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Final thesis

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IN-SITU SOIL AND GROUNDWATER BIOREMEDIATION TECHNIQUES AND APPLICATIONS.

Supervisor: Senior lecturer Viskari, Eeva-Liisa
Commissioned by: Doranova Oy
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In-situ soil and groundwater bioremediation techniques and applications.

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Abstract

Current and previous polluting activities have caused our urban nature to become ever so more contaminated with various pollutants, some natural, some xenobiotic. Many of these contaminated sites today are located within heavily constructed urban areas where activity cannot be seized without economic losses or on areas which are already re-zoned and have pressing need for their development. Old remediation technologies are not able to offer solutions that are environmentally or economically acceptable in many of these cases and therefore the focus has to be placed on novel technologies.

The aim of this thesis is to focus on one field of these novel soil and groundwater remediation technologies, namely in-situ bioremediation, meaning biologically oriented technologies conducted in the site, without excavation and removal. Bioremediation is based on the basic principles of biotechnology and microbiology, with special focus in in-situ with site biogeohydrochemical processes and engineering principles. The technologies themselves require diverse basic background knowledge on many fields of science; therefore a multidisciplinary approach is commonly required in full scale applications.

This paper gives an overview of the main topics in and around bioremediation. Literature survey features a short overview of the role of bioremediation in the field of environmental biotechnology and its global and national economic perspectives, and presents key player in bioremediation, the micro-organisms, and their metabolism and environment requirements. The technical section defines the border conditions for a successful remediation, outlines and defines the currently acknowledged in-situ bioremediation techniques and their applicability and suggests tools for verifying and monitoring the process along quality control. In addition, a documentation of a real-life in-situ pilot scale field trial is included.

TAMPEREEN AMMATTIKORKEAKOULU
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Maan ja pohjaveden in-situ bioremediaatio tekniikat ja applikaatiot.

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Tiivistelmä

Nykyinen ja aiempi ympäristöä pilaava toiminta on aiheuttanut kaupunki-ympäristömme maaperän ja pohjaveden pilaantumisen erinäisillä kemikaaleilla, joista osa on ympäristölle luontaisia, osa vieraita. Monet näistä saastuneista alueista sijaitsee laajalti rakennetuilla kaupunkialueilla, joissa nykyistä toimintaa ei voida keskeyttää ilman taloudellisia menetyksiä tai alueilla, jotka on kaavoitettu uutta käyttötarkoitusta varten ja rakentamisen paine on suuri. Perinteiset kunnostusmenetelmät eivät kykene tarjoamaan taloudellisesti ja ympäristön kannalta kannattavia vaihtoehtoja useissa näistä tapauksista, ja siksi huomio tuleekin keskittää uusiin, innovatiivisiin tekniikoihin.

Tämä lopputyö keskittyy yhteen erityisalueeseen maan ja pohjaveden kunnostukseen keskittyvien tekniikoiden joukossa, nimellisesti in-situ bioremediaatioon, joka sisältää biologiseen toimintaan pohjaavia menetelmiä jotka toteutetaan maaperässä tai pohjavedessä, ilman maaperän kaivamista tai pohjaveden poistoa. Bioremediaatio pohjaa vahvasti biotekniikan ja mikrobiologian perusteisiin, erityishuomio on kuitenkin kunnostuskohteiden biogeohydrokemikaalisten prosessien tuntemisella ja insinöörityön perustekniikoilla. Tekniikat itsessään vaativat laajaa perustietoutta monilta tieteen aloilta ja siksi poikkitieteellistä lähestymistä vaaditaan lähes poikkeuksetta täysmittakaavaisissa sovellutuskohteissa.

Tämän työn tarkoituksena on antaa yleiskatsaus aihepiireihin bioremediaation ympärillä. Kirjallisuuskatsaus kuvaa bioremediaation roolia osana biotekniikkaa ja tarkastelee sen kansainvälisiä ja kansallisia taloudellisia näkymiä, lisäksi se luonnehtii bioremediaation avaintekijöitä, mikro-organismejä, sekä niiden metaboliikkaa ja vaikuttavia ympäristötekijöitä. Teknillinen osuus työstä määrittelee reunaehdot onnistuneelle kunnostushankkeen toteuttamiselle, sekä alustaa ja määrittelee nykyisellään tunnustetut in-situ bioremediaatio-tekniikat ja niiden käyttökelpoisuuden. Teknisen osan päätteeksi esitetään metodeja onnistuneen kunnostuksen todentamiseksi, monitoroimiseksi ja laadun varmistamiseksi. Edellisten lisäksi, lyhyt kuvaus pilot muotoisesta in-situ bioremediaatio kenttäkokeesta on sisällytetty.

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The thesis was supervised on behalf of Tampere Polytechnic by Eeva-Liisa Viskari and on behalf of Doranova Oy by Ari Laitinen. Both have shared significant amounts of their expertise and taken the time and effort to aid me in my questions concerning the thesis. For Ari I owe special thanks for numerous insightful discussions and for giving the confidence on the field that allowed me to learn more and faster.

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Last, I would like to thank my parents, brother, friends and loved one for their understanding and support, without them nothing would be possible.

Thank You!

Jarno Laitinen
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List of abbreviations

Acetyl-CoA	Acetyl Coenzyme A
ATP	Adenosine triphosphate
A_w	Water Activity
BATNEEC	Best Available Technology Not Entailing Extensive Costs
BS	Biosparging
BTEX	Benzene, Toluene, Ethylbenzene, and Xylenes
BV	Bioventing
DDT	Dichloro-Diphenyl-Trichloroethane
DNA	Deoxyribonucleic acid
DNAPL	Dense Non Aqueous Phase Liquid
ESB	Enhanced Saturated zone Bioremediation
FAD	Flavin Adenine Dinucleotide
GAC	Gas Activated Carbon
GW	Groundwater
HDPE	High density polyethylene
K_{ow}	Octanol-Water Partition Coefficient
LC_{50}	Lethal Concetration 50
LD_{50}	Lethal Dosage 50
LF	Land Farming
LNAPL	Light Non Aqueous Phase Liquid
MCL	Maximum Concentration Limit
MNA	Monitored Natural Attenuation
MSW	Municipal Solid Waste
MWW	Municipal Waste Water
NAD	Nicotinamide Ademine Dinucleaotide
NADP	Nicotinamide Ademine Dinucleaotide Phosphate
NAPL	Non Aqueous Phase Liquid
PAH	Polycyclic aromatic hydrocarbons
PCB	Polychlorinated biphenyl
PEP	Phosphoenolpyruvate
POP	Persistent Organic Pollutants
PR	Phyto Remediation
PVC	Polyvinyl Chloride
REDOX	Reduction-Oxidation Reaction
ROI	Radius Of Influence
RTDF	Remediation Technologies Developer Forum
SE EPA	Swedish Environmental Protection Agency
SME	Small-Medium sized Enterprises
SVE	Soil Vapour Extraction
SVOC	Semi Volatile Organic Compounds
SVOC-Cl	Chlorinated Semi Volatile Organic Compounds
SYKE	Finnish Environmental Institute
TNT	Trinitrotoluene
TPH	Total Petroleum Hydrocarbons
UN CDB	UN Convention on Biological Diversity
US EPA	US Environmental Protection Agency
UST	Underground Storage Tank
VOC	Volatile Organic Compounds
VOC-Cl	Chlorinated Volatile Organic Compounds
VTT	Finnish National Research Center
€	1 € = $ 1.19 = £ 0.68 = 138.12 Yen (06.03.2006)

Table of content

1. INTRODUCTION

Biotechnology has been under fast development in the previous decades and expectations towards the benefits it is able to offer are high. In environmental field, both nature sciences and engineering, it is opening new questions and offering new answers. It increases our knowledge of nature and the processes within ecosystems and gives us new tools for working in the environment by using naturally occurring processes.

Bioremediation is one of the new fields of technologies benefiting from the research that has been conducted multidisciplinary. It offers a new, (cost-) effective means for soil and water remediation. Interest towards it has been developing constantly as research and field trials have been able to show its potential in degradating harmful substances.

Thought much is known about the subterranean microbial systems and biogeochemistry, complete and coherent information on bioremediation is not widely available. The multidisciplinary research on various fields has contributed to the development but only some programs and literature have aimed in discussing solely bioremediation, and hardly any on engineering perspective. The combined information on bioremediation and field methodologies would be valuable for engineers dealing with environmental remediation projects.

This study will discuss the issues around bioremediation, from perspectives of economics, natural science and engineering. It will cover the basic theoretical background, give descriptions of feasible technologies and offer solutions for monitoring and quality control. It will also present a case study of an in-situ bioremediation field trial conducted in Finland during year 2005, describing the design and construction process as well as monitoring. Results and discussion on the achievement of field trials goals will also be attached. Some material concerning the field trial is defined classified and not available in the public version of this thesis.

Theoretical aim is to give insight on the historical development biotechnology and explain the terminology used in bioremediation. Processes and systems necessary for the pollutant degradation are to be covered through biogeochemical framework.

The goal is to give insight on the theoretical background of bioremediation and the biogeochemical and engineered processes that undertakes during the remediation process.

Besides clarifying the theoretical background, various in-situ bioremediation technologies are presented and their applicability is discussed more thoroughly. Not all technologies are feasible on all sites, therefore understanding the background behind the biogeochemical processes and pollutant properties is important in selecting the right approach. When remediation is conducted, there is need for monitoring the status and development of the process. Solutions for monitoring as well as discussion on quality control for in-situ bioremediation are presented.

The field trial case study will offer more technical information suitable for engineering purposes. It will present a thorough description of a field trial conducted Finland in summer-winter 2005. In the field trial a permeable biologically reactive barrier was made to study the possibilities to constrain the pollution dispersion of the contaminated site without hindering the natural groundwater flow.

PART I

Environmental biotechnology

2. DEFINING BIOTECHNOLOGY

Biotechnology can be viewed as a group of useful, enabling technologies with wide and diverse applications in industry, commerce and the environment /15/. One of the widest definitions used is the one by the UN CBD (Article 2. Use of Terms), which states that "biotechnology means any technological application that uses biological systems, living organisms, or derivates thereof, to make or modify products or processes for specific use".

2.1. History of biotechnology

Historically, biotechnology evolved as an artesian skill rather than a science /15/. When considering biotechnology through the framework provided by the UN CBD, the history of biotechnology can be traced to 8000 BC, when people begun to collect seeds and domesticate wild animals, thereby starting selective breeding without accurate understanding of the molecular mechanisms. Biotechnology evolved quickly due to lucky errors in the 4000-6000 BC to include brewing of beer, fermenting wine and baking bread with the help of yeast and making yogurt and cheese with lactic-acid producing bacteria /7, 15/.

In latter times, the development of biotechnology has been very rapid. The first micro-organisms were discovered in 1676 by a Dutch plainsman called *Leeuwenhoekusing* who used primitive equipment far from microscopes and opened the amazing world of micro-organism by finding *protozoa* in a sample of pepper-water /4/. By the change of millennium (2000 AD), the human genome was mapped. /42/

2.2. Biotechnology today

The quest in going deeper on details of life itself has kept the development continuous and today the biggest constraints on experimenting arise from legislation. The speed of innovations and new possibilities has lead to a counter effect, causing public criticism on the subject. Biotechnology is usually seen as a sect of genetic engineering, assimilated with the 'artificial' production of new plants and species, or furthest, cloning of animals or even human embryos. Modern consumers and industrialized societies are demonstrating concern, as Ratledge (2001) states, about the 'unknown' health risks, possible deleterious effects on the environment and the 'unnaturalness' of transferring genes between unrelated species.

The difficulties in usage of biotechnology are much a part of the public perception of the issue. In 1997 an *Eurobarometer* study on public perception of biotechnology /15/ noted that (i) the majority of Europeans consider the various applications of modern biotechnology useful for the society. The development of detection methods and the production of medicines were seen to be the most useful and considered the least dangerous. (ii) The majority of Europeans tend to believe that we should continue with traditional breeding methods rather than changing the hereditary characteristics of plants and animals through biotechnology. Less then one in four Europeans think that regulations are sufficient to protect people from any risk linked to modern biotechnology.

The public perception is clearly distorted due lack of knowledge on the scientific basis and history of biotechnological applications and its relation to natural development, e.g. evolution and gene transfer. The Eurobarometer answers show that people are afraid of biotechnology when they have to be dealing with it or it is in contact with their own 'environment' or 'living area'. On the other hand, if applications are developed in laboratories under scrutiny of scientists for the better of humanity the usage of biotechnology is widely accepted.

Today, new legislation is passed nationally and internationally to constrain the fields of research, implementation of applications and sale of genetically engineered products. Governments and international co-operatives have made

legislation on the environmental issues since the 1960s, with main focus on pollution control. In the previous decade also issues on biodiversity have been acknowledged. The strict environmental legislation has opened new possibilities for biotechnology in environmental field, mainly in the fields of 'clean technologies', pollution control and waste disposal.

Modern biotechnology can be divided into three main sectors including medical-, agricultural- and environmental biotechnology. The most advanced fields of study are medical and agricultural, due to the amount of money available for their research.

Agricultural biotechnology concentrates in both traditional and genetic breeding of living organisms to make or modify products and to improve plants and animals. Medical biotechnology concentrates designing organisms to produce xenobiotics for medicine and genetic engineering to increase human health. Environmental biotechnology concentrates on pollution prevention and control by utilizing properties of living organisms and natural systems.

2.3. Legislation

Environmental pollution has been aimed to be controlled by international conventions to minimize the pollution effect to 'global commons' as soil, water and air. A short list of legislation that has been imposed is shown in table 1.

Table 1. List of selected international conventions and protocols concerning pollution and biotechnology (Data from: UN treaty collection)

Year	International convention and protocols
1972	Convention on Prevention of Marine Pollution by Dumping of Waste and Other Matter
1972	Convention Concerning the Protection of the World Cultural and Natural Heritage.
1979	Convention on Long-Range Transboundary Air Pollution (LRTAP).
1982	Law of the Sea
1987	Protocol on Substances That Deplete the Ozone Layer
1997	Protocol on Climate Change
1998	Convention on Prior informed Concent
2000	Protocol on Biosafety
2001	Convention on persistent organic pollutants

NOTE! The 2000 Biosafety protocol is an addition to 1992 Rio Convention on Biodiversity

The conventions presented in table 1 give implications on the developmental path of the legislation in international field. The first international conventions focused around issues as air- and waterborne pollution and aimed at regulating the release

of waste to these common areas. In 1980's and 1990's scientist begun observing changes in the earth's atmosphere, changes in gas composition and holes in the ozone layer.

The 1987 Protocol on substances that deplete the ozone layer (often referred as the Montreal protocol) was a huge victory for environmental lawmaking and international co-operation. Science had been able to show evidence that there were changes in the atmosphere that were man made and threatened life on the earth. Consensus was gained fast, and nearly all nations eagerly signed the protocol and begun working for the common goal.

It was long hoped that the same joint concern would continue in the field of climate change due to increasing greenhouse gas emission, but the hope was useless. The 1997 Protocol on climate change was officially not enforced until 2005 when enough member nations had ratified it, and still, the single largest polluter in total and per capita (USA) has not ratified the agreement and informed that they will not do so.

The new development on the international legislation has been the focus on biodiversity. The year 2000 convention on biological diversity, in addition to 1992 Rio convention states in Article 1. Objectives: "*The objectives of this Convention…are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding*" /23/.

Biotechnological innovations have been extensively researched in the last decades, and as a new development, companies have begun to patent rights for organisms or genes, found in nature /16/. There is a question that has been raised more often these days; what is the individuals right to own something that nature has created? A company might patent a gene found in Amazon from a plant able to produce antibiotics, hence having an overseeing right over that gene. The indigenous peoples of the area have probably used the plant for centuries and possessed

previous knowledge on the healing properties. Today, there are already cases where companies have done so and gained a right for something that they have not created themselves and are not required for any technological or economical compensation.

3. ENVIRONMENTAL BIOTECHNOLOGY

Environmental biotechnology is not as glamorous field of study as medical biotechnology, which promises to cure cancer and remove genetic diseases. Environmental biotechnology in contrast to medical is different as it is dealing with high volumes of low-value wastes, products and services /19/ when the latter deals with low volumes of high value products and services.

Environmental biotechnology is fundamentally rooted in waste, mainly concerned with the remediation of contamination caused by previous use and the impact reduction of current activities Dealing with waste and cleaning up pollution are in everybody's best interest, but most people would rather not recognize the issues, as they are easier to be ignored. They are problems people feel they do not contribute to themselves and rather would have them not exist in the first place. /9/. Even for industry, thought the benefits may be noticeable on balance sheet, the likes of effluent treatment or pollution control are more of an inevitable obligation than a primary goal themselves /9, 19/.

3.1. The scope for use

Key intervention points for environmental biotechnology are waste management, pollution control and manufacturing processes, which are often referred as clean technologies /9, 19/.

3.1.1. Waste management

Waste management is one of the most fundamental and commonly applied fields of environmental biotechnology. The applications can be divided into solid and sludge treatment. High amounts of biologically degradable waste in both forms are produced domestically, industrially and agriculturally /19/. Biowaste is a modern term introduced to simplify the existing terminology used for classifying waste from organic-origin. Before there has been numerous waste labeling schemes

where biodegradable material has been defined as *green, yard, food, manure* and so on, mainly biowaste falls in one of the three categories feces, raw plant matter or process waste /9/, smallest common denominator being their characteristic high carbon content.

Evans (2003) quotes a study by Lemmes (1998), noting that in EU (*note, before the new membernations*) the annual amount of biowaste was 2500 Mt, of which 1000 Mt was of agricultural origin, 550 Mt of garden and forestry waste, 500 Mt of sewage, 250 Mt from food processing industry and only 200 Mt from the MSW.

In waste management commonly acknowledged basic concept is the *Reduce, Reuse, Recycle*. Biotechnology can offer an important step for this process. Firstly it can offer a change to produce more with less, and secondly it can help reusing the once produced organic components. All biowaste can be reused by microbial processes. One popular and effective process is anaerobic sludge treatment, where sludge is anaerobically transformed to energy intensive 'by-products' like biogas and the material is transformed back to soil-like amendment which can be used again.

