

## MINOTAURUS Report Summary

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### **Final Report Summary - MINOTAURUS (Microorganism and enzyme Immobilization: NOvel Techniques and Approaches for Upgraded Remediation of Underground-, wastewater and Soil)**

Executive Summary:

The MINOTAURUS project aimed at improving the effectiveness of depollution and water treatment technologies through precise and reliable biotechnological processes. It addressed classic organic groundwater contamination (CAH, PCB, MTBE) as well as micro-pollutants (pharmaceuticals and personal care products - PPCP, endocrine disruptors - EDC) present in wastewater. These bioprocesses are all based on the concept of immobilization of biocatalysts (microorganisms and enzymes) and their application in a different reactor-based (ex situ) or in-situ technologies such as bioaugmentation, enzyme technology, rhizoremediation with halophytes, and a bioelectrochemical remediation process.

In developing the different technology lines similar procedures were followed entailing:

- Selection of biocatalyst (enzyme, strain, consortium identification)
- Characterisation of biocatalyst in order to elucidate the degradation pathways, products and kinetics
- Prove effectiveness of immobilisation
- Characterise the biodegrading strains / consortia and their response to environmental conditions
- Derive parameters for process modelling and technology up-scaling
- Testing under more realistic conditions in the field

For all these technologies and steps a comprehensive set of analytical tools was applied. Besides molecular biology-based and physico-chemical approaches, isotope fractionation techniques and in situ microcosm systems were tailored to assess process performance. The project developed amongst others new FISH kits and qPCR methods to detect target organisms and to monitor the process.

That way the project delivered a number of new processes and a suite of adapted tools to monitor those.

Targeting PPCP and EDCs in municipal wastewater new strains were identified as potent degraders for phenolic compounds and SMX respectively. Degradation pathways and enzymes involved were elucidated and deploying a set of newly developed specific molecular-biological monitoring tools. Investigating the degradation spectrum of the laccase producing fungus *Phoma* sp. it was found effective for BPA, NP, EE2 and DF and to a lower extent for CBZ.

For the treatment of CAH-contaminated groundwater in a PBR, a very efficient TCE degrading consortium was isolated from real groundwater. The process was successfully developed further selecting suitable carriers and identifying optimum co-substrates as well as oxygen pulse feeding regimes. Experiments and modelling supported the reactor design.

The treatment of MTBE contaminated groundwater in a PBR inoculated with specialised degrading strains was successfully optimised, up-scaled and tested on-site. The process turned out to be robust and suitable also for varying inflow concentrations.

A continuous-flow bioelectrochemical reactor (BER), simulating an in situ treatment system for groundwater was thoroughly investigated in lab-scale with both spiked synthetic groundwater and groundwater from a contaminated site.

Finally, the suitability of the investigated treatment processes was assessed based on their treatment effectiveness yet having particular regard to current and possible future policy frameworks and associated risks

#### Project Context and Objectives:

As a Research and Technological Development initiative, the MINOTAURUS project aimed at delivering an innovative set of novel environmental biotechnologies, which are all based on the concept of immobilization of biocatalysts, in order to eliminate emerging as well as classic organic pollutants. MINOTAURUS made use of both new biocatalysts and well established, tried and proven ones. The project deliberately addressed the elimination of compounds representative of several classes of pollutants and mixtures thereof reflecting the real problem of contamination by organic pollutants.

The proposed technologies apply to both engineered (ex-situ) and more natural (in situ) systems for the bioremediation of groundwater, wastewater and soil. The technologies aim at the improved control and enhancement of degradation reactions by immobilized biocatalysts such as microorganisms and enzymes.

#### Ex- situ

- Immobilization of laccase on nanostructured silica for the removal of endocrine disrupting compounds (EDCs) and residues of pharmaceutical and personal care products (PPCPs) in a membrane reactor for treating wastewater
- Biomimetic titanification of laccase applied to a magnetic retention reactor for the degradation of endocrine disrupting chemicals (EDCs, i.e. nonylphenols (NPs) and bisphenol A (BPA)) as well as pharmaceuticals and personal care products (PPCPs, i.e. sulfamethoxazole (SMX) and carbamazepine (CBZ)) in wastewater
- Immobilization of other relevant enzymes on membranes for the removal of Benzene Toluene Ethylbenzene and Xylene (BTEX) and methyl tert butyl ether (MTBE) and its degradation product tert-butyl alcohol (TBA) in a membrane bioreactor (MBR) treating groundwater
- Bioaugmentation of packed-bed bioreactors for the increased degradation of i) MTBE and TBA by immobilized cells of an enriched microbial consortium in groundwater; ii) low chlorinated aliphatic hydrocarbons (CAH) via cometabolic degradation by immobilized cells of pure strains and microbial consortia in groundwater
- Bioaugmentation of one MBR using isolated strains of bacteria and fungi as well as microbial consortia immobilized on natural and cheap material for the degradation of EDCs and PPCPs in wastewater

#### In-situ:

- Intensified biodegradation of highly chlorinated CAH by microorganisms immobilized on polarized solid state electrodes (cathodes and anodes) in aquifer conditions
- Intensified biodegradation of PCBs and BPA by naturally occurring microorganisms and exogenous ones immobilized on the roots of halophytes in wetlands systems depolluting soil, groundwater or wastewater

The conceptual approach of the MINOTAURUS project is depicted in Figure 1.

#### Figure 1 Conceptual overview of the project MINOTAURUS and technologies applied

The concept covers intensified bio-reactor technologies utilising degradation capacities of micro-organisms and applying amongst others bioaugmentation strategies (WP1) as well as the direct application of enzymes (WP2). In-situ technologies, namely a bio-electrochemical process with electrodes and rhizodegradation systems are investigated in WP3. Up-scaled versions of a number of technologies will be tested under field conditions in work package 4. An evaluation with respect to socio-economic suitability of developed technologies will be carried out in work package 5.

To ensure the optimal development of the technologies, each bioremediation process will be monitored and assessed using a set of technology-tailored tools. The selection and adaptation of cutting-edge physico-chemical and biological methods (e.g. optrodes, metagenomics and isotope fractionation) combined to a rational understanding of engineering and enzymology/microbial physiology aspects is an ambitious approach of MINOTAURUS to "open the black-box" of the proposed environmental bio-processes. Biodegradation kinetics models are applied as to improve the predictability of performances to be achieved with the investigated biotechnologies. This approach is reflected in the work package structure as illustrated in Figure 2 which is characterised by common task in each technology line, which address

- new immobilisation techniques (which organisms, which reactors, how to operate them),
- monitoring tools,
- assessment of reliability, robustness and predictability.

