MinoTaurus

Project Final Report

Grant Agreement number: 265946

Project acronym: MINOTAURUS

Project title:

Microorganism and enzyme immobilization: novel Techniques and Approaches for Upgraded Remediation of Underground-, wastewater and Soil

Funding Scheme: FP7-CP-FP

Period covered: 2011-01-01 to 2013-12-31

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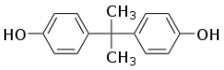
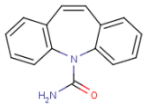
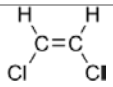
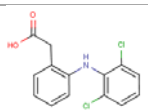
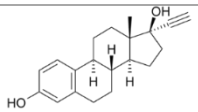
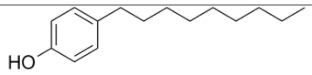
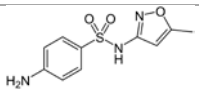
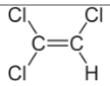
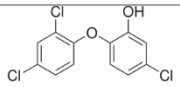
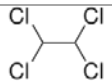
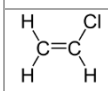
Abbreviations

Abbreviation	Explanation	
BER	bioelectrochemical reactor	
BPA	Bisphenol A	
BZL	Benzotriazole (corrosion inhibitor)	
CBA	chlorobenzoic acid	
CBZ	Carbamazepine (anti-epileptic)	
cis-DCE	cis-Dichloroethene	
CW	Constructed wetland	
DF	Diclofenac (anti-inflammatory)	
EE2	17α-Ethinylestradiol (oral contraception)	
HPLC-ESI-MS	High Performance Liquid Chromatography-Electrospray Ionization-Mass Spectrometry	
FISH	Fluorescence in situ hybridization	
NP	Nonylphenol (industrial chemical)	
PPCP	Pharmaceuticals and personal care prodcuts	
PCR-DGGE	Polymerase Chain Reaction - Denaturing Gradient Gel Electrophoresis	
RD	Reductive dechlorination	
SHE	Standard Hydrogen Electrode	
SMX	Sulfamethoxazole (antibiotic)	
TCE	Trichloroethylene	
TCS	Triclosan (anti-bacterial and anti-fungal)	
TeCA	Tetrachloroethane	
UPLC-QTOF-MS	Ultra Performance Liquid Chromatography-Quadrupole Time of Flight-Mass Spectrometry	
VC	Vinylchloride, Chloroethene	
EDC	Endocrine disrupting compounds	

Final publishable summary report

1 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.

2 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.

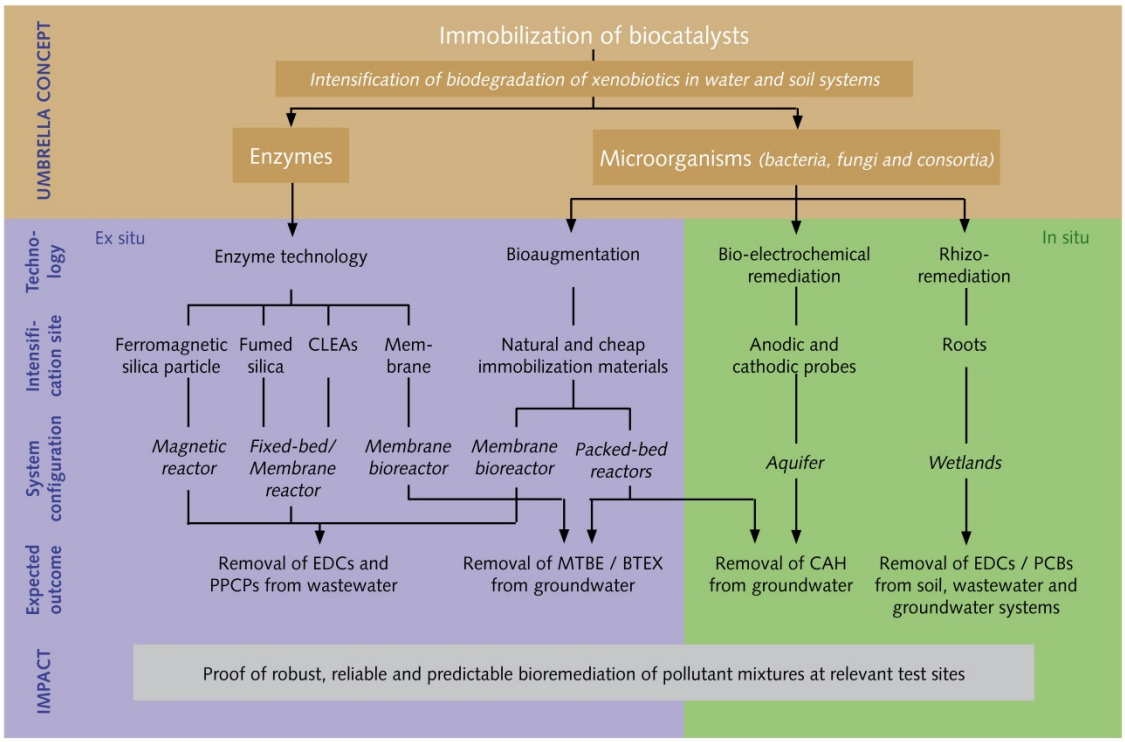


Figure 1 Conceptual overview of the project MINOTAURUS and technologies applied

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.

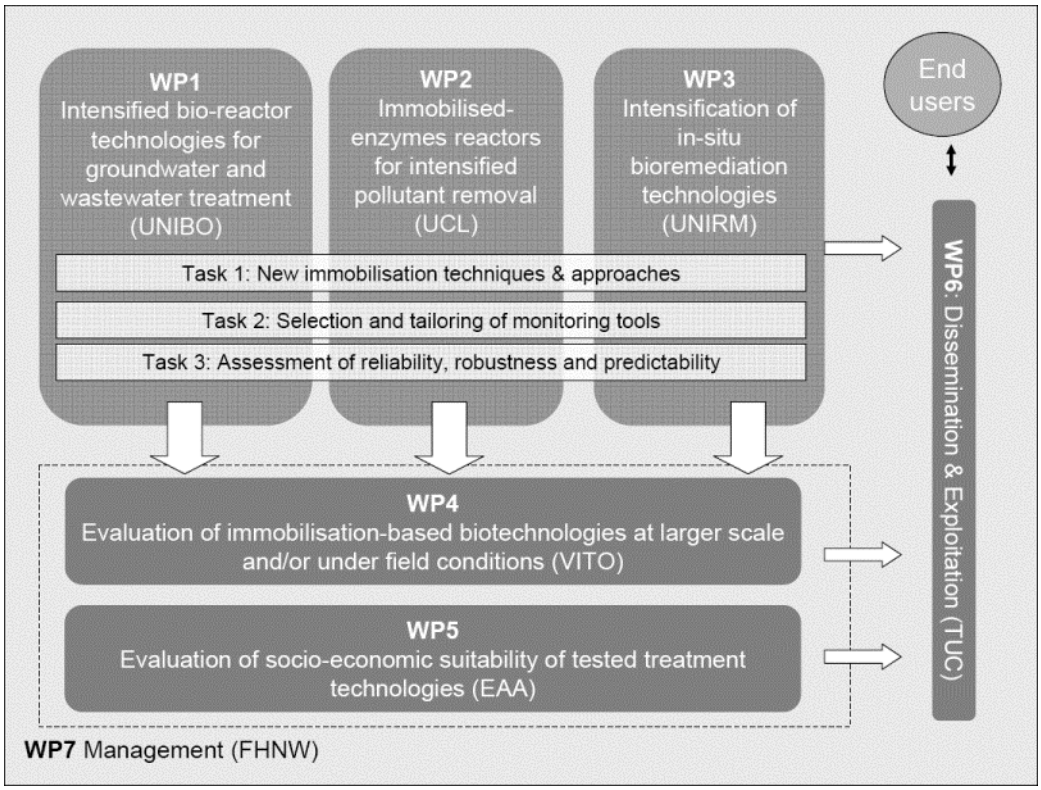


Figure 2 Work Package structure of the MINOTAURUS project

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 1and 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 (Rabin Medical Center)	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/wastwater 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.