The possibilities for environmental biotechnology in dealing with biowaste are enormous and technologies have already found their place in everyday operations of most municipal institutions. There is also constant development in the field of small scale biowaste management. In Finland e.g. there are plans for co-operatives of agroindustries and municipalities to begin collecting human and animal origin sewage combined with agricultural waste for small scale energy and heat production.

3.1.2. Manufacturing processes

Industrial processes always produce some amount of waste, or *scrap*, which on economy wise means extra internal and external costs. An industrial process can be divided into a number of basic stages as Scragg (2005) does; the following figure 1 represents the stages of industrial production process and the possible stages where biotechnology can be applied cost effectively (BATNEEC).

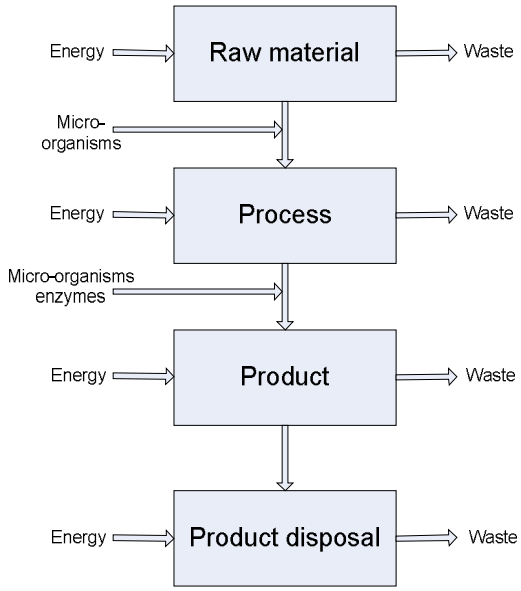


Figure 1. A basic division of an industrial process showing the needs for energy and sources of waste /19/

The main objectives for sustainable industrial process include low consumption of energy and non-renewable raw materials and elimination of waste /19/. The optimization of industrial processes has been a key factor in process and factory design for decades. The main tools and models for this category come from the field of total quality management, a philosophy which mainly originates from Japan and USA.

Main reason for industries to aim for 'clean technologies' is the economical benefits they offer both in reducing *scrap* and in fulfilling the ever tightening environmental regulations that have increased the cost of waste management manyfold.

Biotechnology can be utilized in achieving cost effective results in achieving clean technologies. Renewable energy sources can be used instead of non-renewable fossil energy, and in some cases also the waste can be re-used as energy. Raw material extraction can utilize microbial cultures; e.g. microbes have been used for metal extraction and oil recovery. Raw material processing with biotechnology can also replace the inorganic catalysts with micro-organisms and enzymes /19/.

Compared to chemical processes, manufacturing industries can also benefit from the applications developed on basis of whole organisms. Microbes and enzymes

usually operate with lower temperatures and pressures, hence less energy consumption and safer conditions for workplace and environment /9/.

3.1.3. Pollution and pollution control

Pollution is one of the most prevailing topics in today's environmental discussions and a subject of continuous legislation.

The awakening to existence of chemical pollution took place in the 1960's in the USA through the book of Rachel Carson, entitled "The Silent Spring" (1962), where she wrote about the dangers of chemical usage, especially DDT and it's bioaccumulation in the food chain. Carson concluded that we had already irrevocably harmed birds and animals and had contaminated the entire world food supply.

In the following decades mankind experienced other alarming 'events' that lead to tightening of the legislation. Examples include the Love Canal case in USA, which climaxed in 1978, when the New York State Department of Health announced a medical emergency and President Carter declared a national emergency for the area. The Love Canal area had been used for 50 years as a municipal and industrial waste landfill and in 1950's the current owner sold it to the city for $ 1 which started immediately filling the area with housing projects, schools and industries. It took a while for the inhabitants to notice the effects of living in the area; not the fact that their children started to born defected was enough, but it required the chemicals to start flowing freely into cellars and full canisters of toxic chemicals to surface due to erosion and heavy rains. The company responsible for the operation of the site was sued for $117,580,000 /38/

In 1984 the Bhopal disaster of India, claimed by many as the worst industrial disaster in history. It was caused by the accidental release of 40 t of methyl isocyanate from a pesticide plant located in the heart of the city of Bhopal. The accident lead to the instantaneous death of 4000 people and the gases also injured anywhere from 150,000 to 600,000 people, at least 15,000 of whom later died /41/.

Accidents have been happening constantly, like Chernobyl nuclear reactor 1986 and Exxon Valdez oil-spill in 1989. Thankfully the last decades have been quieter

in the field of pollution accidents, partly due to international legislation and conventions, hence increased knowledge.

A goal of environmental biotechnology is to control existing pollution when another is to aim towards pollution control at source. Industries producing waste or wastewaters that contain high concentrations of organic waste or biodegradable contaminants, have found biological treatment methods very effective in their pollution control operations. "B*iotechnology stands as a particularly cost-effective means of reducing the pollution potential of wastewater, leading to enhanced public relations, compliance with environmental legislation and quantifiable cost-savings to the business*" /9/.

Pollution contaminated land and groundwater is a large concern for today's industrial societies. Previous operations have contaminated sites around the world and at some parts due to economic reasons or lack of knowledge or caring, land and water are still contaminated by poor process operations or by simply dumping.

The short industrial history of mankind has already contaminated large, primary areas. The contaminations are due to previous industrial operations on site, like mining, refining or storage. The usage of these sites is a hot topic today for community planning and construction industries. Most sites are though too contaminated to be usable in construction until large scale remediation has occurred.

Järvinen & Salonen (2004) from Ramboll Finland Ltd. have estimated in their memorandum for Finnish Environmental Institute; "Remediation costs of contaminated sites in Finland", that in Finland there is 20 000 contaminated sites in Finland, which is twice as much as in the previous estimation conducted within the framework of SAMASE-project in the beginning of 1990's /30, 22/.

Previous methods for soil remediation have mainly been focused around removing the contaminated soil and storing it to a landfill elsewhere. Biotechnology has brought new possibilities for completely removing the contaminants from the polluted soils and water. "*Bioremediation technologies provide a competitive and sustainable alternative and in many cases, lower disturbance allows the overall scheme to make faster progress*" /9/.

It is estimated that some 60 000 to 70 000 chemicals are in use today, of which 80 % is actively being used in industrialized countries. Many of these chemicals are classified as *xenobiotics*; substances that are foreign to the biological system they are found from, but can be produced or occur elsewhere naturally. An example is antibiotics, which are naturally produced by bacteria but do not occur naturally in humans.

3.2. Market for environmental biotechnology

Evans (2003) has researched for figures on the biotechnology market size worldwide and in Europe. He writes that the estimated size of environmental biotechnology products and services worldwide is $ 75 billion (63 G€) (2000 est., OECD) accounting for 15% to 25% of the whole environmental sector. He also gives figures from UK's department of trade and industry, which estimated 15-20% of the world environmental market was biotech-based.

Naturally most of the biotech based market is due to medical and agricultural sciences, which can afford the constant R&D operations. Following the line of results that Evans presents, it could be estimated that environmental biotechnology makes approximately 20% share of the global environmental markets. This portion can be expected to increase in relation to traditional environmental sector as well as in total, due to increase in environmental products and service markets.

As noted earlier, environmental biotechnology is a diverse industry, including everything from waste management and manufacturing to pollution control. In Finland the biotech markets are dominated by pharmaceuticals, where 2 out of three employees are working. In 2001 number of Finnish biotechnology SME companies was 106, with sales 141 M€, R&D costs 114 M€ and profits -96 M€. Out of the 106 companies working in the field of biotechnology, only 3 are working primarily in the field of environmental biotechnology /25/.

According to calculations made by Järvinen and Salonen (2004) on the basis of various reports, between 2003 to 2025 in Finland there will be approximately 330 soil remediation projects annually with 60 M€ annual costs. They estimate that the cost structure in a remediation project consists of 5-20 % investigations, < 5 % planning, 30-50 % excavation, transportation and quality control and 30-50 % of

treatment and final disposal. They have only taken in consideration the possibility of excavation as a remediation technique, as according to their memorandum: "*Use of in situ techniques abroad seems to be increasing. In Finland, this will not be the case to the same extent because, for example, of the cold climate. In contrast, isolation of contaminated soils in situ will probably increase. This is likely to reduce total costs even if the costs of surveys and planning will rise*".

A quick calculation, estimating that 10% of these projects were remediated biologically (as in UK 12%, /9/) and that the 50% of the costs which is reserved for excavation, transport, treatment and disposal is used for bioremediation, gives for the market size of bioremediation in Finland 3 M€ annually for the next 20 years. In reality, the total annual in-situ market has been remarkably lower.

PART II

Microbiology

4. TAXANOMY AND STRUCTURE

4.1. Taxonomy of organisms

The sequence of taxonomy runs from domain, kingdom, division, class, family, genus and finally species. The genus/species level of names is the one usually used to identify different organisms.

There are differing methods for classifying living organisms. One of the simplest is the divisions is into domains of *eukaryotes* and *prokaryotes*, where the difference is in the cell structure. Eukaryotes have larger cells, but most importantly their nucleus is formed from DNA molecules and they have a membrane protecting their nucleus. Prokaryotes are smaller in size and do not have a clear nucleus, only a nucleus area with a single DNA molecule, not protected by a membrane /10/.

A more evolutionary division is the three-domain system into *eucarya, bacteria* and *archaea*. Previously both bacteria and archaea were classified as prokaryotes, but in the last decade due to genetic analysis, there has been found differences in the ribosomal RNA, mainly in the 16 S rRNA /19/. The differences found imply that the archaea are in fact closer to eucarya than bacteria, which means that archaea have at some point in evolution diverged from bacteria and developed as a unique domain. See figure 2 for illustration on the development of life on earth.

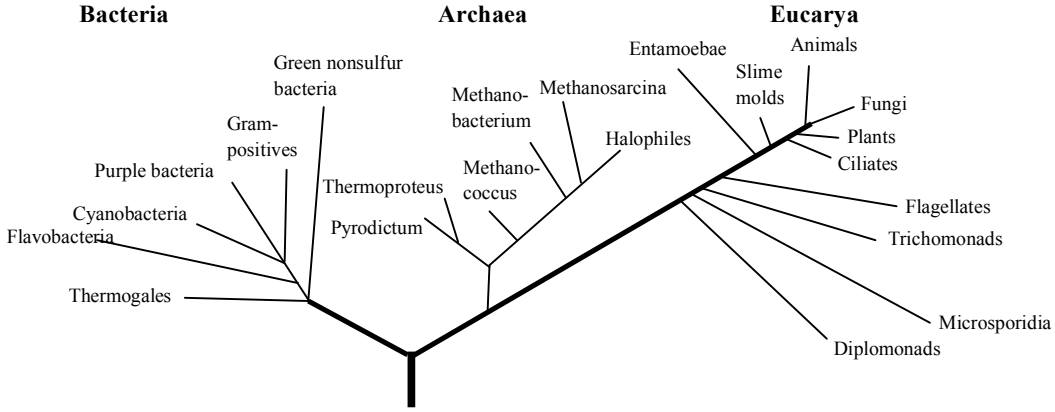


Figure 2. Development of life on Earth and classification into the three domains. (Data from: /2, 15, 27/)

4.2. Microbial structure

Micro-organisms are defined as microscopic organisms that are not visible to naked eye. In reality they include species from all three domains, mainly from bacteria and archaea, but also from eucarya, namely fungi, algae and protozoa.

In terms of structure, archaea are closer to bacteria than eucarya. They both have simple prokaryotic structure; single DNA molecules, no nuclear membrane nor chloroplasts or mitochondria. Eucarya are a more diverse group (shape wise). They have multiple DNA molecules, a protective nuclear membrane and mitochondrion and chloroplasts /10, 19/.

4.2.1. Prokaryotes

Bacteria have three general physical appearances and their sizes range from 0.2 to 250 µm, with normal range of 1-10 µm. The simplest shape is the single bacterial cell, or coccus, which occurs singly, in pairs and in chains. Not all cocci are perfectly spherical and can vary greatly in shape from spherical to almost square. The next common shape is the rod or bacillus, which can also occur singly, in pairs, and in chains. Less common are spiral shapes, the spirallum and organisms that consist of thin threads. The thin threads are called hyphae and the mass of hyphae is known as a mycelium. Typical examples of this group are *streptomyces*, which are found in the soil and often produce antibiotics, such as streptomycin. There are also other shapes, such as the stalked bacteria like *caulobacteria*, but

these shapes are less common /19, 10/. Some bacteria are motile which is achieved by using one or more flagella which are hair like filaments extending from the cell wall. The flagella can be single, bipolar or multiple. Other filaments do occur on bacteria, known as fimbriae and pili. Pili are involved in cell-to-cell adhesion and attachment to surfaces during biofilm formation /19./

Bacteria are classified using physical, chemical, genetic, and metabolic characteristics. Genus and species are assigned on the basis of shape, chemical makeup and genetic characteristics /10/.

Bacteria are the most abundant group of organisms present in the soil. The number of bacteria present and the predominant species present is a function of soil characteristics and the specific environment (e.g. temperature and moisture) /10/. Because of their diversity, bacteria are usually found in heterogeneous communities. Some species will be primary degraders; that is they will initiate the degradation of organic materials in the soil. Other species will grow on compounds resulting from partial degradation of complex organics or waste products of primary degrader's /10/.

4.2.2. Eukaryotes

Eukaryotes are micro-organisms that include the higher plants and animals, namely algae, fungi and protozoa. Their importance for bioremediation is not very high, but in general, yes. Different forms of algae are used as nutrients, fungi and bacteria in food and drink industry and protozoa plays a large part in wastewater treatment. The diversity of eukaryotes in their cellular structures as well as cellular organization and metabolism makes their domain too wide for quick overview here. The main uses of eukaryotes in bioremediation are white-rot fungus and plants used in phytoremediation.

Algae

Algae are photosynthetic eukaryotes that can be both micro- and macroscopic in size and are generally found free living in fresh and salt water. Algae structures vary from unicellular to colonial, and some are filamentous or coencytic. Some algae have plantlike structure, with multi-cellular growth but no real difference

between cells /19, 10/. Algae are generally photosynthetic but some can utilize different organic or inorganic compounds for growth /19/. On land algae are found in the soil and on the surface of plants and rocks in a symbiotic relationship with fungi in form of lichens.

Algae are not important players in the field of bioremediation. In a few cases algae have been used in the bioremediation of aquatic systems either by bioaccumulation of hydrophobic compounds in their lipids followed by harvesting of the algal biomass or by degradation in the presence of sunlight. Algae are also sometimes used in on-site nutrient removal systems but are extremely difficult to separate from the water and often become troublesome contaminants themselves and possibly start forming blooms /10/.

Fungi

Fungi are immotile filamentous organisms that consist of branching structures that are called hyphae, forming networks known as mycelium. They generally have cell walls separating fungal hyphae into individual compartments, hyphae are generally branched and growth occurs in the tip of each hyphae. Hyphae without cross walls are generally known as coencytic /19, 10/.

Some fungi are aquatic, living in freshwater, but most are terrestrial, living in soil or on dead plants. Fungi uses organic compounds for both energy and carbon source. Some of the better known fungi include moulds, yeasts and mushrooms. Relative to bacteria, fungi are generally less numerous, grow at considerably lower rates, and do not compete well in most engineered environments. Additionally, metabolic processes of fungi are generally less diverse than those of bacteria /19, 10/.

Protozoa

Protozoa are eukaryotic predators that mainly feed on bacteria or other organisms, thought some use dissolved organic substances for food. They are heterotrophic motile or non-motile unicellular organisms that lack cell walls. Protozoa require water to carry out metabolic activity. However, there exists many species of protozoa and a high number are typically seen in the microbial communities' /10/.

In biological treatment systems, protozoa play an important role by feeding on and reducing the number of bacteria degradating target contaminants. A rise in bacterial numbers in soil undergoing active biodegradation is often accompanied by a rise in number of protozoa /10/. Protozoa can help control bacterial growth near injection wells in in-situ bioremediation processes, where excessive bacterial growth may cause clogging of porous media and thus decrease hydraulic conductivity /10/.

4.3. Community of organisms

The soil consists of various consortia of micro-organisms and larger organisms up to the level of animals. All have their specific role in the cycles of organic and inorganic constituents that make up the soil system. A consortium can consist of two or more organisms living in close proximity to other ecosystems which are interacting with each other. A consortium generally implies a positive interaction where one group benefits from the actions of the other /10/.

The surface soil, generally entitled the *rhizosphere* is the richest in microbial activity due to the good environmental conditions and the positive influence of the plant roots which excrete organic and inorganic nutrients for the micro-organisms. Still, life is not limited to the top one meter layer of soil, as micro-organisms have been detected at depths up to 600 meters in soil /10/ mainly the amount of micro-organisms depends on the availability of organic and inorganic nutrients to support life.

In biodegradation, several groups of bacteria may be necessary to completely mineralize one compound. Individual species isolated in pure culture and given the target compound as a sole carbon source may be found to be incapable of mineralizing it. Generally, a consortium of micro-organisms is able to carry out the degradation faster and more efficiently compared to a pure culture. Several organisms may be involved in each step and compete for the target compound or breakdown products during mineralization. The species suited to the particular environment will predominate. However a change in the environmental conditions will result in a different species rising to dominance /10/.

5. MICROBIAL METABOLISM

All organisms require energy, carbon and other molecules to grow and divide. The assimilation of these materials through chemical transformations into new cell material is called *metabolism* /10, 15/.

There are different ways that organisms carry out their metabolism, and not all use carbon in its organic forms as some, like plants take the required carbon for cell construction from inorganic atmospheric CO_2 and energy from photosynthesis. There are four generally differentiated methods for obtaining energy and carbon for cell construction; *Photoautothropy, photoheterotrophy, chemoautothropy* and *chemoheterothopy*. The basis on how the classification is conducted is quite straightforward, but the categorization cannot achieve full accuracy due to the metabolic diversity within microbes in reality. Basically, when carbon is derived from inorganic CO_2 the metabolism is termed as autotrophic, when from organic compounds it is termed heterotrophic. Similarly, when energy is derived from chemical compounds, the metabolism is termed chemotropic, and when light is used, phototrophic. By combining the classifications, it is possible to classify most organisms by carbon and energy source (see table 2) /10/.