Figure 2 Work Package structure of the MINOTAURUS project

This thorough lab-testing of the considered processes forms the ground for stepwise up-scaling and eventual transfer of the technologies to on-site testing. As bioremediation technologies obtained at lab-scale are often not successfully proven under real conditions, special effort will be made by MINOTAURUS partners to test the technologies on site at an early stage. This possibility of direct implementation of a number of developed technologies in five reference sites confronted with relevant pollutants thus constitutes a key strength of the project. The sites available for testing are summarised in Table 1 and represent

The MINOTAURUS pre-selected four sites are listed in Table 1-

Table 1 Sites included in on-site studies

Name	Type	Country (city)	Targeted compounds
WWTP Birs	Municipal WWTP	Switzerland (Birsfelden)	EDCs and PPCPs
Golda-Hasharon Hospital	Hospital wastewater	Israel (Tel Aviv)	PPCPs (mainly CBZ)
Rho Site / Modena site	Contaminated groundwater	Italy (Milano)	CAH
Belgian site	Contaminated groundwater	Belgium (City to be selected)	MTBE, TBA, BTEX
Heraklion	Constructed wetland for the treatment of wastewater and groundwater	Greece	PCBs and BPA

Rho (aquifer, Italy), Heraklion (Greece, wetlands for soil/wastewater and groundwater treatment), Birsfelden (Switzerland, municipal wastewater treatment plant (WWTP)), and Tel Aviv (Israel, hospital wastewater). For these sites the partners of MINOTAURUS have previous experience and a sound basis for the conduction of field testing activities. The fifth site was to be selected in Belgium in the course of the project as an aquifer contaminated with MTBE and BTEX. At these five sites technologies will be tested in either small scale or pilot scale.

Project Results:

### 3.1 WP1 Intensified bio-reactor technologies for groundwater and wastewater treatment

Work Package 1 aimed at developing efficient bioaugmentation strategies based on the immobilization of selected microorganisms in membrane bioreactors and packed bed bioreactors for the intensified biodegradation of relevant contaminants in wastewater and groundwater

#### 3.1.1 Bioaugmented membrane bioreactor technology

##### 3.1.1.1 Strain identification

The activities around this reactor technology started with the quest for potential degraders to be identified in and isolated from environmental samples. To this end a lab-scale MBR was operated with activated sludge from a municipal wastewater treatment plant and spiked with the target compounds (SMX, CBZ, NP, BPA and CBZ). Several microorganisms growing on the xenobiotics supplied as sole carbon source could be isolated from lab-scale MBR and activated sludge. Using <sup>14</sup>C labelled compounds it could be shown that, amongst others, *Microbacterium* strain BR1 was capable of mineralising SMX and was thus selected for further studies.

In parallel the search for potential degraders of the recalcitrant target compounds carbamazepine (CBZ) and diclofenac (DF) was continued in a broad screening campaign on known strains which were derived from environmental samples and wastewater treatment plants. A protocol was developed to detect among a total of more than 400 bacterial strains as well as fungi and actinomycetes those capable to grow on medium containing the target substances CBZ or DF. Only few strains were identified, however, none was eventually able to actually mineralise CBZ or DF when cultured in spiked minimal mineral medium.

##### 3.1.1.2 Degradation pathways and products and kinetics

For those strains found capable to degrade target compounds, the metabolic pathways of pollutant degradation were further elucidated. This included insight into SMX degradation via a so-called ipso-substitution reaction and the formation of oligomers

of pollutants by the aquatic fungus *Phoma* sp. UHH 5-1-03 which was able to degrade a broad range of EDCs and PPCPs.

### 3.1.1.3 Immobilisation and viability

Identified strains were immobilised into alginate beads, as other tested carrier for attached growth were unsuccessful. Tested in relevant wastewater samples, it was found that degradation performance for the respective substrates BPA and SMX were completely diminished within one to two days, and that in both cases immobilization in the alginate beads gave more robust activity over time, possibly due to physical shielding from negative influences. However, as of now, both immobilized biocatalysts are only stable for several days, so that continuous respiking of a reactor would be required.

## 3.1.2 Bringing forward packed-bed reactor concepts for the treatment of contaminated groundwater

### 3.1.2.1 PBRs: bioremediation of CAH-contaminated groundwater via aerobic cometabolism

For the treatment of CAH-contaminated groundwater in a PBR, a very efficient TCE degrading consortium was isolated from real groundwater. The process was successfully developed further selecting suitable carriers and identifying optimum co-substrates as well as oxygen pulse feeding regimes. Experiments and modelling supported the reactor design.

The activities around this reactor technology included the selection of the growth substrate and of the biofilm carrier for the subsequent development of the packed-bed reactor (PBR) aerobic cometabolic process. These activities led to the selection of

- butane as substrate
- Biomax as porous ceramic carrier
- an effective CAH-degrading butane-growing microbial consortium, named B4.

Then a preliminary aerobic co-metabolic TCE-degrading process in a packed bed reactor was developed. The objective was to develop and optimise a pulse feeding regime for growth substrate and oxygen in order to obtain the required information for the scale-up of the process to a 31 L Biomax-filled PBR that was developed and tested in the framework of WP4. Crucial aspects to be considered were:

- to avoid an excessive biomass growth at the beginning of the column with the risk of porosity clogging
- to avoid a too low and ineffective biomass concentration in the terminal portion of the column
- to minimize the substrate competitive inhibition on CAH cometabolism (TCE is consumed in the presence of the oxygen but in the absence of butane).

Extensive testing (over more than 100 days) in 1 L PBRs packed with different carriers (including the Biomax) and initially inoculated with consortium B4 in combination with computer simulations identified an optimal pulse-feeding regime. Several batch tests of TCE aerobic cometabolism were aimed at assessing the robustness of the process of CAH aerobic cometabolism developed by UNIBO, by evaluating the variation of the process performances as a result of the variation of a given operational condition. In particular, the tests included the analysis of the influence of temperature, pH and of the presence of TeCA as an additional toxicant on the TCE biodegradation rate. It turned out that the attached-biomass process seems more robust against temperature variations than suspended cells.

### 3.1.2.2 PBR for the treatment of MTBE contaminated groundwater

For the treatment of MTBE contaminated groundwater a PBR inoculated with specialised degrading strains was further developed and successfully optimised, up-scaled and tested on-site (confer WP4). A comprehensive screening of carrier materials and their suitability as growth support in PBR technology was performed and led to the selection of biochips and polystyrene granulate which were then tested in a bench-scale reactor was operated (Figure 3). In general it was concluded that the system is robust and can be operated with a single inoculation event for quite a long period. The system comprising the newly identified biochips as carrier material was found more robust than the system filled with PSG and sponges. The need for an occasional re-inoculation cannot be excluded totally, but, when necessary, it can be made in a relatively easy way.

Figure 3 Overview of tests performed

being batch degradation experiments (left), small column experiments (middle) and bench-scale column bioreactor (7L) tests (right).

### 3.1.3 Tailoring monitoring tools for the developed technologies

Whilst the removal capacity of all reactor technologies was assessed by chemical analysis of the parent compounds also numerous molecular biological tools have been developed in the project to detect the specialised microorganisms and to assess their activity. Some of the approaches applied were:

#### Degradation activity test with radio-labelled compounds

This set-up makes use of the easy detection of  $^{14}\text{CO}_2$  released in biodegradation of radiolabelled compounds. It provides a measure to compare the activity of e.g. suspended and immobilised forms of microorganisms or to verify their activity in a competitive system such as bioaugmented sludge over time.

#### FISH technologies to detect and quantify viable inoculated strains

A set of easy to handle detection kits was developed and used to verify the presence of bioaugmented species in the (upscaled) reactor systems (cf. WP4 and Figure 4).