3 Main S&T results/foregrounds

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 ^{14}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 reacator (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 $^{14}CO_2$ 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).

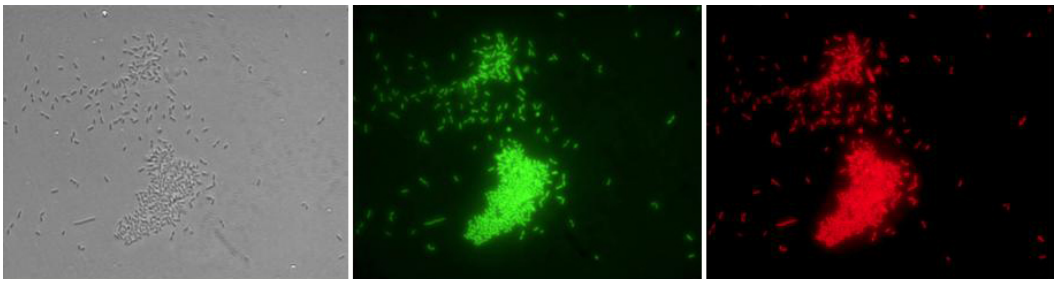


Figure 4 Example of FISH applied to sample detecting Sphingomonas sp. strain TTNP3

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 f**uture 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 *Coriolopsis polyzona*, *Phoma* sp. and *Thielavia* sp. (cf Table 3) which were immombilised 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* *Coriolopsis polyona* Co-immobilised with laccase of Trametes versicolor	Genus *Thielavia*	Genus *Thielavia*	*Phoma* sp.
Immobilisation	Fumed silica nanoparticle	Fe_3O_4-biotitania particles	Silica core particle	No immobilisation
Reactor type	MBR, ultrafiltration membrane	Magenetic 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 Michelis-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

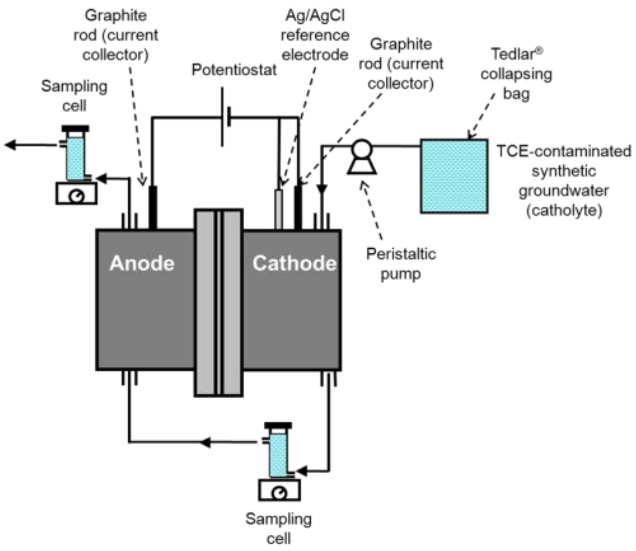
The project also investigated two processes relying on natural systems or in-situ remediation:

- 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_3^- or SO_4^{2-} 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_2 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 *Cypriavidus* 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 →1
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 → magnetic retention reactor*	*WP2*	*x*	*No*	*Birsfelden (CH)*	*2*
1	**FBR-MBR-enzymes**	**WP2**		**Yes**	**Birsfelden (CH)**	**1 →2**
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



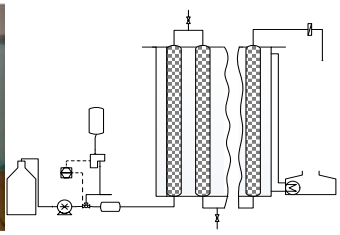
Pilot scale MBR reactor with ceramic flat sheet membranes (System 3)

Installed at a hospital site in Israel



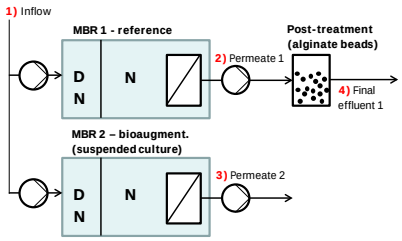
Pilot scale de-ironing unit & Upflow inoculated bioreactor for MTBE/TBA-removal (System 4)

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

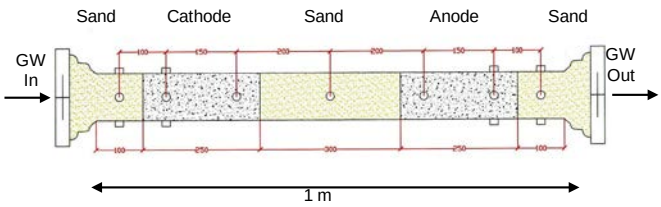
PBR reactor for immobilised microorganisms & aerobic cometabolic removal of CAHs – (System 5)

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^3/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-sconsortium). 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 occour 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.

4 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, particulary 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.

5 Website and contact details

www.minotaurus-project.eu

5.1 Contact details of project partners

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6 Use and dissemination of foreground

6.1 Section A

This section should describe the dissemination measures, including any scientific publications relating to foreground. Its content will be made available in the public domain thus demonstrating the added-value and positive impact of the project on the European Union.

- Template A1: List of all scientific (peer reviewed) publications relating to the foreground of the project
- Template A2: List of all dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

6.1.1 Template A1: List of all scientific (peer reviewed) publications relating to the foreground of the project.

Table 5 List of scientific publications (starting with the most important ones)

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
1	Dechlorination of Trichloroethene in a Continuous-Flow Bioelectrochemical Reactor: Effect of Cathode Potential on Rate, Selectivity, and Electron Transfer Mechanisms 10.1021/es202 262y	Federico Aulenta , Lorenzo Tocca, Roberta Verdini , Priscilla Reale , Mauro Majone	Environmental Science and Technology	Vol. 45/Issue 19	American Chemical Society	United States	01/10/2011	8444-8451			Peer reviewed
2	Development of an attached-growth process for the on-site bioremediation of an aquifer polluted by chlorinated solvents 10.1007/s1053 2-013-9664-z	Dario Frascari, Giacomo Bucchi , F rancesco D oria , Antonella Rosato , Nasrin Tavanaie , Raffaele Salviulo , Roberta Ciavarelli , Davide Pinelli, Serena Fraraccio , Giulio Zanaroli , Fabio Fava	Biodegradation		Springer Netherlands	Netherlands	06/10/2013	0			Peer reviewed
3	Isolation of Bacterial Strains Capable of Sulfamethoxazole Mineralization from an Acclimated Membrane Bioreactor 10.1128/AEM.0 5888-11	H. Bouju , B.Ricken , T. Beffa , P. F.-X. Corvini , B. A. Kolvenbach	Applied and Environmental Microbiology	Vol. 78/Issue 1	American Society for Microbiology	United States	01/01/2012	277-279			Peer reviewed

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
4	An unexpected gene cluster for downstream degradation of alkylphenols in Sphingomonas sp. strain TTNP3 10.1007/s0025 3-011-3451-8	Boris A. K olvenba ch , Hyazinth Dobrowinski, Jan Fousek , Cestmir Vlcek, Andreas Schäffer , Frederic L. P. Gabriel, Hans-Pet er E. Kohler , Philippe F. X.Corvini	Applied Microbiology and Biotechnology	Vol. 93/Issue 3	Springer Verlag	Germany	01/02/2012	1315-1324			Peer reviewed
5	Identification of Bacteria Utilizing Biphenyl, Benzoate, and Naphthalene in Long -Term Contaminated Soil 10.1371/journal.pone.004065 3	Ondrej Uhlik , Jiri Wald , Michal Strejc ek , Lucie Musilova , Jak ub Ridl , Miluse Hro udova , Ce stmir Vlcek , Erick Carde nas , Martina Mackova , T omas Macek	PLoS One	Vol. 7/Issue 7	Public Library of Sc ience	United States	13/07/2012	e40653			Peer reviewed
6	Sorption-assisted surface conjugation: a way to stabilize laccase enzyme 10.1007/s0025 3-011-3534-6	Yannick-Serge Zimmermann , Pat rick Shahgaldian , Philippe F. X. Corvini ,Gregor Hommes	Applied Microbiology and Biotechnology	Vol. 92/Issue 1	Springer Verlag	Germany	01/10/2011	169-178			Peer reviewed
7	Aerobic/anaerobic/aerobic sequenced biodegradation of a mixture of chlorinated ethenes, ethanes and methanes in batch bioreactors 10.1016/j.bio rtech.2012.10.026	Dario Frascari, Serena Fraracci o , Massimo Nocentini , Davide Pinelli	Bioresource Technology	Vol. 128	Elsevier Limited	United Kingdom	01/01/2013	479-486			Peer reviewed
8	CARD-FISH analysis of a TCE-dechlorinating biocathode operated at	Antonella Di Battista , Roberta Verdini, Simona Rossetti ,	New Biotechnology	Vol. 30/Issue 1	Elsevier	Netherlan ds	01/11/2012	33-38			Peer reviewed