Table 2. Classification of organisms on basis of carbon and energy sources /10/

Classification	Carbon source	Energy source
Based on carbon source		
Autotrophs	CO_2	
Heterotrophs	Organic compounds	
Based on energy source		
Chemotroph		Chemical compounds
Chemolithotroph		Inorganic compounds
Chemoorganotroph		Organic compounds
Phototroph		Light
Combined terms		
Chemoautotroph	CO_2	Chemical compounds
Photoautotroph	CO_2	Light
Chemoheterotroph	Organic compounds	Chemical compounds
Photoheterotroph	Organic compounds	Light

The largest classes are photoautotrophs and chemoheterotrophs, and they also have the largest impact on bioremediation. The former use light as energy source and obtain their carbon from inorganic CO_2 as the latter use organic compounds for both energy and carbon source. Main phototrophic species are plants and algae, but also some prokaryotes and photosynthetic bacteria fall into this category. The

chemoheterotrpohic category is the largest, including the majority of bacteria and many eukaryotes /10/. The main focus in this paper is on the heterotrophic micro-organisms, which will be discussed further in the following. The use of autotrophs in in-situ remediation will also be discussed shortly.

5.1. Metabolism of organic material

Metabolism is the biochemical modification of various chemical compounds by synthesis and breakdown, called *anabolism* and *catabolism* /15/. It includes the uptake and assimilation of these compounds, their distribution, *biosynthesis* and *biotransformations* and the elimination of the remaining compounds and metabolites /43, 15/. Metabolism usually consists of sequences of enzymatic steps, also referred to as *metabolic pathways* /43/. Without metabolism no living organisms could survive as it is the fundamental process that supports life.

Anabolic processes are involved with building of new cell material, not only the proteins, carbohydrates and lipids, but also the intermediary products as amino acids, pyruvate, fatty acids, sugars and sugar phosphates /15/. Cells are not able to synthesise all compounds required in metabolism, therefore they require various trace elements for construction which also have to be readily available to complete enzymatic steps. The anabolic 'biosynthesis' is basically and *endothermic* process, as it requires energy for building processes. The energy required for these internal building processes is provided by catabolic processes, which oppositely are *exothermic*, meaning they are energy producing. In simple, carbohydrates are degraded to ultimately give out CO_2, water and generate energy /15, 10, 9/. The energy generated in the exothermic catabolic metabolism is usually chemical energy in the form of ATP (adenosine triphosphate) but other forms exist as NAD (nicotinamide ademine dinucleaotide), PEP (phosphoenolpyruvate) and acetyl-CoA (acetyl coenzyme A) /19/.

Anabolism and catabolism are required to function with each other but not simultaneously as it is counterproductive. There are many signals that switch on anabolic processes while switching off catabolic processes and vice versa. Most of the known signals are hormones and the molecules involved in metabolism itself. /43/.

5.2. Cometabolism

Cometabolism is a biodegradation process during which an organic compound is transformed by the micro-organism but no energy or carbon is derived from the process /1/. As such these organisms would require another substrate as a carbon and energy source on which to grow, thus in cometabolism micro-organisms use other compounds as primary energy and carbon source while metabolizing another compound to utilize the enzymes gained from metabolism to enhance mineralization of the primary substrate /10/. The environmental benefit of cometabolism is that hazardous and recalcitrant chemicals may be altered to structurally less harmful compounds that can be metabolized by other micro-organisms; similarly there is hazard that the compounds may be transformed to more toxic or bounding forms.

The term cometabolism is often debated, since the 'philosophical' reasons for the process are somewhat unclear. Cometabolism occurs not only in the presence of primary substrate, but also when one is not available, therefore it is sometimes called as fortuitous metabolism /10/.

5.3. General metabolic pathways

Chemoheterotrophic micro-organisms metabolize organic material using two different pathways, namely fermentation and respiration. Fermentations are reactions where the final *electron acceptor* is a product of metabolism and not exogenous as in respiration, thereby they could be described as internally balanced oxidation-reduction reactions /10/. Fermentation does not yield as much energy as aerobic respiration, as the compounds cannot be fully oxidized /19/. Fermentation has significant economical importance in food and brewing industry and in futures in bio-energy production.

5.3.1. Respiration

Respiration involves usage of *exogenous* (extracellular) electron acceptors, and is commonly divided into aerobic- and anaerobic respiration, carried out mainly by aerobic, facultative or anaerobic bacteria. The metabolic processes involved in both are essentially the same, but differ at the final steps /10/. The main differences arise

from the compounds used as terminal electron acceptors; as in aerobic respiration the terminal electron acceptor is always oxygen, in anaerobic it is other than oxygen as NO_3^-, SO_4^{2-}, CO_2, S^-, Fe^-. In aerobic respiration, O_2 is the terminal electron acceptor preferred by the organisms due to its high energy yield in complete reactions. The aerobic respiration of glucose can be summarized as

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}$$

It is worth noting that this is exactly the reverse of photosynthesis, another important biological reaction that occurs in many photoautotrophs and is necessary to support most life on Earth.

When oxygen is not available, the organisms will concentrate on the next available electron acceptor. Aerobic respiration is commonly the preferred method of metabolism, as anaerobic metabolism yields only about 8% of the energy that can be produced under aerobic conditions /15/.

Aerobic cellular respiration has three main stages: glycolysis, the citric acid cycle, and electron transport when anaerobic includes only glycolysis and the anaerobic pathway, generally fermentation.

Glycolysis

Glucose, a six carbon sugar, is split into two molecules of a three carbon sugar. In the process, two molecules of ATP and two NADH electron carrying molecules are produced. Glycolysis can occur with or without oxygen. In the presence of oxygen, glycolysis is the first stage of cellular respiration. Without oxygen, glycolysis allows cells to make small amounts of ATP. This process is called fermentation /15, 19, 9/.

TCA

The citric acid cycle or '*Krebs cycle*' begins after the two molecules of the three carbon sugar produced in glycolysis are converted to a slightly different compound (acetyl CoA). Through a series of intermediate steps, several compounds capable of storing high energy electrons as NADH and $FADH_2$ (*nicotinamide adenine dinucleotide* and *flavin adenine dinucleotide*) are produced along with two ATP

molecules. These reduced forms carry the high energy electrons to the next stage. The citric acid cycle occurs only when oxygen is present but it doesn't use oxygen directly /15, 19, 9/.

Electron transport chain

The electron transport chain is a series of electron carriers in the membrane of the mitochondria. Through a series of reactions, the high energy electron carriers pass the hydrogen from NADH to electron acceptor that becomes reduced. In the process, a gradient is formed, and from this ultimately ATP is produced /15, 19, 9/.

5.3.2. Oxidation-reduction reactions

Chemical energy is utilized through oxidation-reduction (*redox*) reactions. A redox reaction is a coupled reaction that involves transfer of electrons from one molecule to another; oxidation describes the loss of an electron when reduction describes the gain of an electron. An *electron donor* becomes oxidized after releasing electrons while *electron acceptor* is reduced after receiving electrons, the two molecules involved in this process are generally called *redox pair* /10/.

The amount of energy release in a redox reaction can be calculated from the standard reduction potentials that are commonly published as electron tower (see table 3, for common redox potentials), where the most likely oxidized compounds are a top and most likely reduced at the bottom. The redox potentials are commonly measured in Volts. The released energy will be higher, the further the two compounds are in the tower. /10/

Table 3. Typical redox potentials measured in bioremediation sites /43/

Process	Reaction	Redox potential (Eh in mV)
aerobic:	$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$	600 — 400
anaerobic:		
denitrification	$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O$	500 — 200
manganese IV reduction	$MnO_2 + 2e^- + 4H^+ \rightarrow Mn_2^+ + 2H_2O$	400 — 200
iron III reduction	$Fe(OH)_3 + e^- + 3H^+ \rightarrow Fe^{2+} + 3H_2O$	300 — 100
sulfate reduction	$SO_42^- + 8e^- + 10H^+ \rightarrow H_2S + 4H_2O$	0 — -150
fermentation	$2CH_2O \rightarrow CO_2 + CH_4$	-150 — -220

In micro-organisms, the energy is generated from the flow of electrons during redox reactions. The process of electron transfer is mediated by electron carriers, which are of two distinct types; freely diffusible and ones attached to enzymes. The most common electron transporters are NAD^+ and $NADP^+$ (*NAD-phosphate*), which are hydrogen atom transporters /10/. Energy released during redox reactions is stored in the cell in the form of high energy phosphate bonds in phosphate containing compounds, of which most important is ATP. The energy from ATP is released in *hydrolysis* and the amount of energy released in hydrolysis of one phosphoanhydridic bond is -30.5 kJ/mol. /10/

5.3.3. Fermentation

Fermentation is fundamentally an anaerobic metabolism. When the oxygen levels are low, the consortia of anaerobic and facultative aerobic micro-organisms are the dominant species. Fermentation begins with an organic substrate, continues to *glycolysis* and finally fermentation of the end product, which can vary from methane gas to lactic acid which is used commonly in food industry. /19/ During fermentation only 2 ATP are generated in the *glycolysis* phase as in the anaerobic conditions the Krebs cycle is not available /15/.

5.4. Development of new metabolic pathways

Micro-organisms, mainly bacteria and archaea are a diverse, old in evolutionary terms and possess the capability for rapid evolution during binary fission. They have survived in the worlds most harsh conditions, mainly due to their capabilities to adapt to new environmental conditions. Generally micro-organisms live in mixed communities rather than groups of cloned organisms. The synergetic benefits from consortia are obvious; it can increase the habitat range, the overall tolerance to stress and metabolic diversity of individual members of the group /19/. The cometabolic features in micro-organisms make possible that a consortia of organisms can more easily degrade a wider spectrum and more recalcitrant compounds than monocultures.

Another consequence of this close proximity is the increased likelihood of bacterial transformation by absorption of extracellular free DNA released e.g. in death of another organism. This process is dependant on the competence of a cell to take up

DNA and is referred to as *horizontal transfer*. In addition to transformation, genes are readily transferred on plasmids. Plasmids are parts of bacterial cells which carry DNA and are also circular and self replicating, and most importantly, often carry the genes that hold the information of metabolic pathways. Plasmids may move between bacteria and by replicating make their DNA transferable /19/.

Often micro-organisms carry within their DNA the information for many metabolic pathways, but only a few are at use at one time. When the organisms encounter new energy and carbon sources, they can attempt to activate an 'old' metabolic pathway that would develop required enzymes or try develop a new pathway from the basis of old pathways that is able to utilise similar carbon sources.

6. CONDITIONS FOR GROWTH

The microbial growth as growth of any living organisms is dominated by environmental factors. The possibility to utilize bioremediation requires specific control over the subterranean environmental conditions to increase the control over the size and composition of the microbial community.

Microbes have been discovered in the extremely hostile environments around the world; from the arctic permafrost to the volcanic oceanic deeps. These bacteria (mainly archaea) are those with the capabilities to degrade the most hazardous and recalcitrant chemicals in our environment and provide the array of vast microbial metabolic pathways /19/ that can be utilized e.g. in developing new enzyme processes or bioaugmentation.

6.1. Environmental parameters

The density and composition of microbial community and the growth rate are a direct function of the environment and available nutrients. Primary environmental factors include temperature, pH, moisture, oxygen- and nutrient availability /10, 9/. Biochemical factors influencing growth include contaminant toxicity, concentration, solubility and volatility, and most importantly, the existence of microbial community with the metabolic pathways to mineralize the contaminant /10/.

6.1.1. Temperature

Temperature influences the growth of micro-organisms as they generally live well in narrow temperature ranges and majority have the optimum growth range from 30-37 ˚C /19, 10/. In bioremediation the majority of the bacteria utilized is from the groups entitled *psychrophilic* and *mesophilic* (see figure 2 for classifications), depending on the used remediation technology.

In general, at temperatures above +40 ˚C the activity decreases due to enzyme and protein denaturation and at temperatures closing 0 ˚C the activity essentially stops /10/. The bacteria are generally more tolerant to low temperature extremes, since they can capsulate and recover, but at high temperatures the population is at increased risk to die. As a rule of thumb, for every 10 ˚C increase in temperature (within the limits presented) the microbial activity increases twofold. /10/

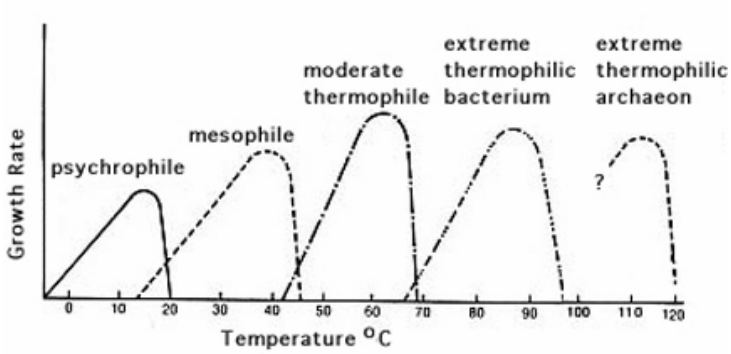


Figure 3. Classification based on temperature range /27/

6.1.2. pH

Micro-organisms usually live in conditions where the pH is close to neutral, namely the optimal growth range of most micro-organisms is within the pH range of 6-8 /10, 19/. As is the case with temperature, thought most micro-organisms favour the near neutral pH range, there are bacteria that can survive in very harsh conditions, but generally, highly acidic or alkalic conditions inhibit growth; for classifying bacteria on basis of optimal pH, see figure 4.

The pH does not only affect the bacterial growth directly, but also by affecting the solubility of nutrients and metals. An important nutrient for microbial growth is

phosphorous, which solubility is maximized at pH 6,5. Similarly metal transport is minimized at pH above 6.

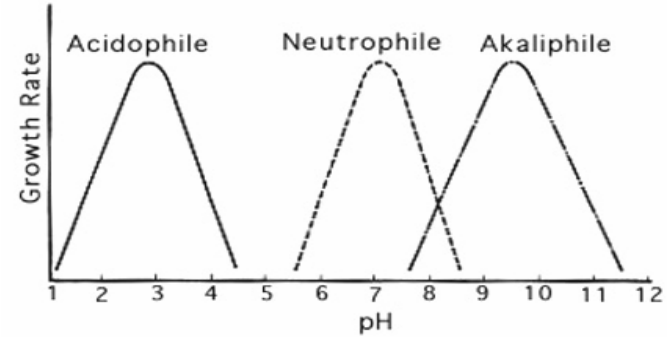


Figure 4. Classification based on pH range /27/

6.1.3. Oxygen availability

Micro-organisms are usually divided into groups depending on their response to oxygen; the division is simply *aerobic* and *anaerobic*. Aerobic organisms are those that require oxygen for growth and for anaerobes oxygen is lethal. In reality, the division is not as straightforward, and within a microbial community different groups with different relation to oxygen availability can exist, for various definitions on microbial groups and their relation to oxygen see table 4.

Table 4. Bacterial classification and response based on O_2 /27/

Group	Environment		
	Aerobic	Anaerobic	O2 Effect
Obligate Aerobe	Growth	No growth	Required (utilized for aerobic respiration)
Microaerophile	Growth if not too high	No growth	Required but at levels below 0.2 atm
Obligate Anaerobe	No growth	Growth	Toxic
Facultative Anaerobe (Facultative Aerobe)	Growth	Growth	Not required for growth but utilized when available
Aerotolerant Anaerobe	Growth	Growth	Not required and not utilized

The largest microbial group is definitely the obligate aerobes that utilize oxygen for aerobic respiration. In the same group with obligate aerobes can grow facultative aerobes, that are microbes which utilize oxygen when available, otherwise use secondary metabolic route with different final electron acceptor. Another common group are the obligate anaerobes, which live only in anaerobic conditions and do not utilize oxygen. There are also different microbial groups that live in the very

low oxygen environments, but their metabolism is restricted and focused usability in bioremediation nonexistent.

6.1.4. Nutrient availability

Micro-organisms are constructed, besides from water, mainly from carbon, oxygen, nitrogen, hydrogen and phosphorus (for full reference on microbial composition, see table 5). All these compounds and other trace elements have to be readily available and obtainable by the micro-organisms from their environment or they have to be able to synthesize them to thrive. The lack of one of the components required will restrict the growth /19/, as is often found in the natural and engineered environments.

6.1.5. Moisture

Micro-organisms consist of 80-90 % water and therefore will only grow in conditions where there is enough free water /19/. Water also serves as the transport medium through which organic compounds and nutrients are moved into the cell and through which metabolic waste products are moved away from the cell /10/. The water content also affects the aeration of the soil and the potential for contaminant solubility.

The availability of water in a solution is called *water activity*. Water activity is defined so that de-ionized water has the reference value of 1.0 and most bacteria require a water activity level of 0.9 /19/ (see figure 5). Water activities in agricultural soils range between 0.9 and 1.0. /27/. In saline environments, salt has a natural decreasing effect on water activity; most marine organisms can tolerate salt levels up to 3 % /19/, but there are some that require salt for growth and grow in salt concentrations above 15%, these organisms are called *halophiles*. The term *osmophiles* is usually reserved for organisms that are able to live in environments high in sugar /19, 27/.

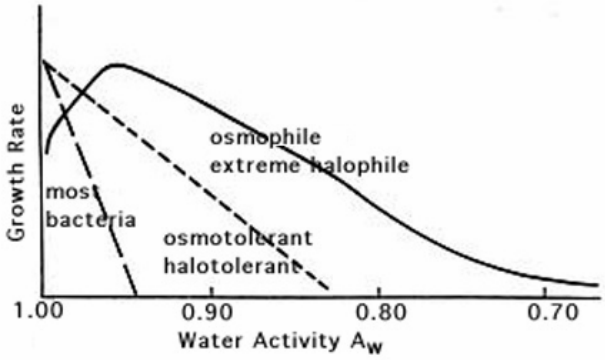


Figure 5. Classification based on A_w. /27/

6.2. Phases of growth

Bacterial growth is controlled by environmental factors as represented previously. Most bacteria cells tend to divide in a process known as *binary fission* when they reach their correct size /10, 19, 27/. The cycle of life for bacterial population consists of a sudden, exponential growth, followed by a short stationary phase and finally after environmental conditions can no longer support the community, the decline in population.