Figure 4 Example of FISH applied to sample detecting *Sphingomonas* sp. strain TTNP3

#### qPCR method for strain-specific identification and quantification

For the monitoring of *Microbacterium* sp. strain BR1, PCR primers based on the 16S rRNA gene sequence have been developed and tested for future application in bio-augmented MBR pilot plant (WP4). Progress has also been made in the isolation of the enzyme responsible for the ipso-reaction. The corresponding genes (a FMN-dependent monooxygenase and an FMN-reductase) have been identified, respectively and their function has been verified by heterologous expression in *E. coli*. This enables future development of molecular tools to detect SMX-degrading activity as opposed to detecting merely the presence of the degrading strain.

Genes identified for *Sphingomonas* sp. strain TTNP3, which is known as a bacterium capable of degrading both NP and BPA via the ipso-substitution pathway, were used to develop molecular monitoring techniques to target functional genes. The most suitable target for monitoring proved to be *hqdB*, the gene coding for the beta subunit of the hydroquinone dioxygenase. The detection tool was later applied in the bioaugmented MBR (cf. WP4).

q-PCR primers were also developed for the M-consortium applied in the bioaugmented PBR for MTBE removal. The primers were used to characterise samples from lab test and pilot tests (Deliverable D4.3). This way it was possible to verify the presence of target organisms in the respective system and to describe its distribution.

#### Application of DGGE to characterize the consortia immobilized on the aerobic cometabolic PBRs degrading CAHs

In monitoring the biomass growth on different carrier material in continuous flow columns, samples from each packed bed column were analysed for the structure and composition of the immobilized microbial community by DGGE to

- to verify the even distribution of the B4 consortium along the column
- to confirm that the composition of B4 consortium does not change with immobilisation

This was proven by the highly similar DGGE fingerprints observed in the 4 columns. This was consistent also with the detection of highly similar biodegradation rates in the three sections of each reactor.

A summary of the most promising combinations of biocatalyst identified, tested and further investigated in the different reactor concepts and modes of their monitoring is given in Table 2

Table 2 Monitoring tools and methods developed and applied for the different biocatalysts and reactor technologies

Envisaged reactor type MBR PBR

Wastewater Groundwater

Target compounds EDCs, PPCP MTBE / TBA CAH

Effective for target compound SMX BPA, NP

Newly identified degraders *Microbacterium* strain BR1 B4 consortium

known degraders *Sphingomonas* strain TTNP3 *Phoma* sp. UHH 5-1-03 M-consortium

Immobilisation Encapsulation in alginate beads Encapsulation in alginate beads Not immobilised Biochips,

Polystyrol granulates 4 porous carriers (Biomax, Biomech, Cerambios, Biopearl)

Monitoring tools for biocatalysts FISH probes

qPCR (for 16s RNA gene FISH probes

qPCR (for metabolic gene FISH probes

Ergosterol assay FISH probes and qPCR primers developed DGGE to track changes in bacterial consortium during immobilisation

### 3.2 WP2 - Immobilized-enzymes reactors for intensified pollutant removal

Work Package 2 was dedicated to the characterisation of the activity and kinetics of individual laccases as well as co-immobilised laccases of different species for the degradation of single target substances or mixtures thereof. This also included attempts to identify transformation products.

Another research objective was the development and optimization of suitable immobilization strategies and application of the biocatalysts in tailored bioreactors.

#### Identification and characterization of suitable enzymes

One of the major tasks in work package 2 was the development of suitable immobilization strategies. In order to be able to carry out this task, the identification of suitable enzymes as well as securing a sufficient supply was necessary. Whilst a number of laccases were screened only a limited set was found suitable for tackling the relevant pollution. Finally, three laccases were selected for further use: Laccases from *Corioloropsis polyzona*, *Phoma* sp. and *Thielavia* sp. (cf Table 3) which were immobilised and applied in different reactor.

Identification and optimization of suitable immobilization strategies and application of the developed biocatalysts in tailored bioreactors

Three different immobilization approaches have been proposed: immobilization on nanostructured silica, crosslinking of enzyme aggregates and immobilization on membranes. The immobilization on nanostructured silica was successful and their application in membrane reactors was successfully tested, up-scaled and transferred to work package 4. However, the other two approaches had to be abandoned. Briefly, cross-linked enzyme aggregates had inferior stability characteristics as compared to enzymes immobilized on nanostructured silica whereas the envisaged use of enzymes immobilized on membranes for the degradation of MTBE / BTEX compounds was not feasible due to insufficient degradation rates as well as problems with the regeneration of cofactors. Instead a completely novel reactor based on the magnetic retention of biocatalysts produced via biomimetic encapsulation of enzymes.

Table 3 Tested enzymes and target substances

Enzyme Technology approach A B1 C D

Wastewater

Target compounds EDCs (BPA, NP), PPCP (SMX, CBZ, DF, TCS)

Better characterisation of known Biocatalyst

Laccase from Genus *Thielavia*

*Corioloropsis polyzona* Co-immobilised with laccase of *Trametes versicolor* Genus *Thielavia* Genus *Thielavia* *Phoma* sp.

Immobilisation Fumed silica nanoparticle Fe<sub>3</sub>O<sub>4</sub>-biotitanium particles Silica core particle No immobilisation

Reactor type MBR, ultrafiltration membrane Magnetic retention reactor Membrane reactor MBR (in WP4)

#### 3.2.1 Enzyme conjugated fumed silica nanoparticle in membrane reactor

FHNW carried out research on the production and characterization of laccase-modified fumed silica nanoparticles (fsNP). A previously established method for the production of fumed silica nanoparticles was optimized and achieved considerably improved binding efficiency and specific activity.

These fsNP were tested in a lab-scale ultrafiltration membrane reactor under varying BPA influent concentration as well as applied enzyme activity. Radio labelling of the compound allowed monitoring the fate of BPA in the system and revealed that it is rapidly converted into higher-molecular compounds which are retained by the membrane, which was to be confirmed in pilot-scale test under WP4.

### 3.2.2 Biomimetic titanification with magnetic retention principle

A methodology to encapsulate laccases in biomimetically synthesized titania particles was successfully developed as alternative approach. This approach overcomes some of the commonly faced problems in enzyme immobilization and in addition allows the implementation of further functionalities e.g. the co-encapsulation of magnetic materials. The optimization of the immobilization protocol and characterization of the biocatalysts was carried out together with the development and application in dedicated bioreactors.

In batch experiments, the bio-inspired laccase particles were able to degrade the majority of the micropollutants analyzed except SMX, following in many of the cases the same trend than the soluble counterparts. Immobilization on biotitania particles resulted in higher pH stability of the enzyme at acidic pHs, but, on the other hand, in lower biocatalytic efficiency for some of the studied compounds. The different structure, hydrophobicity and physico-chemical properties of the micropollutants involved may cause the different degradation behaviour comparing with the soluble enzyme probably due to a different accessibility for the enzyme caused by mass-transfer limitations.

#### The magnetic retention reactor

An operational reactor for the continuous degradation at lab-scale of micropollutants catalyzed by bio-inspired laccase particles were designed and constructed. The reactor used a magnetic retention principle and was tested at different hydraulic retention times and enzyme loads. Though degradation of target compounds was possible, a number of technical operational challenges remained.

### 3.2.3 Silica core biotitania laccase particle in membrane reactor

Additionally, the versatility of the biomimetic titanification was evaluated with the production of silica-core biotitania laccase biocatalysts and application in membrane reactors. The production of silica-core biotitania laccase particles was similar to the one developed for the production of magnetic-core biotitania laccase particles. Instead of magnetic particles, previously silanized porous silica particles of size 200-80 nm were employed as the core.