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
	different set potentials 10.1016/j.nbt .2012.06.002	Biancama ria Pietrangeli , Mauro Majone , Federico Aulenta									
9	Trichloroethylene aerobic cometabolism by suspended and immobilized butane-grow ng microbial consortia: A kinetic study 10.1016/j.bio rtech.2013.07.006	Dario Frascari, Giulio Zanaroli , Giacomo Bucchi , Antonella Rosato, Nasrin Tavanaie , Serena Fraraccio , Davide Pinelli , Fabio Fava	Bioresource Technology	Vol. 144	Elsevier Limited	United Kingdom	01/09/2013	529-538			Peer reviewed
10	Immobilization of defined laccase combinations for enhanced oxidation of phenolic contaminants 10.1007/s0025 3-013-5055-y	Erik M. Ammann , Christoph A. Gasser , Gregor Hommes , Philippe F.-X. Corvini	Applied Microbiology and Biotechnology	Vol. 98/Issue 3	Springer Verlag	Germany	01/02/2014	1397-1406			Peer reviewed
11	Electrochemical stimulation of microbial cis-dichloroethene (cis-DCE) oxidation by an ethene-assimilating culture 10.1016/j.nbt .2013.04.003	Federico Aulenta , Roberta Verdini, Marco Zeppilli , Giulio Zanaroli, Fabio Fava , Simona Rossetti , Mauro Majone	New Biotechnology	Vol. 30/Issue 6	Elsevier	Netherlan ds	01/09/2013	749-755			Peer reviewed
12	ipso-Hydroxylation and Subsequent Fragmentation: a Novel Microbial Strategy To Eliminate Sulfonamide Antibiotics 10.1128/AEM.0 0911-13	B. Ricken , P.F. X. Corvini ,D. Cichocka ,M. Parisi , M. Lenz , D. Wyss , P. M Martinez -Lavanchy , J. A. Muller , P. Shahgaldian ,L. G. Tulli , H.-P. E. Kohler, B. A. Kolvenbach.	Applied and Environmental Microbiology	Vol. 79/Issue 18	American Society for Microbiol ogy	United States	15/09/2013	5550-5558			Peer reviewed

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
13	Plant secondary metabolite-in duced shifts in bacterial community structure and d egradative ability in contaminated soil 10.1007/s0025 3-012-4627-6	Ondrej Uhlik , Lucie Musilova , Jakub Ridl , Miluse Hroudova , Ce stmir Vlcek , Jiri Koubek , Marcela Holeckova , Martina Mackova , Tomas Macek	Applied Microbiology and Biotechnology	Vol. 97/Issue 20	Springer Verlag	Germany	01/10/2013	9245-9256			Peer reviewed
14	Diversity of chlorobiphenyl-metabolizing bacteria and their biphenyl dioxygenases in contaminated sediment 10.1016/j.che mosphere.2013.07.073	Jiri Koubek , Martina Mackova , Tomas Macek , Ondrej Uhlik	Chemosphere	Vol. 93/Issue 8	Elsevier Limited	United Kingdom	01/11/2013	1548-1555			Peer reviewed
15	Purification and characterization of hydroquinone dioxygenase from Sphingomonas sp. strain TTNP3 10.1186/2191- 0855-1-8	Boris A Kolvenba ch , Markus Lenz , Dirk Benndorf, Erdmann R app , Jan Fousek , Cestmir Vlcek , Andreas Schäffer , Frédéric LP Gabriel, Hans-Peter E Kohler , Philippe FX Corvini	Applied Microbiology and Biotechnology	Vol. 1/Issue 1	Springer Verlag		01/01/2011	8			Peer reviewed
16	Determination of Oxidoreductas e Activity Using a High-Throughput Microplate Respiratory Measurement 10.1021/ac302 716j	Gregor Hommes , Christoph A. Gasser , Erik M. Ammann Philippe F.-X. Corvini,	Analytical Chemistry	Vol. 85/Issue 1	American Chemical Society	United States	02/01/2013	283-291			Peer reviewed
17	Production of a robust nanobiocatalyst for municipal wastewater treatment	Gregor Hommes , Christoph A. Gasser , Chaim B.C. Howald , Roland Goers,	Bioresource Technology	Vol. 115	Elsevier Limited	United Kingdom	01/07/2012	8-15			Peer reviewed

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
	10.1016/j.biortech.2011.11.129	Dietmar Schlosser , Patrick Shahgaldian , Philippe F.-X. Corvini									
18	Design-of-experiment strategy for the formulation of laccase biocatalysts and their application to degrade bisphenol A 10.1016/j.nbt .2012.05.023	Philippe Demarche , Charles Junghanns , Nicolas Mazy, Spiros N. Agathos	New Biotechnology	Vol. 30/Issue 1	Elsevier	Netherlands	01/11/2012	96-103			Peer reviewed
19	Formulation and characterization of an immobilized laccase biocatalyst and its application to eliminate organic micropollutants in wastewater 10.1016/j.nbt .2012.12.004	Rakesh R. Nair , Philippe Demarche , Spiros N. Agathos	New Biotechnology	Vol. 30/Issue 6	Elsevier	Netherlands	01/09/2013	814-823			Peer reviewed
20	Application of the growth substrate pulsed feeding technique to a process of chloroform aerobic cometabolism in a continuous-f low sand-filled reactor 10.1016/j.procbio.2011.08.019	Dario Frascari, Martina Cappelletti , Stefano Fedi , Angelo Verboschi , Roberta Ciavarelli , Massimo Nocentini , Davide Pinelli	Process Biochemistry	Vol. 47/Issue 11	Elsevier BV	Netherlands	01/11/2012	1656-1664			Peer reviewed
21	Chloroform aerobic cometabolism by butane-growi ng Rhodococcus aetherovorans BCP1 in continuous-f low biofilm reactors 10.1007/s0044 9-011-0647-3	R. Ciavare li ,M. Cappelletti , S. Fedi ,D. Pinelli , D. Frascari	Bioprocess and Biosystems Engineering	Vol. 35/Issue 5	Springer Verlag	Germany	01/06/2012	667-681			Peer reviewed

