Portuguese Association of Biotech Industries, Portugal), Andreas Loibner (BOKU, Vienna, Austria), Eric Trably (INRA - Laboratoire de Biotechnologie de l'Environnement, Narbonne, France), Korneliusz Miksch (The Silesian University of Technology, Gliwice, Poland), Jan W Dobrowski (AGH University Science & Technology Krakow, Poland), Maria Gavrilescu (Gheorghe Asachi Technical University of Iasi, Romania), Thomas Schafer (Novozymes, Denmark), Jens Aamand (Geus, Denmark), Rainer Meckenstock (Universität Tübingen, Tübingen, Germany), Manfred Kircher (CLIB2021, Germany), Sara Sjoling (Sodertorn University, Sweden), George O’Malley (BioRefinery Ireland, Newport, Ireland), Vladimir Popov (Russian Academy of Sciences, Moscow, Russia), Fazilet Vardar Sukman (Ege University, Izmir, Turkey), Vladimir Elisashvili (Durmishidze Inst. Biochem. Biotechnol., Tbilisi, Georgia), Hector Poggi-Varaldo (CINVESTAV-IPN, Mexico), Jose Osvaldo Beserra Carioca (Federal University of Ceará – UFC, Brasil), Haman Malkawi (Yarmouk University, Jordan), Yasser R Abdel-Fattah (Mubarak City for Scientific Research and Technology Applications, Egypt), Amur Cherif (University of Tunis, Tunisia), Pedro J. Alvarez (Rice University, Houston, TX, USA), Jose Duarte (President of International Association Mediterranean Agroindustrial Waste, IAMAW), Hisao Ohtake (President of Environmental Biotechnology section of the Asian Federation of Biotechnology -AFOB- Japan), Eliora Ron (General Secretary of European Academy of Microbiology -EAM- & Chair of BAM section of International Union of Microbiological Societies –IUMS), James Philp (General Secretariat of Task force on Industrial and Environmental Biotechnology at OECD) and Joanna Dupont (Director at EUROPABIO & Delegate ETP-SUSCHEM).