As said, most bacteria tend to increase their population by dividing their cells. At the start of the division, a cross wall forms, DNA doubles and separates into the new cells and at the end the cell separates into two genetically similar cells. In some cases like the chain-forming streptococci, the cell fails to separate, forming chains. Besides, not all micro-organisms divide by binary fission, some form buds which pinch off from the mother cell, others, like fungi, form elongating hyphaes. Most eukaryotic micro-organisms as yeast, algae and protozoa divide asexually after mitosis /19/.

The bacterial growth rate is defined by the environmental conditions. As long as the bacteria have optimal living conditions, sufficient carbon source and nutrients, the population will continue to grow. After all the food and nutrients have been used, the population will stabilise for a while, but very soon, the population will die.

In enhanced remediation systems, the duration of phases might differ highly from the natural or laboratory scale. The lag (or ‘acclimation’) period, is the time the bacteria require to get used to the new environment and during the lag phase, the

growth is nearly zero /19/. The lag phase can last for hundreds of days /10/ depending on the previous growth history, biodegradability of contaminant, microbial metabolic capabilities and existence of preferential carbon sources /10, 19/. The microbes might require a lengthy exposure to the chemical to induce enzymes or even genetic mutations might be required /10/.

The growth phase is usually exponential; microbes grow faster than they tend to die off, therefore the population doubles at regular intervals. Only when carbon source or a nutrient becomes limiting, the growth slows down but the living cells may stay viable for a long period /19/. When the bacteria reach the upper limit of their environmental sustainability the growth rate decreases to zero and metabolic activity decreases. When the bacteria stop growing, they either physically die or just inactivate metabolic activity and wait for the next growth phase. /10/

In engineered systems, environment and nutrients are controlled at a predefined level to 'host' required microbial population and maintain the ongoing remediation. Usually there is no need to grow the microbial mass very high, because if the system flow is not sufficient, the metabolic wastes may change the environment or the increased bacterial mass may cause clogging in the piping or in decrease the subterranean hydraulic conductivity. It is important to control that the microbial population is not allowed to enter the decline phase as the re-activation of their metabolic activity and re-growth might take a long time.

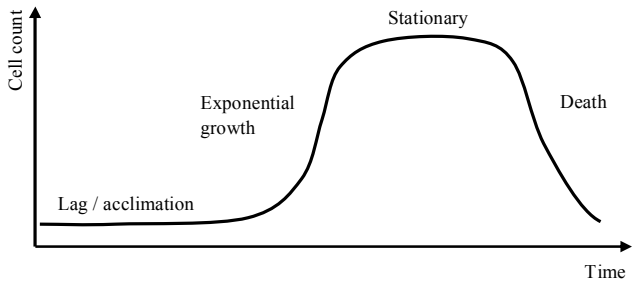


Figure 6. Bacterial growth curve (Data from: /27/)

Table 5. Bacterial composition and elemental functions in cellular metabolism /10, 27/

Element	% of dry weight	Source	Function
Carbon	50	organic compounds or CO2	Main constituent of cellular material
Oxygen	20	H2O, organic compounds, CO2, and O2	Constituent of cell material and cell water; O2 is electron acceptor in aerobic respiration
Nitrogen	14	NH3, NO3, organic compounds, N2	Constituent of amino acids, nucleic acids nucleotides, and coenzymes
Hydrogen	8	H2O, organic compounds, H2	Main constituent of organic compounds and cell water
Phosphorus	3	inorganic phosphates (PO4)	Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids
Sulfur	1	SO4, H2S, So, organic sulfur compounds	Constituent of cysteine, methionine, glutathione, several coenzymes
Potassium	1	Potassium salts	Main cellular inorganic cation and cofactor for certain enzymes
Sodium	1	Sodium	Major cellural inorganic cation
Magnesium	0.5	Magnesium salts	Inorganic cellular cation, cofactor for certain enzymatic reactions
Calcium	0.5	Calcium salts	Inorganic cellular cation, cofactor for certain enzymes and a component of endospores
Chlorine	0.5	Chlorine	Major cellural inorganin anion
Iron	0.2	Iron salts	Component of cytochromes and certain nonheme iron-proteins and a cofactor for some enzymatic reactions
Trace elements: *manganese, cobalt, copper, zinc, etc* ..	0.3	Varies	Confactors in electron transport in specific enzyme catalyzed reactions

PART III

Bioremediation

"The contamination of soil and water with organic and inorganic pollutants is of increasing concern and a subject of legislation. These pollutants include complex organic compounds, heavy metals, and natural products such as oils and are derived from industrial processing, deliberate releases, and accidental releases" /19/.

7. POLLUTION CONTROL AND ROLE OF BIOREMEDIATION

As noted in the previous chapter on environmental biotechnology when the scope for use was discussed, waste is one of the most frequently discussed topics in the field of environmental sciences. Even thought there are an ever increasing amount of xenobiotics released to the environment, not all manufactured chemicals are harmful in nature and some naturally occurring substances may contribute to the pollution or be extremely dangerous when concentrations increase above suitable values.

7.1. Classifying pollution

Classifying pollution is a difficult task and no single classification can exist due to the diverse nature of contaminants. Most commonly contaminants are classified on basis of nature, composition, properties, sources or uses, mainly depending on the topic to be analyzed. In our case the pollution will be classified more in the perspective of a required risk assessment. The classification outline suggested here is based on the is based on Swedish EPA Quality criteria on contaminated sites, Finnish Research institutes (VTT) publication "Pilaantuneiden maiden

kunnostushankkeiden hallinta – Managing remediation on contaminated sites" and on the comments of Evans (2003) from the university of Durham, representing the current views of the UK. The Finnish legislation is in the course of transformation, and due to the fact that there will be a proposal on the new recommendations on contaminated sites which will be published in 2006, which is still in the draft phase, the legislative renewals from Finland will not be considered here.

Mainly pollution classification in the case of contaminated soil and groundwater is conducted though a risk assessment based procedure, where the actual contamination levels are estimated through holistic analysis including site investigations and laboratory analysis. The potential for risk is estimated on the basis of extensive research and using tools and software developed for modelling.

In the case of contaminated soil and groundwater the risk assessment procedure should be done according to well established procedures. There are many national guidelines for conducting risk assessment of contaminated sites, and one should use the national, legally recommended methods. The following subtopics give implication on the issues that are to be considered when classifying sites.

7.1.1. Chemistry and concentration of contaminant

The chemistry and concentration are one the most basic defining factors of contamination. Even moderately harmful substances can cause serious damage if they are present in high concentrations or large amounts /31/. The issue is even more complicated as the initial contamination does not always fully define the whole nature of pollution. Due to chemical or biochemical reactions the contaminant may be transformed into other more hazardous substances that can cause increased risk. Some contaminants also possess synergetic properties, that when found together they may cause an increased risk that is higher than the sum of the two individually /19, 31/.

7.1.2. Toxicity

Toxicity refers to the potential of the contaminant to cause hazard or risk to humans or other living organisms. Toxicity of a substance can be affected by many different factors, such as the contact media; skin, inhalation, injection, the time of

exposure, the number of exposures and the physical form of the toxin; solid, liquid, gas.

Most commonly in environmental remediation cases the contact with the contaminant is not acute, but rather chronic, as the contact with the toxin can last for years, e.g. in the case of contaminated water that is drank or volatile emissions that enter e.g. through the foundation of apartment buildings to the respiratory system of occupants. Cases where there is a risk for acute toxic effect are usually noted early as the volume of the concentration is either high or otherwise obvious.

Toxicity is usually measured in LC_{50} or LD_{50}, 'lethal concentration' or 'lethal dose', notably values that when reached cause and lethal effect in 50% of certain population when consumed in a specific manner. The toxicity test are usually conducted on V*ibrio fischeri, Eisenia fetida*, etc., but the tests are under criticism from animal rights movement and other institutions. More advanced and accurate methods are developed for testing toxicity directly on humans cells. For example the Tampere University Dept. of Medical Science has studied the possibilities to use human tissue cultures for rapid toxicity testing /46/.

7.1.3. Mobility and persistence

Perhaps the most effecting factor in overall risk analysis is the mobility of pollutant. If the pollutant has tendency to disperse and dilute, this has effect on the remediation possibilities and pollution control as the dispersion is rarely uniform. If the pollutant is not mobile, it has a tendency to remain in 'hot-spots' near the origin of contamination /9/. Non-mobile contaminants are easier to control thought their concentrations may be inhibitory to biological remediation methods.

Persistence of a compound is the duration effect. Highly toxic chemicals which are environmentally unstable and break rapidly are less harmful then persistent substances, even thought they may be intrinsically less toxic /9/.

The mobility of a substance depends highly on its chemical stability, polarity, solubility and K_{ow} ratio. Larger compounds are usually more stabile and are biodegraded more slowly, hence their persistence will be higher. Non-polar compounds tend to be hydrophobic and tend to partition to soil surface or form

NAPLs, therefore their mobility is decreased as persistence is increased due to lower solubility /10/. Solubility is the single most affecting parameter for biodegradation as microbes use nutrients from aqueous phase /10, 19/, solubility is usually linked with K_{ow}, which is the octanol-water partition coefficient; the ratio of the concentration of a chemical in octanol and in water at equilibrium /6/.

7.1.4. Bioaccumulation / magnification

Some compounds are not readily biodegradable but instead are accumulated in the tissues of living organisms and concentrated over time. /9/ Examples of such chemicals are DDTs and PCBs which are recalcitrant chemicals that do not only bioaccummulate, but are increased in concentration in the food chain in a process called biomanification /19/.

These recalcitrant xenobiotics do degrade in nature, but their half time is on average 5-10 years /19/. This combined with the slow biodegradation and accumulation effect makes these compounds very dangerous in the environment.

7.1.5. Risk to humans / nature.

What is degree of risk this contamination causes to the surrounding nature, and more importantly to humans? The decision on risk is nearly always qualitative, even thought it might be based on quantitative data. It is impossible to accurately know the subterranean conditions and the behaviour of the chemical contaminant in-situ or ex-situ.

In modern legislature the MCLs (maximum concentration limits) for contaminants in soils and groundwater are defined. Also the latest versions include classification of different contaminants to different ‘risk groups’ that can have differing exposure limits and management restrictions /31/.

Assessing the potential hazard should be fundamentally conducted for the whole ecosystem as the contamination is likely to effect the whole biota, not only humans. It is possible that some contamination is so ‘insignificant’ that it can be left untreated, but when this is not the case, the contamination should be remediated and the threat removed.

7.2. Role of bioremediation

Contaminated soils and groundwater are a common harm and an increasing risk in today's urban societies. They are, according to Netherlands environmental agencies study in 2001, the most important environmental issue in Netherlands /26/. The contaminants are mainly rising from the old industrial sites that have historically been located close to urban habitation centres. The contaminants that are spread in the environment are mainly organics, originating from the chemical and petrochemical industries and inorganics from the metal and mineral extraction and various mixtures from agroindustries. Another thought separate category is the MSW and MWW organic wastes.

7.2.1. Novel remediation strategies

In the past the most common soil remediation method has been excavation and off site disposal. Today the method is not favoured in most environmentally conscious nations, as the basic operating principle and ecological effect is very poor. In this classic technique the contaminated soil is excavated and taken elsewhere for disposal. The void that has naturally remained on-site is then filled with virgin material to replace the contaminated soils and make the site again usable. When the remediation or disposal off-situ costs are calculated, summed with the virgin material costs and the environmental effects of the transportation, the technology does not seem feasible anymore. The technology is in fact not remediation or purification, but instead removal and replacement, with no real treatment. As stated, this is fundamentally false, and many nations, as USA, GB and NL are encouraging to use other real remediation techniques, if not clearly forbidding the use of excavation and disposal.

Other traditional technique for soil remediation is containment and in the case of groundwater, pump-and-treat. Neither of these technologies offers cost effective solution for soil and groundwater treatment, as containment again is no remediation and pump-and-treat is a very time consuming process that can not guarantee the full remediation of the groundwater and the saturated zone as it is dependent on contaminant solubility and K_{ow}.

Today most of the environmental protection agencies recommend the use and research of other, more advanced remediation techniques that can be used for complete mineralization of the contaminants or for collecting the contaminant in a re-usable form or otherwise minimizing the environmental effect.

The US EPA and Department of Defence have been working together with national agencies in a project called Federal Remediation Technology Roundtable, available online, which describes clearly most novel technologies by principle and capabilities. Also US Air Force Centre for Environmental Excellence and other sections working under department of defense have developed programs for testing and working with novel technologies for soil remediation. The probable reason for the high degree of military involvement is the fact that in most nations military is responsible for military sites and their remediation.

Figure 6 is a mind map of novel soil and groundwater remediation techniques that have been tested and recommended by numerous governmental and research institutes. A lot of research is also conducted on the economical benefits of novel technologies, but they are not covered here. For examples of other novel techniques and their expenditures see Scragg (2005), Evans (2003), Eweis (1998) or Penttinen (2001).

As one can note, the remediation techniques presented are categorized as in-, on-, and ex-situ techniques. No generalization has been made on the basis of technology methodology to categorize into biological, chemical, physical or thermal and classical techniques that have been proven functional as excavation and disposal or pump-and-treat are not covered here. Instead, in the following chapters a more thorough view on especially biological remediation techniques is given.

By looking at figure 6 it can be noted that most novel techniques that have been developed can be operated in-situ. It is clear that there are both environmental and economical benefits from not excavating the site and dealing with the problem elsewhere. The in-situ techniques are from numerous categories, including all biological, chemical, physical and thermal methods.

By using these novel techniques, most contamination should be able to be remediated, including radionuclides, explosives and heavy metals. It has to be still

noted, that in some cases the contamination cannot be degraded or detoxified to levels where it would not impose risk, and in these cases the contamination has to be either excavated or isolated from the surrounding nature.

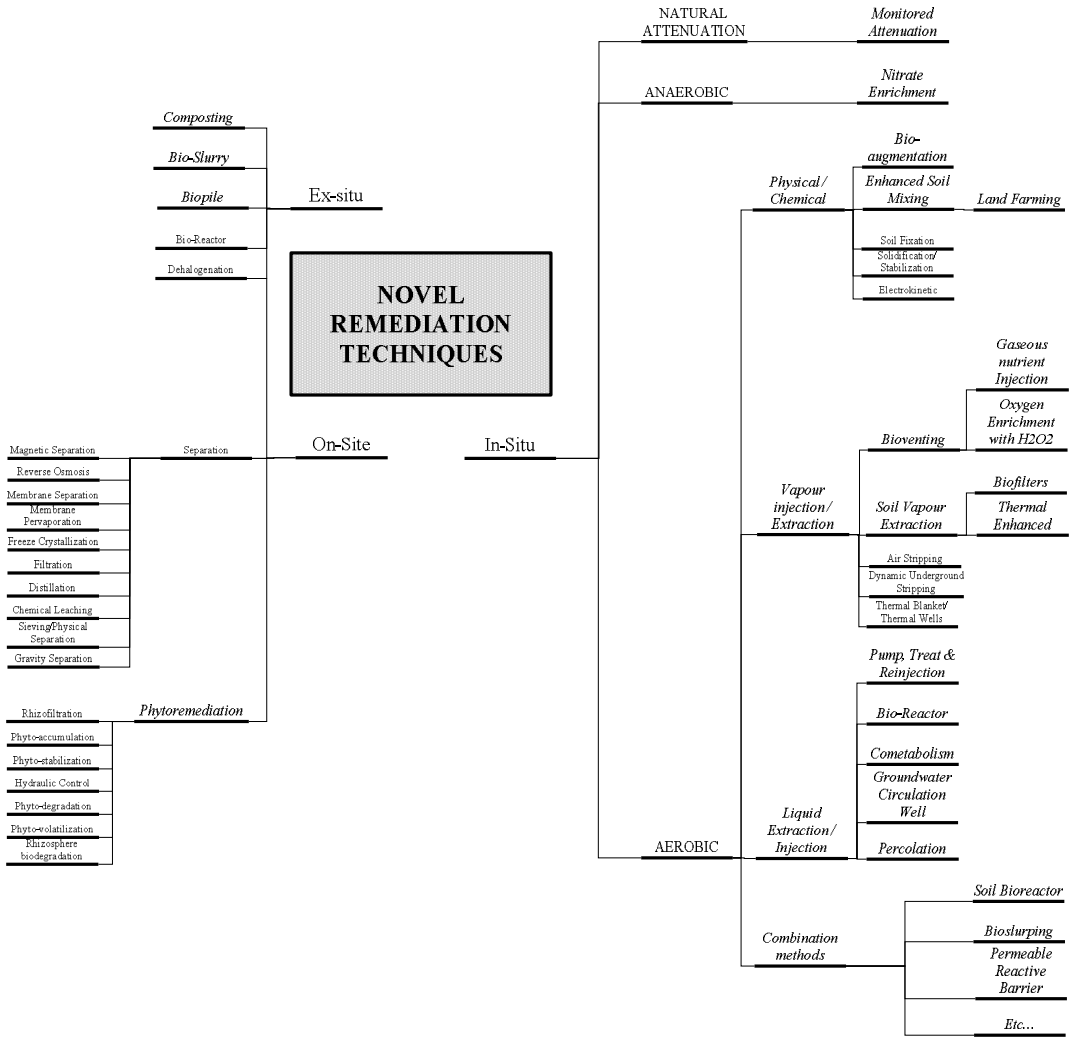


Figure 6. Many modern remediation technologies are incorrectly classified as bioremediation. The following mind map is a generalized overview of the novel remediation techniques used today with highlight on technologies accepted as bioremediation.

7.2.2. Role of bioremediation

Bioremediation is term that is applied to any systems or process in which biological methods are used for transforming or immobilising contaminants in soil or groundwater /10/. It is a set of techniques that uses micro-organisms to remove pollutants from the environment. The principal organisms in bioremediation are bacteria and fungi that have the ability to degrade hydrocarbons such as oil, coal

tar, and various xenobiotics such as pesticides. Although heavy metals can not be degraded they can be accumulated by micro-organisms and therefore removed from the environment /19/.

The role of bioremediation is defined by the environmental factors influencing natural biodegradation capability and the chemical properties of the contaminant. Bioremediation is not a universal answer to soil and groundwater remediation, as it required specific conditions to fulfil and later high tech process optimization and control throughout the process.