Those ones were loaded with different amounts of enzyme as to produce nanoparticles with different specific activities. These biocatalysts were tested in a lab-scale membrane reactor for the degradation of PPCP and EDC. It could be shown that after addition of the biocatalyst, the micropollutant concentration in the outlet decreases in all cases, showing substance specific removal effectiveness of between 10-95% which can be considered the result of both catalytic degradation by the biocatalyst and micropollutant adsorption on the biocatalyst particles. Yet this effect did not last long. After 20 hours of operation the micropollutant concentration came back to the levels of the stabilization phase (no biocatalyst present) which is indicative of a low stability of the biocatalyst.

### 3.2.4 Characterisation of laccase of *Phoma* sp.

Laccase of the aquatic fungus was thoroughly investigated in MINOTAURUS as to describe its degradative properties and to conclude possible applications in wastewater treatment. It was found that extracellular polysaccharides have a stability-enhancing effect of on laccase and a positive effect on the biochemical properties. Moreover it was shown that the presence of additional pollutants has a considerable influence on compound degradation. EE2 and BPA, being most efficiently degraded by laccase, are more slowly degraded in a mixture of compounds than alone; most likely due to competitive inhibition in presence of more than one pollutant representing a laccase substrate. By contrast, all other pollutants (except CBZ which was found to resist laccase attack under any condition) are faster degraded when applied in mixture. All together, these results suggest that more easily degradable pollutants such as EE2 and BPA act as redox mediators thus enhancing/enabling the oxidation of other, more recalcitrant pollutants.

Further, Size Exclusion Chromatography (SEC) was used to observe the formation of degradation products with molecular masses higher than those of the respective parent compounds and could confirm as the formation of such oxidative coupling products as typical for laccase reactions. Considering that for certain target pollutants the formation of products with higher molecular masses has been linked to the elimination of biological activity (Cabana et al., 2007), these results are in favour of a risk-free applicability of target pollutant elimination by laccase.

Kinetic modelling represents a valuable tool to enable the prediction of process performances, and was applied by UFZ-EM to



target pollutant (BPA, NP, EE2, TCS, DF, CBZ, and SMX) degradation using the *Phoma* sp. laccase. Modelling was carried out according to both Michaelis-Menten and first-order degradation kinetics. Overall it was found that deviations of the experimentally determined values from the predicted ones of less than 15% were obtained for moderately to slowly degraded compounds such as DF, NP, and TCS, indicating a considerable robustness of the modelling approach. Larger deviations observed for the most quickly (EE2, BPA) and most slowly degraded pollutants (SMX) still showed values well below 50%.

### 3.2.5 Enzyme activity determination based on oxygen consumption

Oxygen consumption is a common measure to assess enzyme activity and a diligent tool in determining reaction rates. Within MINOTAURUS a prototype of an optotrode system, a 24-well oxygen sensor was developed.

In collaboration with UCL, oxygen sensitive coatings were developed in an iterative process in which the device was installed at the lab of partner UCL and coated vials were produced by CRS.

Tests to verify the sensitivity for detection of oxygen consumption were carried out with the laccase assay developed at UCL. It was found that the system is able to measure the consumption of oxygen by laccase enzymatic action, but that the reaction time is too slow and the experimental set-up will need to be adjusted in order to investigate enzyme kinetics.

### 3.3 WP 3 Intensification of in-situ bioremediation technologies

The project also investigated two processes relying on natural systems or in-situ remediation:

- a bioelectrochemical process for the treatment of chlorinated hydrocarbons (CAHs) in groundwater
- a rhizodegradation process for the degradation of polychlorinated biphenyls (PCBs) and EDCs (like Bisphenol A).

#### 3.3.1 Development of a bioelectrochemical process for in-situ remediation of chlorinated aliphatic compounds (CAH)-contaminated groundwater

A continuous-flow bioelectrochemical reactor (BER), simulating an in situ treatment system for groundwater was thoroughly investigated in lab-scale with both spiked synthetic groundwater and groundwater from a contaminated site. Electrons for the reductive dechlorination were provided by the cathode which was operated under a wide range of potentials. The rate and extent of TCE dechlorination were found to be highly dependent on the set cathode potential. Tuning the cathode potential allows to control the competition for the “electrons” among dechlorinating bacteria and methanogens. In an application with real groundwater praxis-relevant effects of co-contaminants such as NO<sub>3</sub><sup>-</sup> or SO<sub>4</sub><sup>2-</sup> on the process were determined. UNIRM has developed a continuous-flow bioelectrochemical reactor (BER), simulating an in situ treatment system for chlorinated hydrocarbons in groundwater, where the influent was fed to the cathode chamber for anaerobic reductive dechlorination (RD) whereas the effluent of the cathode was fed to the anode chamber for further treatment under oxidizing conditions. The main features and operating conditions of the BER are also shown in Figure 1.

Parameter Value (Unit)

Total (empty) volume of each chamber 0.85 (L)

Hydraulic retention time in each chamber (HRT) 0.44-2.5 (days)

Influent TCE concentration 35 (µmol/L)

Temperature Room (18-22 °C)

Inoculum (cathode) *Desulfitobacterium* and *Dehalococcoides* co-culture

Inoculum (anode) Aerobic enrichment culture

Figure 5 Experimental setup of bioelectrochemical reactor

After having investigated the effect a wide range of applied cathodic potential the effect of influent flow-rate was investigated, in a more narrow range of cathode potentials (from -250 mV to -450 mV, vs. SHE). The reductive dechlorination of TCE was found to be largely affected by the influent flow-rate, particularly at -450 mV vs. SHE, whereby the driving force of the electrochemical reactions and accordingly the rate of biological reactions were higher. It could be concluded that the rate of the biological reaction was limited by mass-transfer processes, thus being dependent on the fluid dynamic conditions. In order to better understand the biodegradation the biocathode of the bioelectrochemical reactor was thoroughly characterised using molecular tools. CARD-FISH analysis revealed that depending on the applied cathodic potential different



genera dominated the consortium changing from mostly Dehalococcoides -550 mV to -750 mV to other members of the Chloroflexi phylum, when the cathode was controlled in the range from -250 mV to -450 mV. Most probably, the observed changes in the microbial composition of the biocathode were driven by changes in the dominant mechanisms of electron transfer to TCE: mediated by the electrolytic production of H<sub>2</sub> gas (in the range from -550 mV to -750 mV), or direct (in the range of cathode potentials from -250 mV to -450 mV). The identity of these Chloroflexi, was further analysed and revealed the presence of other previously described dechlorinating species.

Overall, even though the full conversion of TCE to non-chlorinated end-products was never observed, the RD anyway occurred at least down to cis-DCE or vinylchloride (VC), which often accumulated in the cathodic effluent. The ability of mixed microbial cultures to anaerobically oxidize cis-DCE and ethene with the electrode (anode) serving as direct electron acceptor was investigated in order to achieve further conversion in the anodic compartment of the bioelectrochemical system. In summary, no "anaerobic" cis-DCE removal, with the anode serving as direct terminal electron acceptor, was observed at lower (less-oxidizing) potentials.