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
22	Pilze im Süßwasser und organische Umweltschadstoffe10.1007/s 1226 8-011-0120-5	Dietmar Schlosser	BioSpektrum	Vol. 17/Issue 7	Elsevier GmbH	Germany	01/11/2011	745-748			Peer reviewed
23	Harnessing the power of enzymes for environmental stewardship 10.1016/j.bio techadv.2011.05.013	Philippe Demarche , Charles Junghanns , Rakesh R. Nair , Spiros N. Agathos	Biotechnology Advances	Vol. 30/Issue 5	Elsevier Inc.	United States	01/09/2012	933-953			Peer reviewed
24	Combined cross-linked enzyme aggregates from versatile peroxidase and glucose oxidase: Production, partial characterization and application for the elimination of endocrine disruptors 10.1016/j.bio rtech.2011.03.018	R. Taboada-Puig , C. Junghanns , P. Demarche ,M.T. Moreira, G. Feijoo , J.M. Lema ,S.N. Agathos	Bioresource Technology	Vol. 102/Issue 11	Elsevier Limited	United Kingdom	01/06/2011	6593-6599			Peer reviewed
25	Trichloroethy lene aerobic cometabolism by suspended and immobilized butane-growing microbial consortia: A kinetic study 10.1016/j.bio rtech.2013.07.006	Dario Frascari, Giulio Zanaroli , Giacomo Bucchi , Antonella Rosato, Nasrin Tavanaie , Serena Fraraccio , Davide Pinelli , Fabio Fava	Bioresource Technology	Vol. 144	Elsevier Limited	United Kingdom	01/09/2013	529-538			Peer reviewed
26	Diversity of chlorobiphenyl-metabolizing bacteria and their biphenyl dioxygenases in contaminated sediment 10.1016/j.che mosphere.2013.07.073	Jiri Koubek , Martina Mackova , Tomas Macek , Ondrej Uhlik	Chemosphere	Vol. 93/Issue 8	Elsevier Limited	United Kingdom	01/11/2013	1548-1555			Peer reviewed

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
27	Advanced enzymatic elimination of phenol ic contaminants in wastewater: a nano approach at field scale 10.1007/s0025 3-013-5414-8	Christoph A. Gasser, Liang Yu, Jan Svojitka, Thomas Wintgens , Erik M. Ammann , Patrick Shahgaldian , Philippe F.-X. Corvini , Gregor Hommes	Applied Microbiology and Biotechnology	Vol. 98/Issue 7	Springer Verlag	Germany	01/04/2014	3305-3316			Peer reviewed
28	The degradation of alkylphenols by Sphingomonas sp. strain TTNP3 – a review on s even years of research 10.1016/j.nbt .2012.07.008	B.A. Kolvenbach , P.F.-X. Corvini	New Biotechnology	Vol. 30/Issue 1	Elsevier	Netherlands	01/11/2012	88-95			Peer reviewed
29	Crystallization and preliminary X-ray crystallographic analysis of hydroquinone dioxygenase from 10.1107/S1744 309112012341	Stefano Da Vela , Marta Ferraroni, Boris A. Kolvenbach , Eva Keller , Philippe F. X. Corvini , Andrea Scozzafava , Fabrizio Briganti	Acta Crystallographica Section F: Structural Biology and Crystallization Communications	Vol. 68/Issue 5	International Union of Crystallography	United Kingdom	01/05/2012	588-590			Peer reviewed
30	Stable isotope probing in the metagenomics era: A bridge towards improved bioremediation 10.1016/j.bio techadv.2012.09.003	Ondrej Uhlik , Mary-Cathrine Leewis , Michal Strejcek , Lucie Musilova , Martina Mackova , Mary Beth Leigh , Tomas Macek	Biotechnology Advances	Vol. 31/Issue 2	Elsevier Inc.	United States	01/03/2013	154-165			Peer reviewed
31	MINOTAURUS: microorganism and enzyme immobilization: novel techniques and approaches for upgraded remediation of	Rita Hochstrat , Philippe F. X. Corvini , Thomas Wintgens	Reviews in Environmental Science and Biotechnology	Vol. 12/Issue 1	Springer Netherlands	Netherlands	01/03/2013	1-4			Peer reviewed

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
	underground-, wastewater and soil 10.1007/s1115 7-012-9293-8										
	Numerical Parameters Estimation in Models of Pollutant Transport with Chemical R eaction 10.1007/978-3 -642-36062-6_55	Fabiana Zama, Roberta Ciavarelli , Dario Frascari , Davide Pinelli	System Modeling and Optimization	Vol. 391	Springer Berlin Heid elberg	Berlin, Heidelber g	01/01/2013	547		No	Article
	Removal of micro-pollutants with immobilized bacteria on alginate beads in waste water treatment	Kolvenbach B, Bouju H, Goers R, Yao Y, Svojitka J Wintgens T, Corvini	IWA Micropol & Ecoha zard		IWA		19/06/2013				Conference
	Role of extracellular laccase in the removal of endocrine disruptors and pharmaceuticals by the aquatic ascomycete Phoma sp. UHH 5-1-03	Hofmann U, Schlosser D.	OxiZymes, Marseille 2013		OxiZymes		18/09/2013				Conference
	Rhizodedgradation of Bisphenol A by the halophytes Tamarix parviflora and Juncus acutus,	Christofilopoulos S, Kalogeraki V, Syranidou E, Manousaki E., Kalogerakis N.	5th International Symposium on Biosorption and Bioremediation, Prague		Institute of Chemical Technolog y, Prague		24/06/2012			Yes	Conference
	The effect of plants and natural compounds on bacterial population in the contaminated soil	Prouzová P, Hoskovcová E, Bedrlíková E, Musilová L, Strejcek M, Uhlík O, Demnerová K, Macková M	5th International Symposium on Biosorption and Bioremediation, Prague		Institute of Chemical Technolog y, Prague		27/06/2012				Conference

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
	Microbial electrochemical technologies for in situ soil and groundwater remediation	Aulenta F., Rossetti S., Verdini R., Majone M.	European International Society of Microbial Electrochemical Technologies (EU-ISMET) Meeting", Het-Pand, Belgium,		European International Society of Microbial Electrochemical Technologies		27/09/2012				Conference
	Biocatalytic weapons against micropolluta nts.	Agathos SN.	Environmental Microbiology and Biotechnology in the frame of the Knowledge-Based Bio and Green Economy; Bologna, IT		EMB		10/04/2012				Conference
	Robust biocatalysts for pollution control and environmentally benign bioprocessing	Charles Junghanns, Philippe De marche , Rakesh R. Nair, Spiros N. Agathos	International Biotechnology Symposium (IBS 2012), Daewu, South Korea		IBS		14/09/2012				Conference
	Bio-inspired entrapped laccases for clean-up of micropollutants in wastewater.	I. Ardao, P. Demarche , R. Nair, S.N. Agathos.	h Congress of European Microbiologists (FEMS 2013), Leipzig, Germany		EMS		21/07/2013				Conference
	Application of sulfamethoxazole-degrading bacteria to improve the	Kolvenbach BA, Ricken B, Cichocka D, Bouju H, Kohler	2nd European Symposium on Water		VITO		20/11/2013				Conference

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
	removal of ant ibiotics from wastewater	HPE, Svojitka J, Wintgens , T and Corvini P FX	Technologies & Management, 20-21 November 2013, Leuven, Belgium								
	.										
	Removal of micropollutants from wastewater by suspended and immobilized bac eria in a pilot scale MBR	Svojitka J, Kolvenbach B, Bouju H, Yu L, Yao Y, Corvini P, Wintgens T	Aachen Conference Water and Membranes, Aachen, DE		AVT/ISA		29/10/2013				Conference
	Dynamics of a Rhizodegradation Pilot Unit Treating Groundwater Contaminated with Bisphenol-A.	Petousi E, Christofilopoulos S, Hatas J, Syranidou E, Kalogerakis N.	7th International Conference on Environmental Engineering and Management, 1 8 – 21 September 2013, Vi enna, Austria		ICEEM, EFB		18/09/2013				Conference
	Development of an attached-growth process for the bioremediation of trichloroethylene- and 1,1,2,2-tetra chloroethane-contaminated groundwater	Rosato A, Frascari D, Bucchi G, Doria F, Lei S, Spaggiari V, Tavanaie N, Adrian Potra F, Ciavarelli R, Pinelli D, Fraraccio S, Zanaroli G, Fava F	Proceedings of Ecomondo 2013 (Rimini, Novembre2013)		Maggioli Editore,		06/11/2013				Conference
	Diversity assessment of endophytic bacteria of the halophytic plant Tamarix parviflora and their role in BPA degradation	Syranidou E, Christofilopoulos S, Weyens N, Venieri D, Vangronsveld J and Kalogerakis N	9th IPS Conference, Hasselt, Belgium, Sept 12-15, 2012		IPS		12/09/2012				Conference