As noted, bioremediation is mainly used for removing organic contaminants, thought it has been found to be able to remediate a wider range of contaminants, including inorganic substances as nitrates that are classified as toxic when exceeding certain limits.

7.2.3. Separation of ex-, on- and in-situ bioremediation

The classification of techniques is done here on the basis of the location where the treatment takes place. It is by no doubt an artificial classification, but as the techniques share certain fundamental operational similarities and the classification is widely accepted in industry and literature /10/ the same classification will be used here.

Basically the division means where the treatment is conducted, weather it is done at site of pollution, when the classification is either in-situ, as the soil and groundwater are not removed from origin, or on-site, when the soil and groundwater are excavated or pumped for external treatment above ground usually on the same site. The ex-situ techniques are operated by excavating the polluted soil or extracting the groundwater for external treatment elsewhere, in this case by using biological treatment trains.

7.2.4. Why in-situ?

In-situ is usually selected on sites where the treatment by using traditional methods would lead to extensive costs. In-situ is often the **only** solution on sites where excavation is technically difficult or even impossible. Such areas may be found under or near buildings, under hard surface materials, around sewers, cables or

pipelines, at great depths and in areas of widespread contamination /45, 9/. In-situ remediation is mainly suitable for soils with sufficient hydraulic conductivity, and low to medium contamination concentration /10, 9/.

The techniques are not however without disadvantages and problems. The most chronic is the requirement for thorough preliminary site survey which requires high level of resources. Process optimization of the remediation requires constant monitoring and because the reaction conditions cannot be maintained constant, the end point may be difficult to determine. Finally the methods require extensive monitoring and the process may last for very long time periods.

If in-situ is selected as the feasible remediation technique, there should be a decision process on which technology to use on the specific site. In table 7 most common and proven in-situ bioremediation techniques are listed, with applicability to different soil and contaminant conditions.

Table 7. Representative biological in-situ methodologies and their applicability for various contaminants and soil textures

Technology name	Feasibility							Contaminants										Time
	Groundwater	Bedrock	Morein	Sand	Silt	Clay	Soil	TPH	PAH	VOC	VOC Cl	SVOC	SVOC Cl	Pesticides	Metals	Inorganics	Radionuclides	Explosives	
Natural attenuation	+	-	+	o	o	-	+	+	+	+	o	o	o	o	-	-	-	-	Many years
Enhanced soil mixing	-	-	+	+	+	-	+	o	-	+	+	o	o	-	-	-	-	-	Years
Land Farming	-	-	-	+	+	+	+	+	+	o	o	+	o	+	-	-	-	o	Years
Bioventing / Biosparging	+	-	o	+	+	-	+	+	+	+	+	o	o	o	-	-	-	-	mm -> yy
Enhanced bioremediation	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	-	Years
Groundwater circulation well	+	-	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	Years
Cometabolism	+	-	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	mm -> yy
Percolation	-	-	+	+	-	-	+	+	+	+	o	o	o	o	-	-	-	-	mm -> yy
Phytoremediation	-	-	o	+	+	o	+	-	+	+	+	o	o	+	+	+	o	+	Many years
Bioslurping	+	-	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	mm -> yy
Permeable reactive Barrier	+	-	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	Depends

+) Positive effect, field testing done

o) Effect varies, laboratory and pilot scale results

-) Negliciable effect, havent been able to prove positive results

7.3. Bioremedeable contaminants

As the table 7 shows, most techniques are not applicable on bedrock and clay, and only half can be used in conjunction without existing GW. The technologies are mainly functional with organic contaminants, not with metals, or other inorganic

contaminants, radionuclides or explosives. The typical time span for a bioremediation process is rather years than months.

As the basic principle laying within bioremediation is to accelerate the microbial growth and promote their accessibility to carbon in organic contaminants for nutrition, hence the main pollutants to be removed are organic. The currently acknowledged list of the potential contaminants for bioremediation is listed below in table 8, the table has been quoted from Evans (2003). The list is continuously changing as extensive research is conducted on the field, hence for reference of latest achievements and laboratory scale results one should reference to online sources.

Table 8. Bioremediation potential of selected contaminants /9/

Readily possible	Possible under certain circumstances	Currently impossible
Acids	Chlorinated solvents	Asbestos
Alcohols	Cyanides	Asphalt
Aldehydes and ketones	Explosives	Bitumen
Ammonia	PCBs	Inorganic acids
Creosote	PAHs	
Chlorophenols	Pesticides	
Crude oil	Herbicides	
Petroleum hydrocarbons	Fungicides	
Glycols	Tars	
Phenols	Timber treatments	
Surfactants		

7.4. Bioavailability

Bioavailability is defined as the degree to which toxic substances or other pollutants present in the environment are available to potentially biodegradative microorganisms. The rate and extent to which a compound can be mineralized defines the possible benefits of bioremediation, hence knowing the bioavailability is a key factor /20/. Bioavailability is not a function of the microbial metabolism, the environment or the contaminant alone, but it defines the interaction capability of them, the 'availability' to which the contaminant can be mineralized by the micro-organisms. There is no exact parameter used for measuring bioavailability, it is only a set of factors to be considered when considering bioremediation

Bioavailability of a contaminant is affected by numerous factors, as *environmental parameters, molecular structure, hydrophobicity, desorption, diffusion, dissolution,*

solubility, bacteria characteristics and *aging*. The pre-mentioned are by far not in any order of importance but some do affect the bioavailability more than others. /20, 21/.

The environmental factors influencing bioavailability from the micro-organisms perspective have been dealt in the previous chapters, but not the effects it has in the context of the contaminant. Environmental factors are mainly the environmental pH and temperature; acidic or alkaline conditions can cause the contaminant to precipitate and coagulate or otherwise decrease the possibility to be absorbed inside the micro-organism and temperature can affect the contaminant volatility and mobility. The contaminant chemical structure also has its effect, as for example non-polar compounds tend partition to soil surfaces /19/.

Bioavailability may also be limited due to physical entrapment of the contaminant inside the soil pore structures or due to chemical bonding on surfaces. The chemical bonds are weak and easily broken by micro-organisms, and electrokinetic techniques can aid in this, however the physical entrapment can be more of a problem /21/. According to Valdes (2000) there is a debate in the scientific community concerning the physical state of biodegradable contaminants, while some research states that only dissolved substrates are bioavailable, some say that also solid phase substrates can be degraded directly off surfaces.

Hydrophobicity affects the dissolution of a contaminant. Some compounds are hydrophobic by nature which decreases their bioavailability. In cases where the contaminant is hydrophobic, *surfactants* may be used to increase the solubility of the substance /21, 20/. Diffusion through natural organic matter can be the most important mechanism contributing to the slow release of hydrophobic contaminants /21/.

The topic under most research today in the field of bioavailability is aging, or weathering as it is sometimes called. It has been noted in numerous remediation projects and proven in laboratory experiments that the duration of bioremediation increases as the time that the contaminant is in contact with the soil increases. This means that ‘old pollution’ is more difficult to remediate than fresh pollution. The reasons are mainly due to the previously explained physico-chemical reactions of

absorption and adsorption into soil pores and entrapment into organic material, not only soil but also inside dead micro-organisms /21, 20/.

8. DATA REQUIREMENTS

The data required in designing bioremediation projects is mainly from the fields of geology, hydrology, biology, chemistry and naturally engineering. The interlinked nature of the technology requires understanding on all of the fields to build a holistic view of the site and its properties and possibilities. The requirement for multidisciplinary knowledge causes the working group to have a widespread knowledge.

Geology and hydrology are not often separated but instead dealt as one major field entailing the whole spectrum of properties required in understanding subterranean soil properties and their effect to groundwater flow. *Hydrogeology* is the part of hydrology that deals with the distribution and movement of groundwater in the soil and rocks, commonly in aquifers.

Biology and chemistry are often similarly interlinked to *biochemistry*, where the principle focus is not on chemicals, nor ecosystems, but moreover on the chemistry of living organisms, especially focusing on metabolism, which dictates the degradation of various compounds.

8.1. Hydrogeology

Site geology provides important information that has to be established prior to analysing the hydrological conditions. The geological surveys can be conducted during the preliminary investigations or separately. At some sites the geological data already exists and it only has to be linked with the other available data to form e.g. groundwater flow models.

The layout of the site vertical layout is to be defined as accurately as possible as well as the composition of soil texture in the saturated and unsaturated zones. Important geological information on soil matrix includes particle size distribution, soil homogeneity, permeability, porosity, hydraulic conductivity, humus content and soil moisture.

Geological characterization is needed to assess the uniformity of the subsurface hydrostratigraphy. The average rate of ground water flow can be estimated from the hydraulic conductivity, hydraulic gradient, and porosity. Hydraulic gradient is calculated from ground water elevations measured in monitor wells. Effective porosity and hydraulic conductivity are usually assumed based on ranges of values cited in scientific literature or estimated from pumping tests.

Besides the basic geological parameters on the soil structure and bedrock layout, the hydrological data on groundwater flow paths and speed, other physico-chemical information is to be addressed, as pH, redox and electron acceptors.

It is very important to know the site geology and hydrogeology well to determine and model the possible plume distribution of the contamination and to be able to better design the preferred remediation technology setup in-situ.

8.2. Biochemistry

The contamination source, type and concentration are required information in designing the bioremediation system. Not all contaminants are readily metabolized by micro-organisms and not all sites have rich naturally occurring micro-organism populations; hence pre-feasibility studies have to be conducted prior to system design.

Biochemistry helps selecting the most appropriate strategy to treat a specific site by considering three basic principles: the amenability of the pollutant to biological transformation to less toxic products, the accessibility of the contaminant to microorganisms and the possibilities for optimization of biological activity.

The basic data required for biochemical site characterization include the soil properties; pH, BOD, COD, Redox, electron acceptors, nutrients and most importantly the composition and size of the microbial population. Commonly, when designing bioremediation a biochemical study is made to analyze the rate and requirements for optimizing contaminant mineralization in gathered soil samples.

9. IN-SITU BIOREMEDIATION TECHNIQUES

The fundamental basis of in-situ bioremediation involves introducing nutrients and electron acceptors to the contaminated area by various methods. The main goal of the methods is to induce the natural biological activity to increase contaminant biodegradation. The major benefits from of in situ technologies arise from their low intrusion level as noted earlier.

The following chapters will explain the basic technologies underlying bioremediation in detail and provide information on different system designs. All technologies share common attributes as they are fundamentally one and same technology, based on natural biogeochemical process.

9.1. Monitored natural attenuation

Description

In nature various processes, such as dilution, volatilization, biodegradation, adsorption, and chemical reactions reduce contaminant concentrations /24/. The subterranean soil matrix behaves as a natural bioreactor that even without any augmentation is able to biodegrade most organic compounds in a long timeframe. Even if natural processes are left to degrade the contaminants, it is necessary to constantly monitor the development; hence the name, monitored natural attenuation.

Applicability and operational principles

The applicability of MNA is dependant on sufficient knowledge on the site's biogeohydrochemical properties, the contaminant properties and modelling of the concentrations on the down gradient, especially when the plume is still expanding /24/. MNA is usually not considered as an option for remediation due to the basic passive properties of the technique and the unpredictability of the process. In general, neither US EPA or SYKE encourage the use of natural attenuation, except on case to case basis when by risk analysis it can be shown that there are no possibilities for spreading of the pollution or risk for living organisms, now or in the future /39, 14/.

Target contaminants for natural attenuation are VOCs, SVOCs and fuel hydrocarbons. Pesticides also can be allowed to naturally attenuate, but the process may be less effective and may be applicable to only some compounds within the group. Additionally, natural attenuation may be appropriate for some metals when process results in a change in the valence state of the metal that results in immobilization (e.g., chromium). /24/

The operation of MNA is based on natural processes that dilute, adsorb, volatize and biodegrade the contaminant. Depending on the immobilization level of the pollutant, it is possible that further adsorption or volatilization will not happen, thought if the pollutant is immobilized in the saturated zone, the pollutant can leach, hence diluting the concentration thought spreading the plume. In case of highly mobile pollutants, the spreading of the pollution is an urgent risk but usually more mobile pollutants are also simpler in chemical form and more readily biodegradable /39, 14/.

System design

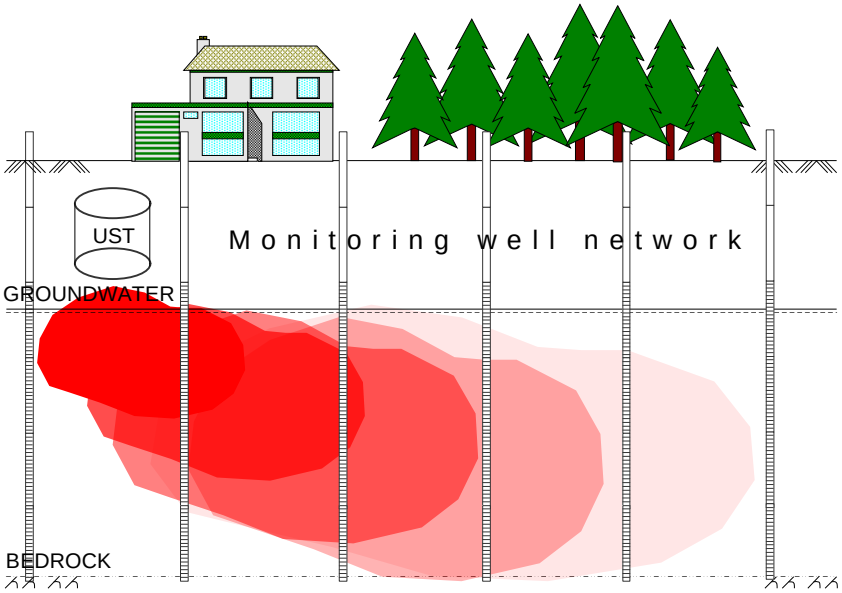


Figure 7. Characteristic system design for MNA

When considering MNA for remediation method, most important phase of the process is the preliminary studies, gathering of data to quantify and qualify the contamination size and spread. Geohydrological data needs to be acquired to sufficiently model the geological matrix and permeability of the area. Biochemical studies on the soil/water samples need to be conducted to measure the

bioavailability. Chemical analysis on the contaminant composition needs to be made to assess toxicity and migration potential should be compared against biodegradation potential to quantify the potential for spreading.

If MNA is selected as the preferred remediation method, extensive monitoring should be arranged to verify that natural attenuation is happening and the pollution does not continue spreading. The results gained from monitoring should correlate with the results from pre-made modelling otherwise re-evaluation is necessary.

As an aim, one should be able to show based on modeling and supported by actual field measurements that natural attenuation is taking place as estimated and remediation to acceptable level can be achieved in a reasonable timeframe.

Limitations and concerns

Natural attenuation has not gained public acceptance as an "active" remediation technology. The timeframe in MNA is very long and results can not be guaranteed only based on laboratory experiments, the failure risk is very high. The costs of MNA can also increase to exceed other more active methods due to intensive monitoring required and if potential risks realize, the costs can increase dramatically.

9.2. Land farming

Description

Land farming is a bioremediation technology, where contaminated soils are mixed with amendments such as soil bulking agents and nutrients, then tilled into earth. Contaminants are biodegraded, transformed and immobilized by microbiological processes and oxidation. /44/ The process is controlled to optimize the contaminant degradation by addition of nutrients and aeration. Land farming can be conducted in-situ for contaminations not deeper than 1,5 meter /10/ and for deeper contaminations the polluted soil needs to be excavated and spread ex-situ, to make the layer depth tillable.

Applicability and operation principles

LF is applicable on sites where the contamination source is close to surface and the on- and off-site leaching problems can be controlled. The soil matrix plays a significant part in LF, all soil types are feasible for processing but there should exist and impermeable layer, weather clay or bedrock, to prevent the leaching of the pollution /24, 37/.

This technology is best used on sites where the contaminants are petroleum hydrocarbons, SVOCs, pesticides, inorganics or explosives. LF should not be used on sites where the contaminants are easily volatized, due to possible vapor phase pollution excretion /14, 37/. The operation of LF is based on natural mechanisms, and when the contaminants are non- or semi-volatile, most of the contaminant degradation is due to biodegradation, not volatilization.

System Design

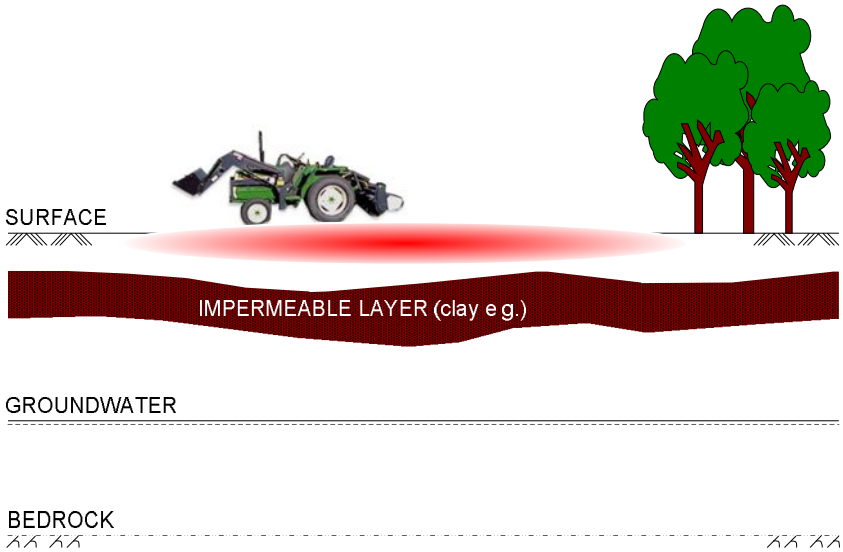


Figure 8. Characteristic system design for LF

In-situ LF systems should be designed to minimize the risk for contamination of groundwater or surrounding soil matrix /10/. If the technology is applied in-situ, it should be made sure that there is no possibility for leaching either by knowing the ground formation or installation of horizontal drainage piping, that can also assist in aeration. Usually the depth of the LF should not exceed 0,5 meters to allow sufficient tilling and aeration.