The anodic oxidation was also observed during the long-term continuous run of the lab-system (see above) and a mass balance for TCE and its dechlorination products was calculated under the different operating conditions (by UNIRM). An example of most relevant mechanisms of TCE removal in the whole process as function of applied cathodic potentials is reported in the Fehler! Verweisquelle konnte nicht gefunden werden.. TCE removal was always more than 90%, but less-chlorinated RD intermediates are formed, whose concentration decreased as the potential became more negative.

### 3.3.2 Development of immobilization-based systems for in-situ bioremediation of groundwater and soil

Among the extensive technologies, the potential of rhizodegradation systems to either decontaminate PCB polluted groundwater or BPA loaded effluents was explored paying particular attention to

- the potential benefits of using halophytes and
- novel bioaugmentation strategies that involve the addition of suitable rhizosphere and endophytic microbes taking into consideration the influence of exudation patterns.

Both actions are expected to result in the intensification of the remediation processes.

Rhizodegradation system for the degradation of BPA involving the use of halophytes coupled to bioaugmentation with suitable microbial consortia

Two test systems were designed and constructed to investigate rhizodegradation systems. One operated as Sequencing Batch Reactors and another simulating a Shallow Aquifer Rhizodegradation Pilot. The main aims of the study were the following:

- Investigation of the ability of *Tamarix parviflora* (SBR Type A), *Juncus acutus* (Shallow aquifer Rhizodegradation pilot) and their associated rhizosphere and endophytic microbial community to degrade BPA from soil and groundwater.
- Investigation of natural attenuation of bisphenol A (BPA) in soil and groundwater in the absence of plants
- Description of root endophytic bacterial diversity & role prior and after bioaugmentation with BPA degraders (*Sphingomonas* sp. strain TTNP3)

The significance of the root system in BPA removal was also confirmed by small scale pot experiments (lasting 6 days) where planted systems removed three times more BPA than mere soil systems.

Endophytic BPA degraders from *T. parviflora* and *J. acutus* to degrade BPA

Endophytic bacteria were isolated from the roots, stem and leaves of *T. parviflora* and *J. acutus* grown on BPA contaminated soil. *Sphingomonas* and *Cypravidus* strain were found in all plant compartments and tested for their ability to grow in rich medium in the presence of BPA. Both strains grew considerably over 3 and 7 days respectively, while reducing the BPA in the medium by 20 to 25%. The presence of BPA degrading endophytic bacteria in the halophytes is in accordance with the lack of visible toxicity symptoms, growth inhibition or chlorophyll content reduction, as these bacteria may provide for a detoxification of BPA taken up by the plants.

Rhizodegradation system for the degradation of PCBs

In another research activity work has been carried out to assess the potential of halophytes to enhance the degradation of polychlorinated biphenyls (PCBs) and to evaluate associated changes in the microbial diversity in the soil. A stimulating effect of plant secondary metabolites (PSM) was observed, which proved their ability to enhance the removal of certain congeners of

PCB, though the microbial diversity was less in amended soil.

Bacterial isolates extracted from long-term PCB-contaminated soil systems were characterised for their PCB degrading capacities. *Achromobacter denitrificans* AD400 was found to be equipped with particular biphenyl dioxygenase genes (bphA) allowing it to metabolise a number of PCB congeners, among them 2,2'-chlorinated congeners, which are rarely degraded by other known strains. It was used to bio-augment the degradation test systems. In these degradation experiments the rhizodegradation systems were set up with long-term PCB-contaminated soil and operated for 15 months. Quantification of microbial biomass clearly distinguishes between vegetated and bulk soil samples. There is a significant increase in living bacteria and all microorganisms with the vegetation. Salinization or bioaugmentation, on the other hand, did not prove a significant positive effect on living biomass. All rhizodegradation systems performed quite similarly removing 33% to 44% of the content of polychlorinated biphenyls and chlorobenzoic acids as the most abundant products of their metabolism by oxidative degradation. The effect of vegetation, however, is obvious when correlating PCBs with their degradation products, chlorobenzoic acids (CBAs). Vegetated soil contained more chlorobenzoic acids pointing out to more effective degradation of PCBs. Salinity and bioaugmentation, on the other hand, do not seem to significantly influence the degradation performance. It is assumed that prolonged cultivation times of the plant in the soil could result in statistically significant depletion of PCB congeners.

### 3.4 WP4 - Evaluation of immobilisation-based biotechnologies at larger scale and/or under field conditions

The aim of WP4 was to evaluate the performance of a number of the technologies developed in WP1-3 under more realistic conditions, using pilot tests in the field or/and larger lab-scale experiments with real groundwater off-site. The monitoring methods developed in tasks 2 of WP1-3 will be used to get a better understanding of the process and to evaluate the robustness, reliability and predictability of the biotechnologies.

In total eight technologies / concepts were tested in an up-scaled version or under more realistic conditions as indicated in Table 4.

Table 4 Overview of technologies considered in WP4

Bold- additional systems selected during the project

Italics- systems not considered

System Type

Linked with Bench scale Pilot scale location phase

8 MBR-bacteria WP1 WP1 Yes Birsfelden (CH) 2

2 MBR-bacteria WP1 Yes Schilde (BE) 1

3 MBR-bacteria WP1 Yes Israel 1

4 PBR-bacteria (MTBE) WP1 WP1 Yes Belgium 1

5 PBR-bact (CAH) WP1 X No Rho site (IT) 2

6 FBR-MBR - new enzymes WP2 x No Birsfelden (CH) 2

7 Perfusion basket reactor-enzymes à magnetic retention reactor WP2 x No Birsfelden (CH) 2

1 FBR-MBR-enzymes WP2 Yes Birsfelden (CH) 1

9 Bio-electrochemical (CAH) WP3 x No Modena site (IT) 2

10 Bio-electrochemie (CAH) WP3 Yes Rho site (IT) 2

11 Inoculated rhizosphere WP3 Yes Heraklion (GR) 2

#### 3.4.1 Selection & description of sites involved

The wastewater treatment plants in Birsfelden, Switzerland (Birs site) and in Schilde (Belgium) were already fixed before the start of the project, for implementing system 1 and system 2, respectively. An arrangement was made by HEFER to install system 3 at a hospital site in Israel. For system 4, based on contacts with consultants and site owners, in total 5 MTBE/BTEX-contaminated sites in Belgium were selected for more detailed evaluation of the available data. A second set of technologies and appropriate sites was selected later in the project Test system 1 was operated at the WWTP Birsfelden, Switzerland (Birs site), while test systems 5 and 9 were linked to the Rho site (Italy). Afterwards, system 9 was relocated to the Modena site for

practical reasons. Finally, the inoculated rhizosphere (system 11) was operated at the Heraklion site (Greece) as planned.

A Short overview of technologies involved in phase 1 is given below.

Pilot scale fixed bed reactor with UF membrane reactor, with immobilised enzymes (System 8)

Operational at WWTP Birs site (CH)

Pilot scale MBR reactor with polymeric membrane (System 2)

Operational at AQUAFIN (BE)

Inoculation with specific bacteria envisioned/pending

Pilot scale MBR reactor with ceramic flat sheet membranes (System 3)

Installed at a hospital site in Israel

Pilot scale de-ironing unit & Upflow inoculated bioreactor for MTBE/TBA-removal (System 4)

Operational at VITO (Belgium) – ready to be transported to location 4.