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
	Development of a Biofilm On-Site Process for the Aerobic Cometabolic bioremediation of a Groundwater Contaminated by Trichloroethylene and 1,1,2,2-Tetrachloroethane	Dario Frascari, Giacomo Bucchi, Francesco Doria, Antonella Rosato, Nasrin Tavanaie , Roberta Ciavarelli, Davide Pinelli, Serena Fraaccio, Giulio Zanaroli , Fabio Fava	7th International Conference on Environmental Engineering and Management (I CEEM, 18 – 21 September2013, Vienna, Austria		ICEEM, EFB		18/09/2013				Conference
	Metabolically active bacteria during bio transformation of aromatic pollutants in contaminated soil.	Uhlík O, S trec#ek M, Musilová L, Wald J, Rídl J, Hroudová M, Vl#ek #, Macková M, Macek T	14th International Symposium on Microbial Ecology, Copenhagen, Denmark, 19–24 August 2012.		Internatio nal Society for Microbial Ecology		19/08/2012				Conference
	Estimation of biphenyl dioxygenase diver sity in soils using gene-targete d metage nomics	Strejcek M, Uhlík O, Musilová L, Macek T, Macková M	4th International Symposium on Microbial Ecology, Copenhagen, Denmark, 19–24 August 2012.		Internatio nal Society for Microbial Ecology		19/08/2012				Conference
	Screening for degraders: Novel identification approach using substrate-specific radiolabeling of cells isolated in micro-compartments	Beck H, Sigrist R, Mosler S, Müller JA, Kästner M.	Annual Conference of the Association for General and Applied Microbiology (VAAM) in collaboratio n with the Royal Netherlands Society for		DGHM, VAAM		10/03/2013				Conference

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
			Microbiology (KNVM), Bremen, March 10 – 13, 2013								
	Hunting for active degraders: Novel screening approach combining substrate-specific radiolabelling of cells and their separation in microcompartments	Beck H, Sigrist R, Mosler S, Müller JA, Kästner M	5th Congress of European Microbiologists (FEMS 2013), Leipzig, Germany		FEMS		21/07/2013				Conference
	Robustness and Reliability of an Inoculated Bioreactor for MTBE/TBA-Rem oval from Groundwater	Bastiaens L., Simons Q., Sterckx H., Borgmans G., Debor L., Gemoets J.	1st European Symposium on Remediation technologies and their integration in Water Management. September 25-26, 2012, Barcelona S pain		VITO		25/09/2012				Conference
	Bio-inspired enzyme entrapment and cross-linkin g approaches as alternative tools for enzyme immobilizatio n	Ardao I; Demarche P, Nair R, A gathos SN	International Workshop on New and Synthetic Bioproduction Systems, Hamburg (Germany). Dec ember 6-7, 2012		NN		06/12/2013				Conference
	Advanced Enzymatic Elimination of Phenolic Contaminants in Wastewater:	Hommes G, Gasser CA, Yu L, Svojitka J, Wintgens T, Ammann	2nd Dissemination Workshop of		nano4wat er		24/04/2012				Conference

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
	A Na no Approach at Field Scale;	E, Shahgaldian P and Corvini PFX	the Nano4water Cluster: Recent Advances in Nanotechnology-based Water Purification Methods ; 24-25 April 2012; Aegean Melathron Hotel, Chalkidiki, Thessaloniki, Greece								
	Laccase-biotitania magnetic biocatalysts produced via bio-inspired entrapment for the removal of micropollutants in wastewater	Ardao, I.; Demarche, P.; Nair, R., Agathos, S.N	BSM Symposium on Microbial Diversity for Science and Industry, 26-27 November 2013, Brussels, Belgium		NN		26/11/2013				Conference
	Selection of Sphingomonas & Cypriavidus endophytic isolates from the halophytic plant Tamarix parviflora for bioaugmentation purposes	Syranidou E., Christofilopoulos S., Weyens N., Venieri D., Vangronsveld J & Ka-logera kis N.	5th MIKROBIOKOSM OS Conference. Athens, Greece, 13-15 December 2012.		N.N.		13/12/2012				Conference
	Enhancing microbial bioremediation of chlorinated hydrocarbons with electrodes serving as	Aulenta F, Verdini R, Reale P, Majo ne M.	3rd International Microbial Fuel Cell Conference, 6-8		N.N.		06/06/2011				Conference

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
	electron donors or acceptors.		June 2011, Leeuwarden, The Netherlands								
	Intensification of chlorinated solvents bioremediatio n using electrodes as electron donors or acceptors	Federico Aulenta, Roberta Verdini, Simona Rossetti, Valter Tandoi, Mauro Majone	5th European Bioremediation Conference, 4-7 July 2011, Chania, Greece		N.N.		06/07/2011				Conference
	Laccase stabilisation as a new and biote chnolog ically relevant function of fungal exopolysaccar ides,	Ulrike Hofmann, Die mar Schlosser	5th European Bioremediation Conference, 4-7 July 2011, Chania, Greece		N.N.		06/07/2011				Conference
	Cross-linking of multiple enzyme aggregates–combi#CLEAS of two laccases with broadened pH spectrum	Charles Junghann s, Philippe Demarche , Rakesh R. Nair, Spiros N. Agathos	5th European Bioremediation Conference, 4-7 July 2011, Chania, Greece		N.N.		04/07/2011				Conference
	solation of bacterial strains capable of mine ralizing sulfamethoxa zole from an acclim ated membrane bioreactor	Ricken B, Bouju H, Corvini PFX and Kolvenbach BA	5th European Bioremediation Conference, 4-7 July 2011, Chania, Greece		N.N.		04/07/2011				Conference
	Degradation of sulfamethoxazole by pure strains isolated from an acclimated membrane bioreactor	Kolvenbach BA; Ricken B; Bouju H; Corvini PFX	IV International Conference on Environmental , Industrial and Applied Microbiology (BioMicroWorl d 2011), 14-16 September 2011, Torremolinos,		N.N.		14/09/2011				Conference

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
			Malag a, Spain								
	Robustness and Reliability of an Inoculated Bioreactor for MTBE/TBA-Rem oval from Groundwater.	Bastiaens L., Simons Q., Steeckx H., Borgmans G., Deblor L., Gemoets J.	8th International conference on remediation of chlorinated and recalcitrant compounds. May 21-24, 2012, Monterey, California.		N.N.		21/05/2012				Conference
	Aromatics-Uti lizing Bacteria and Their Functional Genes in Long-Term PCB and PAH Contaminated Soil	Uhlík O, Musilová L, Strejcek M, Wald J, Vlcek, Rídl J, Macková M, Macek T.	15th International Biodeterioration & Biodegradation Symposium, Vienna, Austria, 19 – 24. 9. 2011.		N.N.		19/09/2011				Conference
	Investigation of bacteria involved in intrinsic bioremediation of PCB and PAH-contaminated soil and their biostimulation	Uhlík O, Musilová L, Strejcek M, Wald J, Vlcek, Rídl J, Macková M, Macek T	5th European Bioremediation Conference, 4-7 July 2011, Chania, Greece		N.N.		04/07/2011				Conference
	Isolation of strains capable of mineralizing sulfamethoxazole from an acclimatized membrane bioreactor for wastewater treatment	Bouju H, Ricken B, Wintgens T, Corvini PFX and Kolvenbach B	6th IWA Young Water Professional s Conference, 10-13 July 2012, Budapest, Hungary		IWA		11/07/2012				Conference
	Removal of micro-pollutants with Laccase-conjugated nanoparticles in advanced	Yu L, Hommes G, Wintgens T, Corvini PFX	Proceedings of the 2nd European		L. Bastiaens / VITO		20/11/2013				Conference