The systems operation depends on maintaining sufficient environmental conditions for the microbial population to grow. The main parameters to be controlled are: temperature, moisture content, aeration, pH and nutrients /37, 14, 24/. Moisture content is usually controlled by irrigation, aeration by tilling the soil and pH and nutrient with addition of agricultural amendments.

Limitations and concerns

In-situ LF is limited to sites where an impermeable layer exists below the contaminated layer and prevents leaching to groundwater. The system requires large 'open' system to be monitored and controlled. Temperature is difficult to control due to daily variations, as is moisture due to rainfall. These variables can effect the time required for remediation and cause risks for surrounding environment due system distortion.

If the contaminant concentrations are high enough, they can inhibit the microbial activity. LF is not suitable for soils with concentrations above 100 g/kg of hydrocarbons /10/ or 2,5 g/kg of metals /37/, hence the technology is not useful e.g. for oilfields or large accidents.

9.3. Phytoremediation

Description

Phytoremediation is a set of processes that uses plants to remove, transfer, stabilize and destroy organic and inorganic contamination from ground water and surface water /44/ Phytotechnologies have been used with good results as protective barriers or in remediation of contaminated zones. The use of the technology is more widely encourages today as there is more information on the applicability and data on positive results. Phytoremediation offers an aesthetic and low-cost remediation technique to sites with low to moderate contaminant concentrations where the pollutants are not located very deep. /19, 37/

Applicability and operational principles

PR is an applicable technology on sites where the pollution is located close to the surface. The technology is able to remediate both soil and groundwater from low-

to midlevel contamination. The technology takes a very long time to sufficiently remediate contaminated sites futures usage, and this can hinder the usage of the sites. Sufficient risk analysis should be conducted on all sites prior to beginning operation.

There are over 400 different species considered suitable for use as phytoremediators /9/. There are different mechanisms for PR to remove the contaminants; some are applicable in-situ and some only ex-situ, like artificially constructed wetlands providing on-site rhizofiltration for effluent or wastewater treatment. In the following a short survey on the possible in-situ PR techniques is given.

1. *Phyto-accumulation / Phyto-extraction* are names for basically one and same process where plants roots absorb the contaminants along nutrients and water. Mainly metals and inorganic substances that are water soluble are taken up by this process. Commonly the contaminant is not degraded, but stored in the plant roots, leaves and stems. /44, 19/

   "*As a general rule, readily bioavailable metals for plant uptake include cadmium, nickel, zinc, arsenic, selenium, and copper. Moderately bioavailable metals are cobalt, manganese, and iron. Lead, chromium, and uranium are not very bioavailable. Lead can be made much more bioavailable by the addition of chelating agents to soils. Similarly, the availability of uranium and radio-cesium 137 can be enhanced using citric acid and ammonium nitrate, respectively*." /32/

   Thought some plants can accumulate heavy metals in their tissues, some, called *hyperaccumulators*, are able to accumulate as much as 1,5% of dry biomass concentration /9/. These are the plants that are the focus of current technological genetic investigations. The benefits of using plants in accumulating close surface metal concentrations is obvious; they could be used for roadsides, industries, 'green' city centers, etc.

2. *Phyto-stabilization.* In this process, chemical compounds produced by the plant immobilize contaminants, rather than degrade them. Green plants have been used for ages for prevention of erosion and stabilization of soil.

Basically, green plants are able to excrete chemical compounds that immobilize the contaminants in the rhizosphere, either on- or in- the roots. Plants do not have the capability to biodegrade metals, but studies have shown that the rhizosphere bacteria can convert heavy metals into less toxic forms, e.g. Cr(VI) to Cr(III) /19/.

3. *Hydraulic Control* is a process where plants act as hydraulic pumps and lift the water from the groundwater table with their roots to bring up water and nutrients. The drawing of water upwards through the soil into the roots and through the plant to atmosphere decreases the movement of soluble contaminants down- or forward. Plants have the capability to affect groundwater flow; especially trees have large root biomass and *transpiration pull*. Evans (2003) among others quotes that poplars for example have very deep roots extending to even 15 meters of depth and transpire up to 1100 litres per day. In an EPA study /37/ a riparian corridor 'a buffer strip' engineered from poplars showed to decrease the nitrate concentration in ground water at the edge of a corn field from 150 mg/L to 3 mg/L after the buffer zone, while also retaining toxic herbicides and pesticides.

4. *Phyto-degradation* which is sometimes alternatively known as *phyto-transformation* involves the biological breakdown of contaminants, either internally or externally, using enzymes /9/. In this process, plants actually metabolize and destroy contaminants within plant tissues or biodegrade them to simpler substances that are then incorporated in the plant vacuoles /9, 19/.

   Plant degradation of herbicides and pesticides has been studied in agriculture for a long time. In the 1990's the focus has transferred to plant metabolism of TCE, TNT, PAHs, PCBs and other chlorinated substances and recently some plant cell structures have been shown capable of degrading nitro-glycerin /19/. This suggests that plants have the potential to degrade various environmental pollutants and through biotechnology these properties could possibly be isolated.

The phyto-degradation capabilities of TCE by poplars has been studied extensively /19, 2/, it has been shown that poplars are able to absorb TCE in water and biodegrade it almost fully, only respirating less than 5%.

5. *Rhizosphere biodegradation* is a process where the plant releases nutrients through its roots as a product of photosynthesis that enhance natural microbial biodegradation in the rhizosphere. The biodegradation is mainly applicable for organic contaminants at low concentrations. This is a form of enhanced natural bioremediation.

6. *Phyto-volatilization* is property of some plants to convert metal ions and organic contaminants to more volatile forms and release them through the stomata /19, 44/. Commonly known phyto-technological pairs (besides poplars) are MTBE and eucalyptus, selenium by Indian mustard, methyl mercury and tobacco and the list continues. /19/

System Design

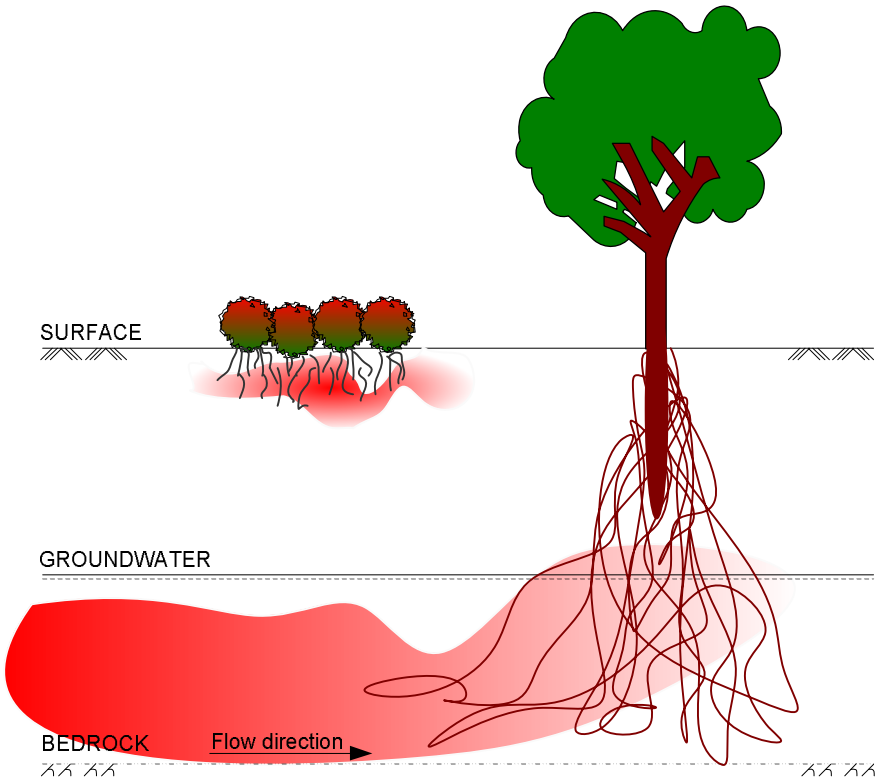


Figure 9. Characteristic system design for PR

The phyto-technological remediation systems are usually designed for specific contaminants and environmental conditions. The major criteria for plant selection are the desired remediation method and the nature of the contaminants /19/

On some sites planting grass varieties with trees to protect the soil, maybe the best route since they generate a tremendous amount of fine root near surface. This particularly suits the transformation of BTEX and PAH compounds /9/. Similarly, on some sites selecting deep rooting plants that grow fast and are easy to maintain and are capable to degrade e.g. chlorinated compounds planting trees or willows might be the choice.

Phytoremediation is considered as a 'novel and innovative' technology that has not yet established its status /9/. Many pilot scale studies has been conducted and some full scale successful remediation projects have also been carried out, there still is not enough data gathered to exactly predict the performance prior to actual remediation.

Limitations and concerns

Phytoremediation has not gained public acceptance as a active remediation technology, but is has a very 'green' image and could be very well used e.g. in last phase treatment in a treatment train combined from biological and non-biological remediation technologies.

It is still unknown what ecological effects hyperaccumulator plants may have if ingested by animals. If these contaminants start bioaccumulating in the foodweb, what is their effect to the ecosystem. Also fallout of plant tissues in autumn may re-enter the food chain or contaminate the soil again, depending on site. There are open questions on phyto-volatilization, as do volatilized contaminants remain at 'safe' levels in the atmosphere or are they possibly at toxic levels already when volatized. Most importantly, sufficient risk assessment has to be made prior to selecting phytoremediation as favored method, as due to the timeframe required by the technology, the exposure of the ecosystem to contaminants is prolonged.

9.4. Bioventing and biosparging

Description

Bioventing and biosparging are techniques where oxygen is pumped to the unsaturated or saturated zone, respectively. The principles are similar to widely used soil vapor extraction (SVE) as all aim at stimulating the underground airflow. However, when SVE is designed to maximize contaminant volatilization, BV and BS are designed to maximize contaminant oxygen contact to increase microbial enhanced mineralization /2/.

Applicability and operational principles

BV and BS are applicable on sites where the contamination is located deeper in the subsurface. Bioventing is used for treatment of contaminants in the unsaturated zone and biosparging for contaminants in the saturated zone. The main geological factor affecting their usage is the soil matrix, mainly the hydraulic conductivity which affects the aeration potential.

The benefits of BV and BS are that the systems can be installed inside buildings at already constructed areas and they do not cause distortion to the current activities on site. When designing the same precautions as with SVE should be considered so the operation does not cause volatized contaminants to spread around. e.g. in basements, which can be controlled by combining the method with SVE wells.

These technologies are best suited for contaminants that are not easily volatized, as the aim is to biodegrade the contaminants. The technology has been effectively used for treatment of petroleum hydrocarbons, PAHs and semi-volatile compounds. /2, 10, 37, 24/. Bioventing and biosparging are the most used bioremediation technologies worldwide and the US AFCEE has studied them widely and determined them as the most feasible technologies for the treatment of UST leakages. /24, 34/.

The main principle of the technology is to lead air underground through an aeration pipe network through the contaminated zone to provide sufficient oxygen delivery for mineralization. Biodegradation capability is also affected by moisture and

nutrient content, thereby both are often added in both bioventing and to a degree also in biosparging, thought moisture is hardly a factor with the latter.

System Design

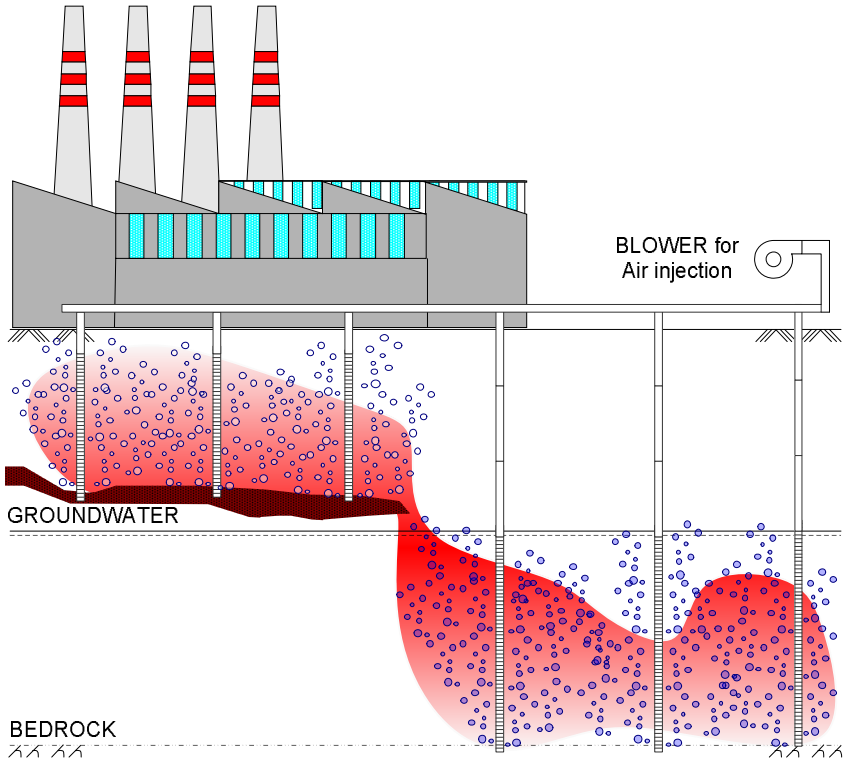


Figure 9. Characteristic system design for BV and BS

Bioventing

Bioventing systems are used on soils with hydraulic conductivity more than 10e-5 cm s-1 /10/. The principal design factor is to verify that oxygen, moisture and nutrients are provided throughout the contaminated zone /10/. Oxygen is forced with a high pressure blower into the soil matrix on low injection rates to prevent possible volatilization but to maintain sufficient aeration. If the contamination is located on shallow sites and the site typography is compatible, percolation can be used for nutrient and moisture controlling, if not nutrients can be added.

The required oxygen flow rate can be calculated mathematically on the basis of preliminary studies on microbial community structure, namely maximum microbial oxygen demand has to be prior analyzed. Second important factor in the design layout of BV system is the design of well layout. Well layout is usually determined

by dividing the horizontal area of contaminated zone by the influence area of a single venting well. The radius of influence (ROI) for a single bioventing well can also be determined either quantitatively by measuring airflow at surrounding wells, or mathematically. Usually the well spacing on field are 1 to 1,5 times the ROI and the radius can vary from 1,5 to 30 meters /2/.

BV vent wells are constructed by either installing them horizontally or vertically, depending on depth of polluted zone and site geology and above ground constructions. The wells are installed as standard procedure groundwater (GW) well installations. The installation piping used is usually 10,2 cm-diameter slotted PVC pipe used in landfill applications /2/ in Finland the normal piping is 52/60 mm HDPE piping used in GW monitoring wells. The sloth size of the piping varies based on soil texture, but most commonly used is 0,3 mm slots. The installation holes are drilled to desired depth and slotted piping is installed to the whole length of the contaminated zone and extended a meter deeper when applicable. The interstice is filled with installation sand (*silica*) and near the ground level bentonite is used to seal the hole to prevent blow-by in the operation phase. The above ground installed piping manifolds are usually hided into shallow covered trenches or connections are drawn so that normal site operation can continue undisturbed. US EPA has developed accurate instructions for BV and BS well designs and installation procedures, which can be found e.g. from Atlas (2005).

BV is a technology that lends itself to combination with other soil remediation technologies. The complexity of the subsurface sometimes dictates that no single technology is suitable on its own /2/. As BV increases the subterranean airflow it increases the possibility that contaminants are volatized.

Biosparging

Biosparging systems are similar on construction and operational principles to Bioventing. The main difference is the operation zone; when bioventing is used above GW-level in the unsaturated zone, biosparging is oppositely used in the saturated, below GW-level zone. The airflow rate should be higher in BV systems to increase the oxygen saturation in water, but not as high as in normal *air sparging* systems where the aim is to volatilize the contaminant compounds. There has been

evidence showing that increasing the airflow in biosparging systems from the moderate required for sustaining microbial activity to doubled flow increased the cumulative mass removal by a factor of two to three /2/. Atlas (2005) references a study where air had been supplied with high flow in pulsed injections for a short while and then shut off, but the air had still continued to be supplied to the aquifer for a day. This method delivers both the high flow advantages of air sparging and satisfies the lower airflow requirements of bioremediation.

Biosparging is a new technology and before air sparging has been the dominant method of removing volatile contaminants from the subsoil saturated zone /10/. Biosparging is able to mineralize more contaminants than air sparging as the technology is not only confined to volatile compounds, but also petroleum hydrocarbons, PAHs and SVOCs can be remediated.

BS wells are installed similarly to previously explained BV wells. The installation can be conducted either horizontally or more commonly vertically, as usually is due to installation depths. The installation depths are often deeper and the existence of groundwater causes the pumping pressures to be increased due to increase in hydrostatic pressure. Also due to more variable subterranean conditions than in BV, the individual injection wells can be equipped with pressure gauges and valves to individually control the spread of oxygen. Well spacing is similar to BV, namely from 1,5 to 30 metres, depending on hydraulic conductivity and pollution concentration /2/.

BS is often used in conjunction with other techniques as SVE and enhanced bioremediation techniques as pump, treat and re-inject. Alternatively, methane can be used as an amendment to the sparged air to enhance cometabolism of chlorinated organics /24/. Nitrate is often used as an injection gas to produce anaerobic conditions instead of oxygen. That has been evaluated benefits of anaerobic conditions in the degradation of certain compounds, especially chlorinated solvents. /24, 45/

Limitations and concerns

When conducting BV or BS based remediation on sites with nearby basements or similar constructions the possibility of vapor phase contaminants spreading should

be analyzed and minimized. A common technique is to install SVE extraction wells close to the constructed areas or surrounding the contaminated and remediated zone. The extraction of air underground increases the borderline airflow and catches the non bioremediated contaminant vapors to above ground treatment, either by biofiltration or more commonly GAC.

BS should not be used on sites where there is a free phase contaminant as the risk of spreading the contaminant in the whole water body increases.

The most limiting factor for both BV and BS technologies is the soil matrix and heterogeneity. Neither can function on soils where the hydraulic conductivity is less than 10e-5 cm s-1 /2/, hence the technologies are constrained to sandy soils. If impermeable soil layers exist in the treatment area it can be that those cannot be treated, and depending on the estimated pollution and risk assessment, it might be necessary to excavate them which would lead to re-consideration of the technological feasibilities of BV and BS.