PBR reactor for immobilised microorganisms & aerobic cometabolic removal of CAHs – (System 5)

Being prepared in labs of UNIBO – to be operated with GW from Rho site (IT)

Bacteria inoculated MBR with ceramic membrane to treat municipal wastewater

(system 1)

Operational at WWTP Birs (CH)

BEARD treatment technology for in-situ removal of CAH-contamination (System 9)

Being prepared in labs of UNIRM to be operated with GW from Modena site (IT)

Inoculated rhizosphere for removal of micropollutants

(System 11)

Being prepared at Heraklion site (GR)

### 3.4.2 Results from pilot testing

#### 3.4.2.1 System 8 - MBR with immobilised laccase enzymes (FHNW) – WWTP Birs (CH)

The system consists of a fixed bed reactor tandem ultra-filtration unit in which immobilized enzymes are dosed. Earlier (pilot test 1), the system was operated with raw wastewater (after sand trap) at the Birs site (Aeration: 20 – 25 Nm<sup>3</sup>/h, Treating ≈ 200 L/h). With laccase containing nanoparticles (conjugates in the μm size range) dosed in the UF reactor at 2g/L, BPA was removed by 50% as shown during a 42 days lasting pilot test.

During the current reporting period, new tests were performed where the fixed bed was used as a polishing step for treated wastewater effluent from WWTP Birs. The effluent from the fixed bed was treated with genus *Thielavia* laccase immobilised on fumed silica nanoparticles. In a first trial (Pilot test 2, 18°C, pH not adjusted (7.8-8.2)) the enzyme activity dropped from 2200 Unit/L to 477 Unit/l in 41days. No contribution of the laccase was observed to reduce the micropollutants benzotriazole (BZL), sulfamethoxazole (SMX), carbamazepine (CBZ) nor bisphenol A (BPA). Next, a third pilot test was performed with pH adjustment to create conditions more favourable for the laccase. The adjustment to pH 6 in the second pilot trial barely contributed to enhancing the elimination of trace organic contaminants. It can be concluded that detrimental ambient conditions and the lacking sensitivity of laccase-NP towards trace concentration of micro-pollutants in the treated wastewater are the major obstacles that hinder the application of laccase in real scale wastewater treatment.

#### 3.4.2.2 System/pilot 2 – Bioaugmented MBR with polymeric membrane to treat municipal wastewater (AQF) – Schilde site (BE)

Earlier, a pilot with polymeric membranes was assembled and installed at the Schilde test site in parallel to a full scale treatment system. Target compounds are bisphenol A, carbamazepine, diclofenac, sulfamethoxazole. The system was started without inoculation in spring 2012 and a monitoring plan was compiled. The inoculation of the system was delayed as the cultivation and/or immobilization of the strains in WP1 took longer than expected.

Eventually, the following strains were selected for bioaugmentation: *Phoma* spp. able to degrade carbamazepine, diclofenac, sulfamethoxazole, bisphenol A.; and a newly isolated *Microbacterium* strain (see WP1) degrading sulfamethoxazole. The scope of this study was to check: (i) whether the specialized strains can survive inoculations in pilot scale; (ii) to evaluate its activity towards the target compounds. Different process schemes have been tested.

During pilot inoculations no consistent proof of SMX removal facilitated by *Microbacterium* BR1 was observed. Its capability to

survive both in post treatment and in secondary treatment was found scarce. Since Microbacterium population was diminished to a tiny % of the total viable biomass, it is likely that it would be washed out during long term operations.

Phoma sp. instead was able in the tested conditions to keep its share of population in comparison with the autochthon biomass during the test periods. CBZ and DF were convincingly removed during the secondary treatment test. A CBZ removal was temporarily achieved also during post-treatment.

#### 3.4.2.3 System/pilot 3 – Bio-augmented MBR with ceramic flat-sheet membranes to treat hospital wastewater (HEFER) – Israel site

A pilot system was designed for an on-site treatment of hospital wastewater. In spring 2012, the pilot system was relocated to a hospital site in Israel selected within the project where carbamazepine (2-12 µg/L) was found to be the major compound of concern. The plant has been equipped with all necessary devices for online and remote control and the system was being stabilized without inoculation. After optimising the system, a very good removal of organic material, ammonia, total nitrogen (nitrification) was obtained. Up to 50% phosphorous removal was also reached. The MLSS did not go up more than 4000 - 5000 mg/L and the system ran at the 2000 – 4000 mg/L MLSS level. Without inoculation, the background micropollutants analyses showed that between 2000-8000 ng/L of CBZ can be found in the wastewater although up to 12000 ng/L CBZ also was analyzed. The CBZ analyses after the MBR operation showed that no significant removal in the MBR treatment. Some CBZ accumulation may occur in the sludge taking into account that the sludge age in this case is higher than 40 days. Efforts were made by FHNW and UFZ to find carbamazepine degrading biomass. WP1 work revealed that carbamazepine is a challenging compound to degrade. No strains could be isolated within the timeframe of the MINOTAURUS project. Consequently, no specialized biomass was available to inoculate system 3. Therefore, also attention was given to natural enrichment of carbamazepine degraders in the pilot. After the installation of the GAC column and from June to end October 2013, all CBZ concentrations that entered the GAC column were completely removed, leaving a possibility that some CBZ consuming bacteria may grow on the column, but which could not be confirmed by experiments.

#### 3.4.2.4 System/pilot 4 – Inoculated bioreactor to treat MTBE/TBA/BTEX-contaminated groundwater (VITO) – Belgian site Pilot test 1

A first pilot test was performed with a bioreactor containing a mixture of polystyrene granulate (PSG) beads and sponges and system. The system was uploaded at VITO (10 mg/L MTBE and 5 mg/L TBA, inoculation with MTBE /TBA-degrading M-consortium). Next, the pilot was relocated (2/2012) to the selected site in Belgium (Location 4), to be fed with real contaminated groundwater. Monitoring efforts revealed that: (1) the system degraded the pollutants below the discharge limits, (2) the system was found more robust than anticipated as extreme pH-fluctuation and temperature increases to > 30°C did at most only temporarily reduce the removal efficiency. Re-inoculation was not performed since the start of the bioreactor, (3) the de-ironing process, needed as pre-treatment, was the limiting step for increasing the flow rate of the groundwater. In collaboration with VER who had developed specific FISH probes (WP1), the inoculum in the system was monitored. The analyses confirmed the presence of the M-consortium in the system. The system was operated at the site till 7/2012. After a non-operational period of 4 months, the activity of the inoculated bioreactor was evaluated by following the degradation of MTBE and TBA spikes under recirculation modus. Within a few days, the bioreactor restarted degrading MTBE and TBA without a need for re-inoculation. Subsequent respikes of MTBE and TBA were degraded within a few days. This indicated the system is robust.

#### Pilot test 2

This testing used a different biomass carrier material. An improved filling material to retain the biomass in the bioreactor was selected based on lab scale tests (see DL1.3). Therefore, again, the reactor was uploaded off-site. Firstly, the bioreactor was operated in recirculation mode (no influent & effluent) and spikes of MTBE were regularly given as indicated in. Once good degradation was obtained, MTBE was dosed continuously (5-30 mg/L/day) for another 30 days. All added MTBE was immediately degraded as proven by the low MTBE-concentration measured in the bioreactor. Next, the system was tested under continuous flow conditions (50 L/h) with artificially contaminated groundwater (5000 µg/L MTBE). The data indicated a good performance of the system with effluent concentrations below 100 µg/L.