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
	wastewater treatment		Symposium Water Technology and Management, 20-21 November 2013, Leuven, BE								
	Micro-pollutant Oxidation by a Laccase from an Aquatic Ascomycete	Hofmann U and Schlosser D.	2nd European Symposium on Water Technologies & Management, 20-21 November 2013, Leuven, Belgium		L. Bastiaens / VITO		20/11/2013				Conference
	Aerobic cometabolic bioremediation of trichloroethy lene- and 1,1,2,2-tetrachloroethane - contaminated groundwater in a packed bed bioreactor	Frascari D, Bucchi G, Doria F, Salviulo R, Rosato A, Tavanaie N, Ciavarel li R, Pinelli D, Fraraccio S, Zanaroli G and Fava F	2nd European Symposium on Water Technologies & Management, 20-21 November 2013, Leuven, Belgium		L. Bastiaens / VITO		20/11/2013				Conference
	Robustness and reliability of an Inoculated Bioreactor for MTBE/TBA-Rem oval from Groundwater – pilot test	Simons Q, Sterckx H, Borgmans G, Gemoets J, Bastiaen s L.	2nd European Symposium on Water Technologies & Management, 20-21 November 2013, Leuven,		L. Bastiaens / VITO		20/11/2013				Conference

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
			Belgium								
	Electrochemically-assisted biodegradation of chlorinated solvents with electrodes serving as electron donors/acceptors.	Majone M., Verdini R., Uccellet ti D., Pallesch i C., Aulenta F., Rossetti S., Zanaroli G., Fava F., Beck H., Mueller J. A., Kästner M.	2nd European Symposium on Water Technologies & Management, 20-21 November 2013, Leuven, Belgium		L. Bastiaens / VITO		20/11/2013				Conference
	Micropollutants clean-up by bio-inspired entrapped laccases.	Ardao I, Demarche P, Nair R and Agathos SN.	2nd European Symposium on Water Technologies & Management, 20-21 November 2013, Leuven, Belgium		L. Bastiaens / VITO		20/11/2013				Conference
	Life cycle analysis of MINOTAURUS wastewater treatment technologies	Steiger O, Niewersch C and Hugi C	2nd European Symposium on Water Technologies & Management, 20-21 November 2013, Leuven, Belgium		L. Bastiaens / VITO		20/11/2013				Conference
	Removal of micropollutants from wastewater by suspended and immobilized bacteria in a pilot scale MBR	Svojitka J, Kolvenbach BA, Bouju H, Yu L, Zenker A, Corvini PFX, Wintgens T.	2nd European Symposium on Water Technologies & Management, 20-21		L. Bastiaens / VITO		20/11/2013				Conference

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
			November 2013, Leuven, Belgium								
	A halophyte suitable for remediating contaminated groundwater with organics Juncus acutus	Christofilopoulos S, Syranidou E, Petoussi M, Kalogerakis N.	2nd European Symposium on Water Technologies & Management, 20-21 November 2013, Leuven, Belgium		L. Bastiaens / VITO		20/11/2013				Conference
	Rhizodegradation of bisphenol A by the halophyte Juncus acutus: Effect of bioaugmentation	Syranidou E, Christofilopoulos S, Corvini PFX, Kalogerakis N.	10th IPS Conference, 1-4 October 2013, Syracuse, N Y, USA		N.N.		01/10/2013				Conference
	Biodegradazione aerobica cometabolica di cloroformio in un reattore a letto impaccato : calibrazione del modello cinetico e fluidodinamico tramite applicazione del me todo di Gauss-Newton	D. Frascari ,R. Ciavarelli, D. Pinelli, F. Zama,	Atti del Convegno GRICU 2012 - Ingegneria Chimica: dalla nanoscala alla macroscala, Montesilvano (PE), 16-19 Settembre 2012		N.N.		16/09/2012				Conference
	Biocatalysis for environmental Clean-up: Past, Present and Future Developments.	Philippe Demarche , Rakesh R. Nair, Spiros N. Agathos.	Biocatalysis Conference 2 012, Xcaret, Mexico, December 4-7, 2012		N.N.		04/12/2012				Conference

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
	Bacteria utilizing aromatic pollutants in long -term contaminated soil as revealed by stable isotope probing and gene-targeted metagenomics	Uhlík O, Strejcek M, Musilová L, Wald J, Rídl J, Hroudová M, Vl#ek #, Macková M, Macek T.	5th International Symposium on Biosorption and Bioremediation, Prague, June 24 - 28, 2012		Institute of Chemical Technology, Prague		24/06/2012				Conference
	Enhanced biodegradation of lower chlorinated ethenes with electrodes serving as electron acceptors	Federico Aulenta, Roberta Verdini, Marco Zeppilli, Dario Frascari, Roberta Ciavarelli, Mauro Majone	5th International Symposium on Biosorption and Bioremediation, Prague, June 24 - 28, 2012		Institute of Chemical Technology, Prague		24/06/2012				Conference
	Modelling of lab-scale packed bed reactor for remediation of groundwater contamination with MTBE/TBA	Margarita Petoussi, Leen Bastiaens, Linde Debor, Nicolas Kalogerakis	5th International Symposium on Biosorption and Bioremediation, Prague, June 24 - 28, 2012		Institute of Chemical Technology, Prague		24/06/2012				Conference
	Towards an intensified biodegradation of micro-polluta nts in membrane bioreactors for wastewater treatment	Helene Bouju, Boris Kolvenbach, Benjamin Ricken, Philippe Co rvini, Thomas Wintgens	5th International Symposium on Biosorption and Bioremediation, Prague, June 24 - 28, 2012		Institute of Chemical Technology, Prague		24/06/2012				Conference
	Robustness and reliability of an inoculated bioreactor for MTBE/TBA-rem oval from groundwater	L. Bastiaens, Q. Simons, H. Sterckx, G.Borghmans, C. Beimfohr , L.Debor, D. Van Houtven, J. Gemoets	5th International Symposium on Biosorption and Bioremediation, Prague, June 24 - 28, 2012		Institute of Chemical Technology, Prague		24/06/2012				Conference
	Treatment of Micro-Pollutants with Free and Immobilized Ascomycetous Laccases	Dietmar Schlosser, U lrike Hofmann, Philippe Demarche , Charles Junghanns,	5th International Symposium on Biosorption and Bioremediation,		Institute of Chemical Technolog		24/06/2012				Conference

No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers (if applicable)	Is open access provided to this publication?	Type
		Spiros N. Agathos	Prague, June 24 - 28, 2012		y, Prague						
	Formulation of defined multienzymatic nanobiocatalysts for environmental applications	Gregor Hom mes, Erik Amman, Phi lippe Corvini	5th International Symposium on Biosorption and Bioremediation, Prague, June 24 - 28, 2012		Institute of Chemical Technology, Prague		24/06/2012				Conference
	TAMARIX PARVIFLORA endophytic bacteria community: are there any potential BPA degraders	Evdokia Syranido u, Stavros Christofilopoulos, Nele Weyens, Danae Venieri, Jaco Vangronsveld and Nicolas Kalogerakis	5th International Symposium on Biosorption and Bioremediation, Prague, June 24 - 28, 2012		Institute of Chemical Technology, Prague		24/06/2012				Conference
	Diversity of BPHA genes in contaminated and pristine soils	Michal Strejcek, Ondrej Uhlik, Lucie Lusilova, Tomas Macek and Martina Mackova	5th International Symposium on Biosorption and Bioremediation, Prague, June 24 - 28, 2012		Institute of Chemical Technology, Prague		24/06/2012				Conference
	Aerobic cometabolic bioremediation of an aquifer polluted by chlorinated solvents: preliminary investigation of the feasibility of a packed bed reactor on-site process.	R. Ciavarelli,G. Bucchi, F. Doria, R. Salviulo , G. Zanaroli, S. Fraraccio, D. Frascari , D. Pinelli, F. Fava	5th International Symposium on Biosorption and Bioremediation, Prague, June 24 - 28, 2012		Institute of Chemical Technology, Prague		24/06/2012				Conference