9.5. Bioslurping

Description

Bioslurping is a remediation process that combines elements from bioventing and vacuum enhanced pumping of LNAPLs. Bioslurping lifts LNAPLs off the water table and from the capillary fringe without lowering the oil-laden water table into clean soil /36/. Bioventing is achieved in the unsaturated soils as air replaces the soil gas that is removed via the recovery well and stimulates aerobic bioremediation /44/. When LNAPL removal is finished the system is easily transformed to a regular bioventing system to complete the remediation.

Applicability and operational principles

Bioslurping systems are applicable on all sites where the contamination is found as a LNAPL on top of the groundwater, which can be collected individually. Usually the removal of LNAPLs is done by oil skimmers, but bioslurpers generally result more then two times the LNAPL removal volume /33/. Bioslurping is used to remediate soils, as well as groundwater. It can also help to remediate soils

contaminated with VOCs and SVOCs. It is applicable at sites with water tables deeper than 10 meters /32/.

System Design

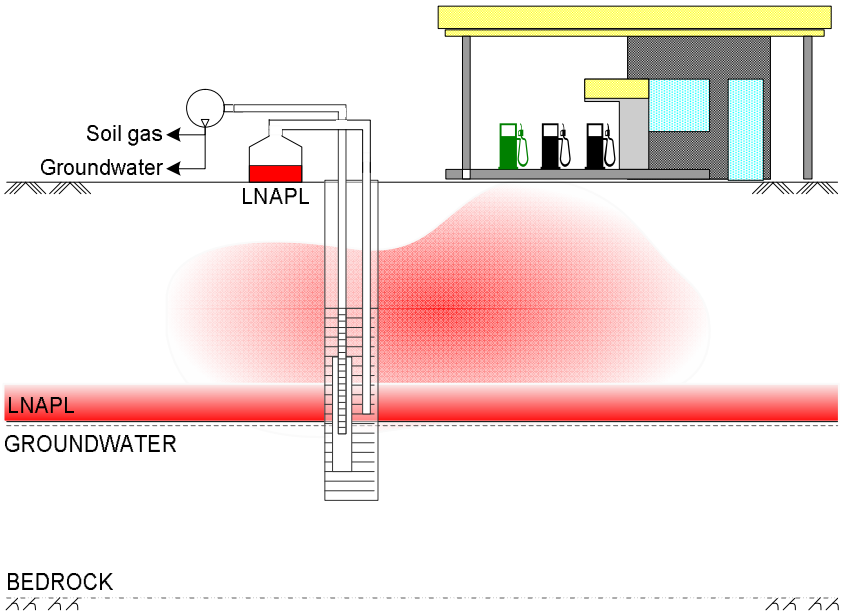


Figure 10. Characteristic system design for bioslurping

There are different techniques for the Bioslurping system, mainly with the difference being the collection method of LNAPLs, water and soil gas. The older system are constructed on basis of a single 'slurp' tube that collects all three into a single influent pipe which leads to the aboveground treatment facility. There first the air and liquid are separated after which the water and oil are separated. Next step in the process is to clean both air and water to reach dischargeable contamination levels. /24/

More modern implications of the bioslurping technology have been developed in the last years to increase the simplicity and operational certainty of the system. Emulsion usually forms in the vacuum piping of the old 'single drop-tube system' as the oil and groundwater are subject to the mixing. The potential for the production of these solids and emulsions should be significantly reduced if LNAPL and groundwater can be separated in the well prior to vacuum extraction. The in-well 'dual drop-tube system' provides an effective means to achieve this goal. A single aboveground vacuum pump is used to enhance the subsurface migration of LNAPL to the extraction well, which is similar to the conventional single drop tube

design. However, with the dual drop tube design, LNAPL and groundwater are extracted from the well in separate streams through two separate drop tubes. /2, 29/.

The benefits of bioslurping system compared to normal pump-and-treat or oil skimming are the increased recovery rate, hence decreased treatment time /29/. Bioslurping system also separates the oil and water already in-well, so there is not need for as effective treatment of water as in pump-and-treat. The decreased amounts of lifted liquids also contribute to decreased expenses. The effectiveness of bioslurping can be increased by addition of normal bioventing or biofiltration techniques in the process.

Limitations and concerns

In bioslurping, as in all remediation techniques, the preliminary data gathering is important. The geohydrological conditions on site affect the effectiveness of the bioslurping as the bioventing and SVE effect are not as effective in low hydraulic conductive soils. The biochemical properties of soil, as pH, moisture and nutrient concentration effect the biodegradation, and aerobic biodegradation of many chlorinated compounds may be limited unless there is a co-metabolite present /2, 24/.

Bioslurping systems have a difficulty establishing a vacuum on deep, high permeability sites /32/ and in reference to all previous concerns, the accurate placement of extraction point is a key to the success of bioslurping.

9.6. Enhanced saturated zone bioremediation

Description

Enhanced bioremediation is technique which aims at enhancing the natural conditions to optimize the contaminant degradation in-situ. The method utilizes the naturally occurring microbial populations, but *bioaugmentation* can also be applied when necessary /14/. The principal aim of the technology is to make the subterranean soil matrix function as a bioreactor. In typical enhanced groundwater bioremediation systems, groundwater is extracted using one or more wells and if necessary, treated to remove residual dissolved constituents. The treated

groundwater is then mixed with an electron acceptor, nutrients and other constituents if required, and re-injected upgradient of or within the contaminant source /37/. In an ideal system, the EB would operate as a closed-loop where no external microbes or water is required and everything that is extracted, is also re-injected.

Applicability and operational principles

ESB can be applied to sites where the hydraulic conductivity of the aquifer is high and homogenous enough to enable the even distribution of electron acceptors and nutrients in the subsurface. If the soil matrix is not homogenous, it is difficult to estimate the flow paths of the GW. The contaminant has to also be dissolved in groundwater or adsorbed to onto saturated soil matrix within the aquifer to be readily available for microbial degradation.

The technology is feasible for contaminations at any depth within the aquifer, and mainly for organic compounds, including petroleum hydrocarbons, PAHs, Cl and non Cl-VOCs and SVOCs.

The basic operational principle of EB includes extraction of groundwater and re-infiltration after the water has been treated and enriched with nutrients and electron acceptor. The method can be applied in a number of treatment modes, including: Aerobic (oxygen respiration); anoxic (nitrate respiration); anaerobic (non-oxygen respiration); and co-metabolic /37/. The aerobic method is usually most efficient with petroleum hydrocarbons and PAHs, when anoxic, anaerobic and co-metabolic are used in remediation of other compounds, such as chlorinated solvents /37/.

The groundwater oxygenation is usually done by direct sparging as in BS or the water is aerated prior to re-infiltration by bubble saturation or addition of hydrogen peroxide H_2O_2 /37, 10/. The nutrients are also added prior to re-infiltration, mainly nitrogen and phosphate. A key design factor is to verify the even distribution of nutrients and oxygenated water to the remediated zone.

System Design

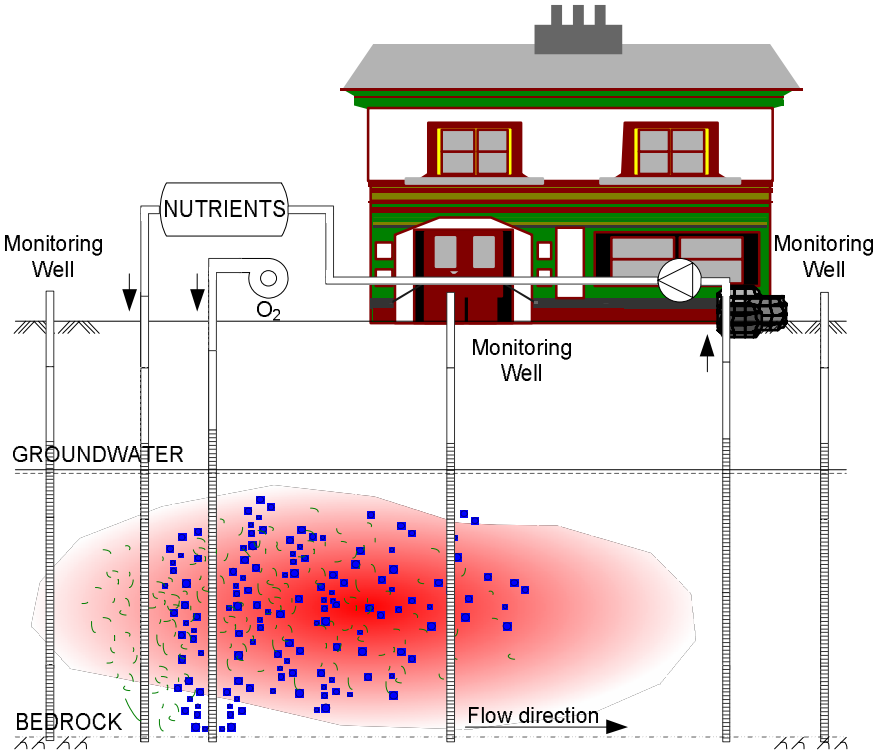


Figure 8. Characteristic system design for ESB

EB systems should be designed very carefully to confirm the applicability of the remediation method to the site in question. Prior to considering EB as remediation method, extensive geo-, hydro- and microbiological testing has to be made. The site has to be geologically evaluated and the groundwater flow mapped. Water and soil samples should be taken for biological testing of biodegradation capability of the naturally occurring microbial population.

The system layout is constructed on the basis of the preliminary data gained from the pre-feasibility studies. The system should be dimensioned to accommodate the GW volume, the contaminant concentration and location and spread. Operational principle requires in the minimum system a setup of extraction and infiltration wells installed at designed locations and aboveground system that facilitates pumping and nutrient and electron acceptor addition. The system can be modified to facilitate various conditions and increased to treat larger areas if necessary, due to modular design. The technology can be combined with in-situ biosparging to inject the oxygen in-situ instead of aerating it aground.

The extraction and injection of water affects the subterranean groundwater flow. To make sure that the contamination plume is not spread due to the changed hydraulic conditions, it is necessary to maintain sufficient control over the system flow. Without adequate hydraulic control, this situation can lead to worsening of the original condition and complicate the cleanup or extend it /37/.

The system should be designed so that the extraction wells are located downstream from the contamination and injection wells upstream. The location of the wells should be designed so that the injected, enriched water is able to freely flow to all sections of the contaminated site, similarly, the extraction wells should be located so that the natural flow direction will not change. The pumping and infiltration volumes should be minimized to prevent changing the natural system.

Limitations and concerns

The location, distribution, and disposition of petroleum contamination in the subsurface can significantly influence the likelihood of success for bioremediation. This technology generally works well for dissolved contaminants and contamination adsorbed onto higher permeability sediments. If the concentration is located in the unsaturated zone, in clay or other low hydraulic conductivity fractions of the soil or outside the 'enriched' zone, the technology will not function properly and other solutions should be considered /37, 10/.

A current field of research is in system functioning under excessive concentrations of calcium, magnesium, or iron in groundwater. These metals can react with injected nutrients, namely phosphate or oxygen and form precipitate. The precipitate has been noted to cause scaling that clogs the infiltration pipes and can damage pump systems which causes distortion and extra costs for the operation /37/.

9.7. Permeable reactive biobarrier

Description

Permeable reactive biobarriers have been successfully used for containment and remediation of pollutant plumes. The aim of these technologies is to constrain the pollution at source to protect surrounding areas or groundwater from effects of

other remediation techniques /2, 37/. Novel technologies have been developed for cases where there are large, widely distributed plumes that are not easily accessible. Enhanced bioremediation systems may be configured as permeable reactive biobarriers that intercept and treat contaminant plume Biobarriers typically consist of substrate injection wells or a solid substrate injection trench located perpendicular to the direction of groundwater /35/.

Biobarrier technology has been applied only on few sites and is not commonly documented technology. Usually these reactive barriers have been constructed by using zero valent iron or other inorganic reactive material /28/ that has been dug underground and through which polluted GW is then diverted. Biological barriers give possibility to protect and manage larger plumes with less costs and can be more easily built and operated on areas otherwise unacceptable.

A more detailed description is offered in the case study enclosed as part four of this thesis.

10. MONITORING AND QUALITY CONTROL

For bioremediation to be successful there has to be sufficient proof of the detoxification of the contaminants, preferably proven by complete mineralization /20/. Rigorous, well documented and successful remediation projects lay the foundations in building societal confidence and practitioner competence. Until the technology is tested and proven, careful emphasis on monitoring the functioning of bioremediation systems is required as they are, in some situations, inappropriate or unreliable /21/.

As Valdes (2000) notes "*The fundamental paradigm for verifying bioremediation technology begins by modestly admitting that both micro-organisms and their habitats are incomplete puzzles*".

10.1. Verifying bioremediation

As stated above, the expected endpoint of bioremediation is the de-contaminated site. This we can be verified only at the end of the process, and only indirectly by measuring the contaminant concentrations. Still, we cannot be sure how it is remediated or has it just disappeared (e.g. volatized or by abiotic means). How do

we know something is happening in the soil matrix, how do we verify that bioremediation is in action?

The NRC (national research council) released in 1993 “In Situ Bioremediation: When Does it Work?” where it recommended a three issues for verifying bioremediation, which are as follows:

1. documented loss of contaminants from the site,

2. laboratory assays showing that micro-organisms in site samples have the *potential* to transform the contaminants under the expected site conditions, and

3. one or more pieces of evidence showing that the biodegradation potential is *actually realized* in the field.

The principles recommended coincide well with the commonly recognized and applied methodologies and conceptions on bioremediation verification, and no doubt that the report has had significant impact on the development of monitoring and quality control of bioremediation. Generally, the process has to make sense; there needs to be consistency, redundancy and convergence of all types of evidence from many scientific disciplines /21/.

Documented loss of contaminants

The site has to be continuously and vigorously sampled to establish an empirical track record of the development of the remediation process. The samples are usually analyzed in analytical laboratories according to proper international, national or internal standards.

Very often this is the only issue in bioremediation that is considered as important, but as mentioned, this only provides information on the current contaminant concentration, not on the process itself nor can it be used to measure the effectiveness of bioremediation. It is understandable that the main concern in the context of legislation is the decrease of contaminant concentrations to legally acceptable levels, but it does not verify that the compound is mineralized /20/.

Laboratory assays on the contaminant bioavailability

As a part of preliminary feasibility studies on site considered for bioremediation, laboratory scale bioavailability studies should be conducted. This means sampling the site soil and groundwater to replicate with fidelity the in-situ conditions in laboratory, because if the research is done with optimal conditions, the results will not apply to the site as conditions which will significantly differentiate from the laboratory setup.

General methodology for the experiments is quite simple. A known weight or volume of the sample is measured in similar containers, the container are equipped with measuring apparatus to monitor the respiration rate (O_2/CO_2 ratio) and incubated in laboratory conditions /20/. Samples can be provided with differing concentrations of nutrients and electron acceptors to quantify and qualify their effectiveness in the engineering measures on remediation. For a good listing of available estimation methods for biodegradation potential, please refer to 20, 2003.

When the bioavailability and microbial potential for bioremediation has been shown, the samples should still be imposed to chemical and biochemical test assays to verify that required level of mineralization and full detoxification has occurred.

10.1.1. Realization of biodegradation process in-situ

The final step in verification of bioremediation is to show based on multidisciplinary evidence that the estimated biodegradation potential is actually realized. In gathering data to ultimately proof and intermediately to optimize the bioremediation process, various techniques can be used, some more resource intensive than others. Commonly the measuring techniques are based either on (i) detailed knowledge on specific microbiological processes, (ii) computer modeling or (iii) mass balancing the contaminants, reactants and products.

Analyses based on specific microbial processes

The measurements based on microbial processes include methods from simple evaluation of the size of bacterial population (MPN, Plating, BIOLOG, etc.) to adding isotopic tracers for evaluation in respiration products. The methods offers generally a very good picture of the extent of the in situ biodegradation, but are

very laborious to conduct. Modern biotechnology is developing constantly new tools for simplified measurement of bacterial capabilities without extensive sample pre-treatment. One of the upcoming techniques is 'bacterial sensors' /20/ which are based on using reporter genes for characterization.

Modelling

Modelling can be used both as a design tool as well as a verification tool in bioremediation. Models consider quantitative aspects of fluid flow, dilution, sorption, volatilization, mixing, microbial growth and metabolic action rates /21/ to predict the development of remediation in the subterranean. It should be remembered that a model is never a true image of reality as it is a generated on the basis of measured data. The main usage of a model in bioremediation verification is that when the model is generated and the process development is inputted to the model, real-life measurements can be compared against the model and recognized weather the bioremediation is proceeding as designed.

Mass balancing

Under well defined hydrogeologic regimes, fluxes of water, contaminants and electron donors and acceptor can be quantified between sampling stations /21/. Site specific gradients of electron acceptors and metabolic end products can be observed inside the remediated site. Thought, the stoichiometric amounts should be high within the zone of increased microbial activity and smaller outside the contaminated area /21, 20/.

10.1.2. Simple field measurements for analysis

As most of the previously mentioned methodologies for verifying bioremediation require laborious analytical measurements and preparations, there are some field measurement methods that can be used to analyze the biodegradation potential in-situ.

Commonly taken measurements in-situ include redox, O_2, pH and temperature. The most important results from these are gained from the redox and oxygen concentration, which tell very straightforward weather there is on-going remediation. The redox potential shows indirectly the remediation activity

(depending on method, naturally). In aerobic respiration the redox values are always positive, and the higher the redox, the more microbial activity is present. As the redox value decreases, it indicates that the biodegradation is also slowing down and the microbial population is decreasing in size. When there redox values are negative, commonly the micro-organisms are utilizing fermentation as metabolism.

Oxygen concentration verifies weather there is, or isn't, sufficient oxygen levels for aerobic or anaerobic metabolism. The oxygen concentration can be also used as an indicator for microbial activity, similarly to BOD method. In aerobic systems, either samples are collected or the air sparging is stopped and the probe is inserted to the sample or ground water wells to measure the rate of oxygen consumption. To distinguish oxygen used by contaminant-degrading microbes from oxygen used by ordinary microbial activity, background oxygen uptake rates should be measured in adjacent uncontaminated wells. Relatively rapid oxygen loss in the contaminated area compared to the uncontaminated area, coupled with a drop in the contaminant concentration, suggests successful bioremediation. /40/

10.2. Quality control

The general aim of quality control is to control the remediation so that everything is done according to plans. Proper quality control realizes the possible faults in plans or lapses in realization and provides a possibility to tackle these issues if necessary. The quality control makes sure that the set goals are met and the result is sustainable. Also the possible harms to humans and nature during the process are controlled and eliminated. /13/

The tools for quality control are standardising, planning, trained and professional employees, high quality supply chain and external supervision.