In a next step, the system was transported and operated at a petrol gas station (Belgium, location 5) treating groundwater mainly contaminated with MTBE. Although high concentrations of MTBE, and locally also for TBA, were found in the piezometers, the existing extraction wells delivered lower pollution concentrations (100-600 µg/L). This is explicable by the

different depths and larger filter screens of the extraction filters as compared to the monitoring filters, which led to dilution of the pollution. MTBE and TBA were removed efficiently in the system (100L/h, HRT = 3h) with bioreactor effluent concentrations below 25 µg/L. The system was operated for 40 days under these conditions.

Afterwards, it was decided to turn off the bioreactor system and make some modification to the groundwater extraction system with the aim to increase the influent concentrations. After a non-operational period of 2 months the system was restarted. In recirculation mode, it was observed that 8 mg/L was removed within 5 days, showing that the bioreactor did not lose its activity. Finally, the system was operated again in continuous mode at 150 L/h (HRT = 2h) with an MTBE influent concentration of 1000 to 1200 µg/L. An MTBE removal of 98% was obtained in the whole system (de-ironing unit + bioreactor), and 94% of the influent in the bioreactor (after de-ironing) was removed in the bioreactor.

#### 3.4.2.5 System 5: (lab-)PBR reactor for immobilised microorganisms & aerobic cometabolic removal of CAHs (UNIBO) – Rho site (IT)

The packed bed reactor (PBR) process developed by UNIBO relies on aerobic cometabolism, which occurs when an enzyme, produced by the cell for the metabolism of a growth substrate, incidentally catalyses the biodegradation of a non-growth substrate. UNIBO's PBR process is aimed at biodegrading the CAHs present in the Rho site, namely trichloroethylene (TCE) and 1,1,2,2-tetrachloroethane (TeCA). A crucial element of the PBR aerobic cometabolic technology developed by UNIBO is represented by the pulsed supply of growth substrate and oxygen. Thanks to this technique, as a result mainly of hydrodynamic dispersion, the over-lapping of substrate and oxygen occurs at low concentration, over a wide carrier portion while moving through the column and, in each point, in a discontinuous way. In this way substrate consumption and biomass growth occur rather homogeneously throughout the column yielding a long and well-developed bioreactive zone. The tests conducted by UNIBO in the framework of WP4 had the following goals: (a) to optimize the pulsed supply of substrate and oxygen; (b) to evaluate the TCE biodegradation rate and conversion attainable at 30°C, by feeding the plant with TeCA-free groundwater; (c) to evaluate the TCE and TeCA biodegradation rate and conversion attainable at 15°C (yearly average temperature of the studied site), by feeding the plant initially with TeCA-free groundwater, and then with groundwater containing both TCE and TeCA.

To achieve these goals, the 31 L PBR (16 m long, 5 cm diameter) was operated in continuous mode for 220 days, with an HRT of 4-4.5 days. The experimental work was articulated into 9 operational phases, characterized by variations of temperature (15-30 °C), TCE inlet concentration (0.3-1.2 mg/L), TeCA inlet concentration (0-0.3 mg/L), type of groundwater (synthetic / real) and schedule of butane and oxygen pulsed supply.

The gradual optimization of the pulsed feed of butane and oxygen, performed by operating the 31 L PBR in continuous mode at 30°C, led to the design of an innovative type of pulsed supply, characterized by the alternation of two operational modes: a biomass growth mode, during which the bioreactor is fed with a sequence of short (8-hour) substrate pulses alternated to oxygen pulses, and a CAH degradation mode, during which the bioreactor is fed with CAH-contaminated groundwater enriched with oxygen; this type of pulsed substrate/oxygen feed requires the operation of 2 or more bioreactors (depending on the ratio of the duration of the two operational modes) in parallel.

When the bioreactor was operated at 30°C with TeCA-free synthetic groundwater, the optimized schedule of pulsed substrate/oxygen feed allowed the attainment of an 81% TCE conversion and a 0.23 1/day TCE normalized degradation rate. When operated at 15°C with TeCA-free real site groundwater, the optimized schedule of pulsed substrate/oxygen feed allowed the attainment of a 71% TCE conversion and a 0.19 1/day TCE normalized degradation rate. Switching to TeCA-contaminated real site groundwater (15°C), the optimized schedule of pulsed substrate/oxygen feed allowed the attainment of a 57% TCE conversion, a 0.11 1/day TCE normalized degradation rate, a 49% TeCA conversion and a 0.07 1/day TeCA normalized rate. Overall, the CAH degradation performances obtained through the aerobic cometabolic process developed by UNIBO under conditions as close as possible to the real site ones are considered satisfactory.

#### 3.4.2.6 System/pilot 1 – Bacteria inoculated MBR with ceramic membrane to treat municipal wastewater (FHNW) – WWTP Birs (CH)

Two identical 230 L submerged MBR pilot systems comprising anoxic and aerobic zones, were taken into operation by FHNW. Target compounds to be removed from wastewater were BPA, NP and Sulfamethoxazole from wastewater. *Sphingomonas* sp. strain TTNP3 and *Microbacterium* sp., were planned to be used as inoculum.

The bioaugmentation by *Sphingomonas* sp. strain TTNP3 was tested in a pilot scale experiment for a period of 21 days. Higher

BPA removal was achieved in bioaugmented reactors compared to reference, both with suspended and immobilized form. However, clear improvement of BPA removal after the bioaugmentation could not be confirmed. Real-time qPCR measurement enabled detection of *Sphingomonas* sp. strain TTNP3 in the activated sludge for up to 2 days after the bioaugmentation. This implies a need for nearly continuous dosing of the inoculum.

Due to difficulties in growing enough sufficiently active biomass of *Microbacterium*, bio-augmentation aimed at SMX degradation could not be performed.

#### 3.4.2.7 System(lab) 9: Bioelectrochemical treatment technology for in-situ removal of CAH-contamination (UNIRM & ISRA) – Modena site (IT)

Bioelectrochemical remediation relies on the use of electrodes to supply the electron donor or acceptor required for contaminant biodegradation in a controlled way and possibly in situ. Colonizing the cathode surface, dechlorinating microorganisms reduce the contaminant using the cathode as an electron donor. The highly chlorinated compound, such as PCE or TCE which are more amenable to reductive dechlorination (RD), is biologically converted into less-chlorinated products which are either nontoxic (such as ethene) or more amenable to following aerobic oxidation (such as cis-DCE or VC). Firstly, the test systems described in WP3 were operated with real groundwater. For the first time bioelectrochemical RD of chlorinated solvents has been verified to be possible in the presence of a real groundwater instead of using a well-defined and simpler synthetic medium. In the system both nitrate and sulphate reduction also occurred, in spite no inoculation of specialised consortia was performed. Hence, the indigenous consortium contained in the groundwater was effective enough to quickly colonize the bioelectrochemical reactor. The RD occurred simultaneously with the sulfate reduction whereas it required that nitrate had previously been consumed. The relative rate and extent of RD and competing reactions has been quantified as function of applied cathodic potential, in the range from -550 to -750 mV vs. SHE. The RD rate increased as the potential became more negative. On the other hand RD always represented a very minor fraction (down to less than 1% at the most negative potential) of overall coulombic efficiency that in turn decreased as the potential became more negative. The application of the system to the field will require that the needed electric power is calculated by taking into account both nitrate and sulfate, whereas methanogenesis can be mostly excluded at potential less negative than - 750 mV vs. SHE.