Table 6 Conference posters of MINOTAURUS partners

	Author, Tile	Year	Partner
	Roberta Ciavarelli, Dario Frascari, Martina Cappelletti, Stefano Fedi, Davide Pinelli. Aerobic cometabolism of trichloromethane by a butane-growing pure culture in continuous-flow biofilm reactors fed with pulsed injections of electron donor and acceptor, IWA Specialist Conference 2011 "Water & Industry", 1-4 May 2011, Valladolid	2011	UNIBO
	Bastiaens L; Simons Q., Debor L., Gemoets J. Use of the MTBE/TBA-degrading M-consortium for groundwater remediation by bioaugmentation. 5th European Bioremediation Conference, 4-7 July 2011, Chania, Greece	2011	VITO
	Bastiaens, L, Simons Q.,Sterckx H., Debor L., Van Houtven D., J. Gemoets. Robustness and reliability of an inoculated bioreactor for MTBE/TBA removal from groundwater. Sustainable approaches to remediation of contaminated Land in Europe (SARCLE-2011) & Contaminated Site Management in Europe (CSME-2011). 24-26 October, 2011, Gent, Belgium	2011	VITO
	D. Frascari, M. Cappelletti, S. Fedi, R. Ciavarelli, M. Nocentini, D. Pinelli, 2011. Chloroform aerobic cometabolism in a continuous-flow sand-filled bioreactor fed with alternate oxygen and growth substrate pulses. 8th Euroepan Congress of Chemical Engineering / 1st European Congress of Applied Biotechnology, Berlin, 25-29 September, 2011.	2011	UNIBO
	R. Ciavarelli, G. Bucchi, F. Doria, R. Salviulo, G. Zanaroli, S. Fraraccio, D. Frascari, D. Pinelli, F. Fava, 2012. Aerobic cometabolic bioremediation of an aquifer polluted by chlorinated solvents: preliminary investigation of the feasibility of a packed bed reactor on-site process. 5th International Symposium on Biosorption and Bioremediation, Prague, June 24 - 28, 2012	2012	UNIBO
	G. Bucchi, R. Ciavarelli, F. Doria, R. Salviulo, G. Zanaroli, S. Fraraccio, D. Frascari, D. Pinelli, F. Fava, 2012. Bioremediation of aquifers polluted by chlorinated aliphatic hydrocarbons: selection and characterization of an indigenous microbial consortium for a packed bed reactor on-site process. Environmental Engineering and Management Journal, 11, n. 3 (Supplement), special issue "Environmental Microbiology and Biotechnology in the frame of the Knowledge-Based Bio and Green Economy, Bologna, April 10-12, 2012: Conference Abstracts", p. S15 (only abstract).	2012	UNIBO
	Hofmann U, Schlosser D. Reactions contributing to micropollutant removal by the aquatic ascomycete Phoma sp. UHH 5-1-03. 5th International Symposium on Biosorption and Bioremediation, Prague, June 24 - 28, 2012	2012	UFZ
	Ardao I; Demarche P, Nair R, Agathos SN. Bio-inspired enzyme entrapment and cross-linking approaches as alternative tools for enzyme immobilization. International Workshop on New and Synthetic Bioproduction Systems, Hamburg (Germany). December 6-7, 2012	2012	UCL
	Bastiaens L., Simons Q., Sterckx H., Borgmans G., Debor L., Gemoets J. 2012. Robustness and Reliability of an Inoculated Bioreactor for MTBE/TBA-Removal from Groundwater. 1st European Symposium on Remediation technologies and their integration in Water Management. September 25-26, 2012, Barcelona Spain	2012	VITO
	Hommes G, Gasser CA, Yu L, Svojitka J, Wintgens T, Ammann E, Shahgaldian P and Corvini PFX (2012) Advanced Enzymatic Elimination of Phenolic Contaminants in Wastewater: A Nano Approach at Field Scale; 2nd Dissemination Workshop of the Nano4water Cluster: Recent Advances in Nanotechnology-based Water Purification Methods; 24-25 April 2012; Aegean Melathron Hotel, Chalkidiki, Thessaloniki, Greece	2012	FHNW
	Strejček M, Uhlík O, Musilová L, Macek T, Macková M. Estimation of biphenyl dioxygenase diversity in soils using gene-targeted metagenomics. 14th International Symposium on Microbial Ecology, Copenhagen, Denmark, 19. – 24. 8. 2012.	2012	ICTP

	Author, Tile	Year	Partner
	Syranidou E., Christofilopoulos S., Weyens N., Venieri D., Vangronsveld J & Kalogerakis N., "Selection of *Sphingomonas* & Cypriavidus endophytic isolates from the halophytic plant Tamarix parviflora for bioaugmentation purposes", 5th MIKROBIOKOSMOS Conference. Athens, Greece, 13-15 December 2012.	2012	TUC
	Uhlík O, Strejček M, Musilová L, Wald J, Rídl J, Hroudová M, Vlček Č, Macková M, Macek T. Metabolically active bacteria during biotransformation of aromatic pollutants in contaminated soil. 14th International Symposium on Microbial Ecology, Copenhagen, Denmark, 19 – 24 August 2012.	2012	ICTP
	Ardao, I.; Demarche, P.; Nair, R., Agathos, S.N. Laccase-biotitania magnetic biocatalysts produced via bio-inspired entrapment for the removal of micropollutants in wastewater. BSM Symposium on Microbial Diversity for Science and Industry, 26-27 November 2013, Brussels, Belgium	2013	UCL
	Beck H, Sigrist R, Mosler S, Müller JA, Kästner M. Hunting for active degraders: Novel screening approach combining substrate-specific radiolabelling of cells and their separation in microcompartments. (FEMS 2013), Leipzig, Germany, July 21-25, 2013	2013	UFZ
	Beck H, Sigrist R, Mosler S, Müller JA, Kästner M. Screening for degraders: Novel identification approach using substrate-specific radiolabeling of cells isolated in micro-compartments, Annual Conference of the Association for General and Applied Microbiology (VAAM) in collaboration with the Royal Netherlands Society for Microbiology (KNVM), Bremen, March 10 – 13, 2013	2013	UFZ
	Bouju H, Corvini N, Nastold P, Corvini PF-X, Wintgens T. Evidences of diclofenac biotransformation by MBR activated sludge under continuous and cyclic aeration. Micropol and Ecohazard 2013; Zürich, 16-20 June 2013 (???or only SNF?)	2013	FHNW
	Christofilopoulos S, Syranidou E, Petoussi M, Kalogerakis N. Juncus acutus: A halophyte suitable for remediating contaminated groundwater with organics. 2nd European Symposium on Water Technologies & Management, 20-21 November 2013, Leuven, Belgium	2013	TUC
	Corvini PFX, Hochstrat R, Wintgens T, Microorganism and enzyme immobilisation for upgrading water: goals & major outcomes of the FP7 MINOTAURUS project. 2nd European Symposium on Water Technologies & Management, 20-21 November 2013, Leuven, Belgium	2013	FHNW, all partners
	Steiger O, Niewersch C and Hugi C (2013). Life cycle analysis of MINOTAURUS wastewater treatment technologies. 2nd European Symposium on Water Technologies & Management, 20-21 November 2013, Leuven, Belgium	2013	FHNW
	Svojitka J, Kolvenbach BA, Bouju H, Yu L, Zenker A, Corvini PFX, Wintgens T. Removal of micropollutants from wastewater by suspended and immobilized bacteria in a pilot scale MBR. 2nd European Symposium on Water Technologies & Management, 20-21 November 2013, Leuven, Belgium	2013	FHNW
	Syranidou E, Christofilopoulos S, Corvini PFX, Kalogerakis N. Rhizodegradation of bisphenol A by the halophyte Juncus acutus: Effect of bioaugmentation. 10th IPS Conference, 1-4 October 2013, Syracuse, NY, USA	2013	TUC
	Syranidou E, Christofilopoulos S, Politi M, Corvini, P & Kalogerakis, N, "Rhizodegradation of bisphenol A by the halophyte Juncus acutus: effect of bioaugmentation", 10th IPS Conference, Syracuse, NY, USA, October 1-4, 2013	2013	TUC