Before remediation project is engaged, a quality control document should be established and agreed upon amongst all parties. The document should contain information on the materials used, their handling and protection during construction. The key components of the equipment have to be tested and quality control log recorded. All employees have to be informed on the content of the quality control document and the issues have to be taken in consideration during all phases of installation and operation phase work /17/.

Sampling has to be designed and a detailed document on the procedures and locations to be sampled is to be produced. The plan has to include detailed information on sampling times, locations, used methods, sample handling, analysis methods, quality control and reporting. Detailed guidelines for sampling are provided in various literatures, thought generally the sampling should only be conducted by a certified person. /13/

The final chain in the series of quality control tools is the independent, external supervision. The process should supervised from planning to sampling, with special emphasis on the construction period, as this is the time when most critical flaws are made. The supervisor should not be economically interconnected with the companies conducting the remediation nor to the owner of the site.

Quality control is an important part of the everyday operations for most large companies today. Especially in the environmental field, when working with novel technologies, special emphasis should be given for these issues.

PART IV

Case study: Biowall -pilot

11. PROJECT INFORMATION

11.1. Introduction

In year 2004, Ratahallintokeskus and Kapiteeli Oyj with Ramboll Finland Oyj as consultant, begun discussions with selected Finnish high-tech soil and groundwater remediation companies to develop and test new methodologies for soil and groundwater remediation; the selected companies were Doranova Oy, Nordic Envicon and Envitop Oy with Niska & Nyyssönen Oy. The aim of the discussions was to develop a cost effective strategy (BATNEEC) for remediation of a creosote contaminated site located in the same groundwater region that is extracted by Pursiala municipal water plant in the city of Mikkeli, Finland.

The contaminated site had been sold by RHK (Ratahallintokeskus) to Kapiteeli Oyj (both government owned institutions) in 2000, and with the sales all rights and liabilities were transferred. All involved parties, including Kapiteeli, City of Mikkeli and Road Administration hold as their opinion that the legal and economical liabilities concerning the remediation of the site fall under the respective responsibilities of VR who had been the owner during the time polluting occurred. The approximated amount of costs for the full scale remediation (that were already taken in notice when the property was sold in 2000) are approximately 3,2 M€.

During the years 2003-2004 a preliminary survey on the type and extent of the pollution on the site was conducted. The survey focused on locating and quantifying the amounts of PAHs in the soil and groundwater in the area. The survey was conducted by Ramboll Finland Oy (previously known as SCC Viatek

Oy). During this period also a biodegradation study on the soil and groundwater samples acquired from the site was conducted by the VTT Technical Research Centre of Finland.

After the preliminary surveys were conducted, the aim was to test and analyze novel remediation methods in a pilot field trial during year 2005, before selecting the best technologies (BATNEEC) for the full scale remediation. Three different methodologies were selected for pilot scale operations, of which Doranova tested a "Biowall" technique, Nordic Envicon "In-situ bioremediation enhanced with electric-osmosis" and Envitop Oy tested "pump and treat" with various reactive filter materials.

In the selection of the remediation technologies used for the full scale remediation planned for 2007-, emphasis is on the end results, feasibility, risks, environmental effects, duration and costs of the technologies. A special focus is on protecting the water quality, as the city of Mikkeli has a legitimate fear on the security of their municipal water supply.

11.1.1. Historical information

An old wood preservation facility has been operated in Pursiala, in the city of Mikkeli (site n:o's. 491402134, 49140251M602 and 49140251M603) during the years 1905…1920-1982. The facility has been owned and operated by RHK and has mainly processed railway sleepers with creosote. Today the old site is located in an important area for Mikkeli, as it is in the groundwater flow path to their municipal raw water pumping station (see map 1). The operations that have been conducted on site for decades have caused widespread pollution of soil and groundwater. The pollutants are mainly derivates of creosote; mineral oils and PAH compounds.

During the final years of operation, the factory impregnated wood approximately 14-15000 m3/a, and the amount of creosote used was about 1300 m3/a. In year 1959, phenol pollution was found on the site, which alarmed the owners to modify the site by covering the sleeper trickle plane and storage facilities with asphalt which had previously been gravel surfaced. During the following years of 1960-

1961 tens of thousands of cubic meters of polluted soil was removed. Later in 2002 more soil was removed by excavation from the opposite side of V5.

11.2. Site background

As presented in map 1 the site is located near the central are of city of Mikkeli and the property is currently zoned for industrial use. In the location of the old impregnation facility area today is located highway 5 an important connection road and the eastern railroad. The 'hot-'spot' has not been constructed yet but there is pressure toward utilizing this property due to its central location.

The area which has not been remediated (north side of highway 5) has elevated contaminant concentrations in the top layers to the depths of 1-3 meters, but also as deep as 25 meters, bound to the soil matrix and as DNAPL on top of the bedrock.

Highest amounts of contaminants have been found from the swamp located close to the old impregnation facility (see map 1, black box). It has been suggested that during the operating period of the facility, waste creosote has been lead to the swamp.

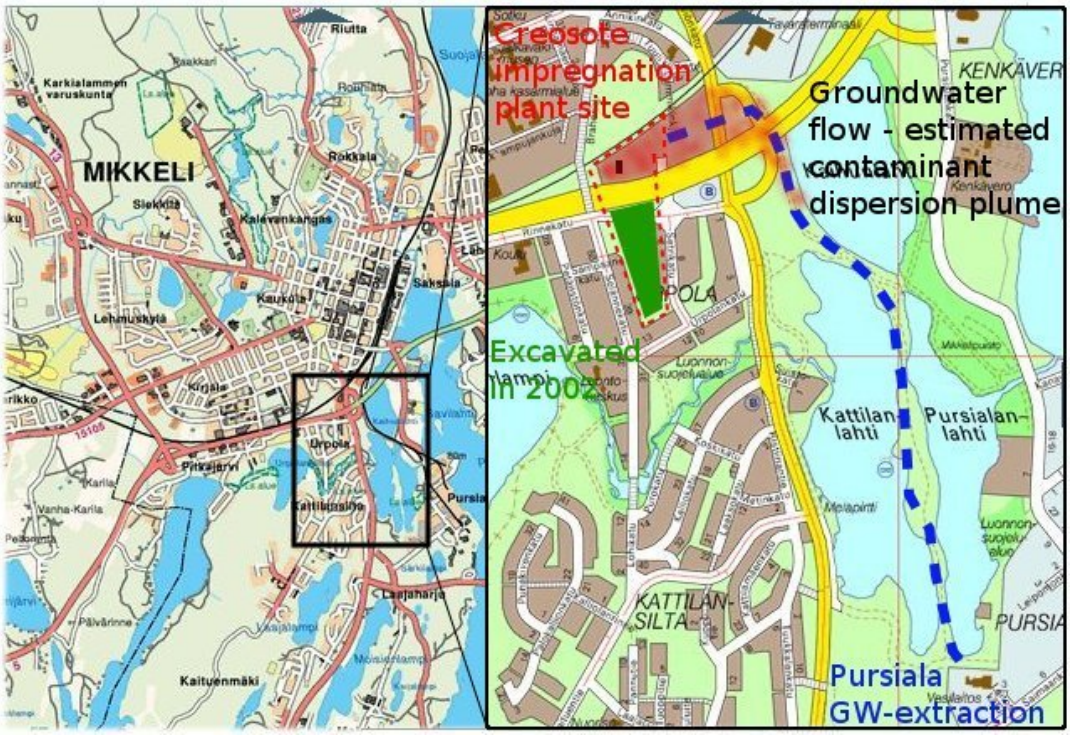
Due to the difficult location of the contamination, it is not possible to excavate the creosote contaminated soils. Railroad, roads, plumbing and buildings inhibit this and therefore other technical solutions are necessary for successful remediation.

11.2.1. Geohydrological data

The contaminated site is located in the pathway of groundwater flow which ends up in the Pursiala water utility extraction site approximately 1,5 kilometres south-east of the old impregnation plant. The groundwater flows through the so called 'northern route' by first heading east and then turning south toward the Kaijunharjun-Kaijanniemi eskers through which it continues straight to the water utility. By modelling, it has also been estimated, that it might be possible that there is another route, the 'southern route', which would basically be direct south-eastern pathway to the utility.

The site geology is not uniform and there are ruptures in the bedrock which can divert and collect the creosote DNAPL flow. The soil in the area is mainly sand

and moraine, and it can be expected to continue similarly in the esker formations leading to the water utility. The ground water level is expected to follow the Saimaa lake water levels with small delay.



Map 1. The project site and more general location in the city of Mikkeli. The detailed map shows the site of the impregnation facility, previously remediated area and the estimated groundwater flow path 'northern route' and pollution dispersion route.

11.3. Application process

Due to the nature of the project being a pilot scale operation instead of a full scale remediation, no environmental permit was acquired. Instead a notice of experimental action was given for the environmental office of Etelä-Savo, which gave a positive decision for all parties to go through with the planned pilot experiments (Doranova Oy permit Dnro ESA-2005-Y-101-18).

12. OPERATIONAL PRINCIPLES AND CONSTRUCTION

12.1. Aim

The Biowall method piloted by Doranova was designed to prevent the flow of pollutants dissolved in water from the contaminated area. As the polluted water is flowing from the hot-spot towards the raw water pumping station in south-east, the 'biowall' forms a biologically reactive barrier perpendicular to the groundwater flow which induces a full mineralization of organic pollutants by aerobic bacteria.

The pilot phase aim of the Biowall construction was to find out the effectiveness of the method in practise by measuring the changes in pollutant concentrations in the upstream and downstream monitoring wells and learn to produce an optimum process status in the biowall operation with minimal site disturbance.

12.2. Principle

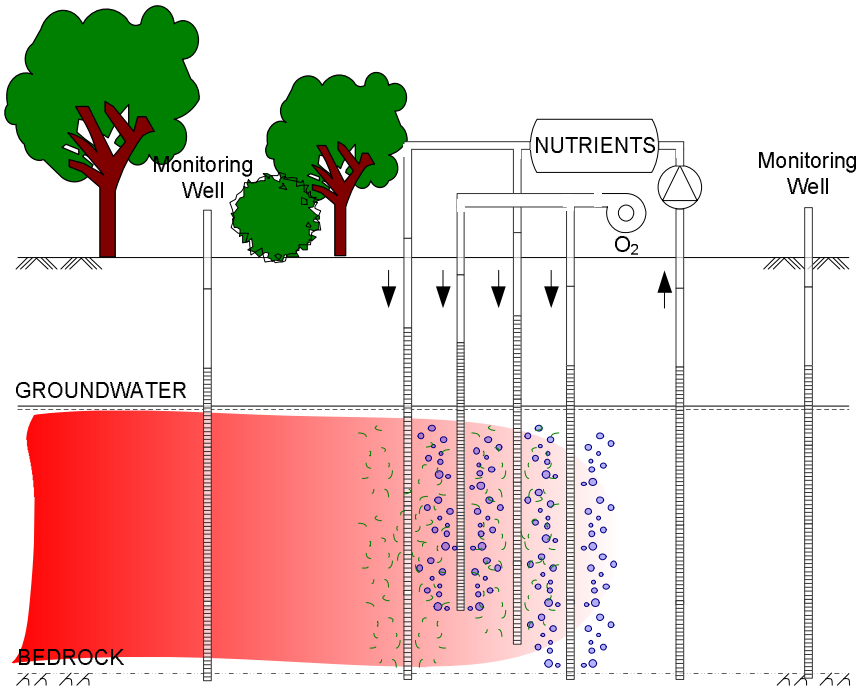


Figure 9. Characteristic system design for biowall

The biowall is an underground in-situ aerobic bioreactor, which is operated by infiltrating nutrients and air at different levels of groundwater. The specific amounts of nutrients and air is generally dependant on the amount of pollutants and the time wanted for total mineralization of the pollutants, which in this case it was basically a function of the groundwater flow rate and the biowall length.

The construction does not aim in functioning as a 'bio-barrier' which would prevent or divert the groundwater flow by changing the underground hydraulic conductivity by maintaining a high density microbial culture, instead it aims at minimizing the disturbances to the natural groundwater flow and nutrient balance outside the active zone.

In the pilot phase the aim of the Biowall was not to treat the whole amount of polluted water. The designed construction width was 25 m and length 5-10 m. according to notice of experimental action.

Biowall's have been constructed earlier e.g. in Netherlands with good experiences on the functioning and results. Doranova Oy has a collaboration agreement with a Dutch expert organisation, namely Biosoil B.V. who's experiences and knowledge was also used in the design and operation phase.

12.3. Construction work

Doranova Oy begun site operations by installing monitoring wells on southern and western sides of the contract area in the beginning of July '05 (11.-15.7.2005), titled M1, M2, M3 and M4 (see appendix 2 installation locations, appendix 3 for installation data and appendix 1 for pictures 2). The monitoring wells were installed perpendicular to the Biowall so that at both sides of the Biowall there would be monitoring wells dedicated for shallow and deep water sampling. During the installation of the monitoring wells information on the geohydrological parameters was acquired; as soil texture, bedrock type and depth and level of water table.

The installation work begun with the removal of the top (~1m) of the soil layer from an area sized 25 x 15 m (Appendix 1, pic. 3). The removed soil was piled next to the excavation and sampled for contamination by Ramboll Finland Oy to resolve possibilities for locating.

The final design planning for the Biowall construction was made at this phase prior to installation of the required infiltration and pumping wells, and air sparing points. The design was made on-site by visualizing and measuring correct installation locations (Appendix 1, pic. 3).

The well installation was done during weeks 30-31. The procedure was to drill to the bedrock level and simultaneously install protective iron casings for the wells. Inside these casing, HDPE pipes were later installed with different configurations on the locations of the slotted sections to enable nutrient infiltrations at different levels underground. After the HDPE pipes were installed the iron casings were removed by welding a hole in their upper part and lifting them with a crane. Prior to removal the interstice of the iron casings and HDPE pipes was filled with sand to prevent later vertical cross flow. Pictures of the installation procedure are found from the appendix 1, pic. 4, 5, 6, 7 & 8. Specific locations of the wells can be found from appendix 1 and information on the installation depths from appendix 2.

During week 32 all the infiltration- and extraction wells, with aeration points were linked. All installed plumbing were individually insulated and equipped with electric heating cable. Underneath and above the installations, sand was provided to further protect the connections (see appendix 1, pic 9, 10, 11 & 12). The extraction wells were equipped with a curb and a covering to make later maintenance possible (see appendix 1, pic 13 & 14). After all installation work was finished the site was re-filled with the same soil material which was removed during start-up (appendix 1, pic 15 & 16). The material had been analyzed during the operations and no pollution exceeding base or limit values was found.

The container providing the technology for the circulation of water and air injection arrived in the week 33 when the final connections between the plumbing that was now underneath the soil and the container were made (appendix 1, pic 17, 18, 20 & 21). During the on-site earthwork, the container containing process instrumentations was pre-built ex-situ (appendix 1, pic 19).

The final installations were made to the container in-situ including electric connections, checking all plumbing and installing a remote on-line monitoring system. Also an additional monitoring well M5 was installed based on the hydrogeological data acquired during installation procedure (location in appendix 2, details in appendix 3).

The biowall was in process preparedness in the change of August-September 2005, and the pilot remediation was initiated accordingly.

12.4. On-site measurements and modelling

Before installation, the site geology was analyzed by using ground penetrating radar technology. The system uses high frequency electromagnetic pulses to analyse the soil and bedrock structure by mathematically estimating the reflection effect of different density materials. The analysis was done on east-north axis in between the monitoring wells M1, M2 & M3, M4 to establish ground formations in the site of the designed biowall.

During the installation of process wells constant monitoring of the soil structure, bedrock depth and fractures were recorded. Similarly sensory observation on the location and degree of pollution were conducted.

Similarly, during the installation the groundwater flow within the designed biowall was measured by using a Phrealog method. The technology is based on observation and optical recording of the movement of omnipresent suspended particles in water, and therefore does not require introduction of any artificial tracers. Based on the recorded movement of particles, the system mathematically calculates the direction based on point of compass and the horizontal flow velocities.

12.5. Process instrumentation and parts lists

See appendix 4.

13. MONITORING

As the remediation was operated as a pilot scale field trial, the process measurements were done often to gather sufficient data on the forming of the subterranean in-situ bioreactor and the changes in pollutant concentrations to quantify and qualify the necessary process adjustments to optimize the functioning of the system and incrementally aim toward the system equilibrium.

In the environmental permit, the agreed minimum monitoring scheme suggested for the pilot was to analyse every two weeks PAHs, phenols, mineral oils (TPH) and nickel from the established monitoring wells M1 - M5. The results were to be reported periodically (after each consecutive measurement period) to the environmental officials and the customer.

13.1. Monitoring program

Doranova Oy carried out an extensive monitoring scheme including sampling from the border areas of the biowall (points M1 – M5) and within it (E20, E22, E10) (see appendix 2 & 3). Besides the required parameters, also BTEX, TAME, MTBE, total phosphate, nitrite, nitrate, total Kjheldhal nitrogen, TOC and occasionally iron were analyzed. Besides the analytically analysed results, the process was measured on-site by using field measurement device (WTW Multi 350i) to measure pH, redox, conductivity and oxygen content.

13.2. Methodologies

Sampling was carried out according to internal guideline for groundwater sampling (DN-i001-2005) which is designed in reference to Doranova Oy's quality management system and in accordance with ISO 9000:2000.

The samples were analysed in an accredited environmental laboratory *Analytico Milieu B.V.* located in Netherlands. Samples were delivered by global express (24 h delivery) via UPS and were stored throughout the delivery chain in airtight, dark and cooled containers.

13.3. Results

See appendix 5.

13.4. Budget

See appendix 6.

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APPENDICES 1, 2, 3, 4, 5 & 6

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