#### 3.4.2.8 System/pilot 11: Inoculated rhizosphere for removal of micropollutants (TUC) – Heraklion site (GR)

A halophyte constructed wetland (CW) was operated with primary treated wastewater spiked with Bisphenol-A over a period of several months during 2013. Overall, the pilot was able to remove BPA by about 90% (HRT = 1.8-3.43 days) except a short period when the flow rate was doubled (removal was only 55%, HRT = 0.9 days).

Bioaugmentation with *Sphingomonas* sp. strain TTNP3 showed no statistically significant increase in the removal of BPA. It appears the indigenous BPA degraders in the rhizosphere and endophytic to halophytes were able to perform equally well. It is noted that the performance of the CW with respect to COD/BOD removal was not satisfactory and hence, it could be better used as a tertiary treatment technology or be used in combination with other technologies for example an MBR.

In order to probe for BPA degrading microbes in rhizosphere reactors, a BACTRAP experiment was carried out. In agreement between partners TUC and UFZ, this was done at the Chania instead of the Heraklion site. The experiment did not provided information on BPA degrading microbes.

### 3.5 WP5 Evaluation of socio-economic suitability of tested treatment technologies

Work Package 5 focuses on describing the investigated treatment processes with regard to their operating windows, i.e. their applicability with respect to legal framework conditions, sustainability, environmental and socio-economic impacts. The goal was to assess the environmental impact of various MINOTAURUS technologies by means of Life Cycle Analysis. Eventually the operational window for the new developments i.e. characterisation of their applicability under current or future boundary conditions were defined.

This included developing a supporting framework for end-users and policy-makers to evaluate the proposed bioremediation strategies in terms of socio-economical acceptability and technological performance.

A framework and methodology for the risk characterization was developed. The approach includes a systematic and process-related hazard identification based on fault-event-trees for the various sub-processes of the technologies and potential exposure pathways (water, soil, air) with human health impacts and impacts on aquatic and soil systems as endpoints. Additionally, a matrix was elaborated to systematically collect data on the performance of all technologies, such as removal



efficiency, energy use, chemicals usage etc. The information gained varied with the scale at which the technologies are investigated. From these data indicators describing the effectiveness and cost-efficiency of the technology options were developed. The specific removal performance achieved in various levels of technology testing (WP1-4) was compiled in a comparative overview. This fed the descriptive judgement of the operationability of the types of technologies.

Based on the analysis of policy frameworks, risk characterization and characterization of treatment effectiveness the suitability of tested treatment technologies was evaluated regarding

- the feasibility of implementation (technological and financial aspects),
- general acceptability (legal and risk aspects) and
- eco-efficiency (environmental improvement and intensity).

It turned out that some technologies (bioaugmented MBR) were compromised in their effectiveness due to low levels of pollution (micro-pollutants) and their narrow target-substance spectrum, which is a drawback in treating highly diluted streams as they occur in municipal wastewater.

Technologies addressing heavily contaminated sites/compartments (e.g. CAH or MTBE in groundwater) could more easily achieve satisfactory removal at acceptable environmental impact.

#### Potential Impact:

Overall, MINOTAURUS was to contribute to proving and improving the effectiveness of bioremediation processes. In that respect the project features a number of potential impacts:

##### Scientific impact

New knowledge was generated with respect to understanding of biotechnological processes utilising immobilised bio-catalysts for the de-pollution of wastewater and groundwater particularly in relation to the removal of priority compounds and micropollutants. The project consortium developed and transferred detection methods for the purpose of process characterisation and control. This enhanced the insight into the microbial communities involved, the degradation processes and the fate of the pollutants.

At the end of the project, a number of ready-to-use FISH test kits and dedicated qPCR methods are at hand. Even more sophisticated protocols for pyrosequencing of metagenomes and stable isotope probing have been applied. The work has underlined the applicability of molecular-biological tools in monitoring and understanding the process performance.

MINOTAURUS has delivered a total of 31 peer reviewed journal papers and numerous conference presentations. The research work contributed to a number of PhD studies (around 6).

##### Technical impact

MINOTAURUS aspired to bring technologies which have so far been mainly of academic interest into the field application and make them more available for end-users. Those impacts can be verified by successful piloting and technology offers arising from the projects.

This objective has majorly been achieved for groundwater remediation technologies.

The bioaugmented packed-bed reactor with a specialised consortium to degrade MTBE was successfully tested on-site with real groundwater over relevant periods. As a result the system qualified for larger-scale implementation as remediation option for contaminated sites. It constitutes a feasible alternative to the stripping and adsorption onto activated carbon step in pump-and-treat systems.

Further process innovation and its up-scaling tackled the treatment of chlorinated aliphatic compounds (esp. TCE) in groundwater. MINOTAURUS partners developed a new and targeted process for the co-metabolic aerobic biodegradation of TCE.

Another promising perspective in treating TCE contaminated groundwater was opened up by the bioelectrochemically assisted reductive dechlorination. Next to being a chemical-free approach it allows for in-situ restoration of groundwater within the aquifer.

##### Environmental impact

MINOTAURUS tested new options to address the pressing issue of (micro-)pollutants in the environment in a more sustainable



way. A comprehensive and reliable assessment of environmental impacts was not fully feasible for all technologies due to the novelty of some processes and/or the limited performance on pilot scale which complicated estimation for upscaled systems. However, some specific features could be identified as decisive contributors to environmental impacts and potential targets for process improvement. Overall, the further development of these technology is advisable, as ecotoxicological tests confirmed that many of the (environmental) water samples did not show significant acute effects, and that the treatment process as such did not increase the acute toxicity which was a matter of concern in relation to transformation products.

#### Economic impact

MINOTAURUS will have direct and indirect economic impacts. The first being mainly related to an improved cost-effectiveness of wastewater and groundwater treatment processes which will make them more widely applicable and offer business opportunities for the technology providers involved. The latter being realised through improved opportunities of previously polluted water sources, which may have a huge local relevance, particularly in drought-prone areas. MINOTAURUS also offers employment opportunities in those companies and partners which can provide viable new services and products built on the project outputs. It is expected that the total additional revenue gained by the project partners will be in the area of several million EUR in the first five years after the project completion, leading to the generation of some tens of new jobs, generally for well-educated employees.

#### Social impact

Through the impacts stated above MINOTAURUS bears also the opportunity to achieve positive social impacts through an improved quality of life in a healthier environment and a more acceptable environmental biotechnology not utilising genetically modified organisms. Social cohesion will be fostered by new jobs and better water resources as well as the positive international and intercultural relations established during the project.

#### Policy impact

As stated above, MINOTAURUS addresses some key implementation questions related to the Water Framework Directive, the Groundwater Directive and the Priority Substances Directive as well as the Thematic Strategy on Soil protection. Project results will equip decision makers with new options for the mitigation of micropollutant impact on eco-systems and human health.

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<b>Result In Brief</b>	<a href="#">New ways to combat water pollution</a>
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