6.1.2 Template A2: List of all dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

Table 7 List of dissemination activities

No.	Type of activities	Main Leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
1	Web sites/Applications	FACHHOCHSCHULE NORDWESTSCHWEIZ	Minotaurus website	18.04.2011	www.minotaurus-project.eu	Scientific community (higher education, Research) - Industry - Civil society - Policy makers - Medias	0	NA
2	Articles published in the popular press	FACHHOCHSCHULE NORDWESTSCHWEIZ	Schadstoffe abbauen	01.11.2011	http://www.euresearch.ch/fileadmin/bilder/SME_Campain/UP_6_11_Schadstoffe.pdf	Scientific community (higher education, Research) - Civil society - Policy makers		german speaking countries
3	Articles published in the popular press	FACHHOCHSCHULE NORDWESTSCHWEIZ	Startschuss zum EU-Projekt MINOTAURUS	01.04.2011	Newsletter of FHNW, Report on Kick-off	Scientific community (higher education, Research) - Industry - Civil society - Policy makers		Switzerland
4	Presentations	FACHHOCHSCHULE NORDWESTSCHWEIZ	MINOTAURUS project overview and highlights of the first year	09.11.2011	RECLAIMexpo / ECOMONDO, Rimini, Italy, session on EU-projects	Scientific community (higher education, Research) - Industry - Policy makers - Medias		various, depending on audience
5	Exhibitions	FACHHOCHSCHULE NORDWESTSCHWEIZ	Cleantec City - Swiss platform for sustainable development in	19.03.2012	Bern, CH (booth with exhibit of pilot plant - MBR for bioaugmentation)	Scientific community (higher education, Research) - Industry - Civil society - Policy makers - Medias		Switzerland

No.	Type of activities	Main Leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
			communities , cities and enterprises					
6	Publication	UNIVERSITE CATHOLIQUE DE LOUVAIN	es enzymes, chasseurs des micropolluants	01.07.2011	article in Le Vif-L'Express 6/11. based on interview with project partner UCL	Civil society		Belgium / french speaking
7	Interviews	HELMHOLTZ-ZENTRUM FUER UMWELTFORSCHUNG GMBH - UFZ	Der Pilz - ein Sanierungskünstler	09.04.2012	Interview at German radio station Deutsche Welle (broadcasting abroad)	Civil society - Medias		international
8	Exhibitions	AQUAFIN N.V.	Open door event on Biotech day at Aquafin, exhibiting the pilot plant for bioaugmentation	23.06.2012	Aartselaar / Schilde	Civil society - Medias	250	Belgium
9	TV clips	AQUAFIN N.V.	ATV-report "Aquafin tests pilot installation"	18.06.2012	Aartselaar	Civil society		Belgium
10	Flyers	TECHNICAL UNIVERSITY OF CRETE	Minotaurus project flyer	01.06.2011	NA	Scientific community (higher education, Research) - Industry - Civil society - Policy makers	1000	NA
11	Organisation of Workshops	ALMA MATER STUDIORUM	Novel approaches to the	08.11.2012	Rimini	Scientific community (higher education, Research) - Industry	45	various, IT,

No.	Type of activities	Main Leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
		- UNIVERSITA DI BOLOGNA	sustainable bioremediation of groundwater and wastewater developed in the frame of the FP7 project MINOTAURUS					
12	Organisation of Workshops	UNIVERSITA DEGLI STUDI DI ROMA LA SAPIENZA	Contaminated site remediation - Application of advanced tools to control biological processes	27.05.2013	Rome	Scientific community (higher education, Research) - Industry	20	various, attendees from all over Europe and abroad
13	Articles published in the popular press	FACHHOCHSCHULE NORDWESTSCHWEIZ	Risk characterization of innovative bio-processes for water purification	01.02.2013	Muttenz	Scientific community (higher education, Research) - Industry - Civil society	1000	Switzerland, countries of project partners
14	Exhibitions	FACHHOCHSCHULE NORDWESTS	Micropollutants in Human	25.09.2013	Wisla, PL	Scientific community (higher education, Research) - Policy makers	100	Poland, evnt held in the frame of conference of the

No.	Type of activities	Main Leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
		CHWEIZ	Environment					Gdansk Water Foundation
15	Oral presentation to a scientific event	FACHHOCHSCHULE NORDWESTSCHWEIZ	Microorganism and enzyme immobilisation for upgrading water: goals & major outcomes of the FP7 MINOTAURUS project.	20.11.2013	2nd European Symposium on Water Technologies & Management, 20-21 November 2013, Leuven, Belgium	Scientific community (higher education, Research)	150	various, mostly European countries
16	Oral presentation to a scientific event	VLAAMSE INSTELLING VOOR TECHNOLOGISCH ONDERZOEK N.V.	Innovaties in groundwater sanering	29.03.2013	Seminarie: Bodemsanering anno 2013: evoluties in kennis en beleid, Ghent University	Scientific community (higher education, Research)	40	Belgium, Netherlands

Table 8 Conferences attended by MINOTAURUS partners

NO.	Type of activities	Main leader	Title	Date/Period	Place	Type of audience	Size of audience	Countries addressed
	Conference	VITO, UNIRM, UNIBO, FHNW, UCL, UFZ-EM, ICTP	5th European Bioremediation Conference	4-7 July 2011	Chania, Greece	2011	300	International
	Conference	FHNW	IV International Conference on Environmental, Industrial and Applied Microbiology (BioMicroWorld 2011)	14-16 September 2011	Torremolinos, Malaga, Spain	2011	200	Europe
	Conference		8th International conference on remediation of chlorinated and recalcitrant compounds.	21-24 May 2012	Monterey, California	2012	220	International, North America
	Symposium		15th International Biodeterioration & Biodegradation Symposium,	19-24 September 2011	Vienna, Austria,	2011	190	International, Europe
			SARCLE-2011,	24-26 October 2011	Gent, Belgium	2011		
2	Symposium	UFZ-UM	OxiZymes 10th International Symposium on Peroxydases	16-19 September 2012	Marseille, France.	2012	200	Europe
3	Symposium	VITO, UNIRM, UNIBO, FHNW, UCL, UFZ-UM	5th International Symposium on Biosorption and Bioremediation, ,	24 - 28 June 2012	Prague	2012	140	International, Europe
	Conference	VITO	3rd Sede Boquer Conference on Water technologies,	15-17 October 2012	Be'er Sheva, Israel,	2012	150	Israel, Europe
		VITO, FHNW, TUC,	Ecomondo fair, November 9th, Rimini, Italy. (oral presentation)	9 November 2012	Rimini, Italy	2012		
			(EMB2012).,	10-12 April 2012	Bologna	2012		
	Symposium	UCL	International Biotechnology Symposium (IBS 2012),	14-21 September 2012	Daewu South Korea,	2012		International, Asia

NO.	Type of activities	Main leader	Title	Date/Period	Place	Type of audience	Size of audience	Countries addressed
			Daewu South Korea,					
	Conference	UCL	Biocatalysis Conference 2012,	4-7 December 2012	Xcaret, Mexico,	2012	220	International, North America, Latin America
	Conference	TUC	9th IPS Conference,	12-15 September 2012	Hasselt, Belgium,	2012	100	International, Europe
		UNIBO	Atti del Convegno GRICU 2012 - Ingegneria Chimica: dalla nanoscala alla macroscala,	16-19 September 2012,	Montesilvano Italy	2012	50	Italy
	Symposium	ICTP	14th International Symposium on Microbial Ecology,	19 – 24 August 2012.	Copenhagen, Denmark,	2012	100	International
	Symposium	VITO	1st European Symposium on Remediation technologies and their integration in Water Management.	25-26 September 2012	Barcelona Spain	2012	90	Europe
	Workshop	UCL	International Workshop on New and Synthetic Bioproduction Systems,	6-7 December 2012	Hamburg (Germany).	2012	60	Europe
	Workshop	FHNW	2nd Dissemination Workshop of the Nano4water Cluster: Recent Advances in Nanotechnology-based Water Purification Methods	24-25 April 2012	Chalkidiki, Thessaloniki, Greece	2012	50	Europe
	Conference	TUC	5th MIKROBIOKOSMOS Conference	13-15 December 2012	Athens, Greece,	2012	90	Greece, international
	Conference	FHNW	6th IWA Young Water Professionals Conference,	10-13 July 2012,	Budapest, Hungary	2012	140	International
	Meeting		European International Society of Microbial Electrochemical Technologies (EU-ISMET) Meeting",	27-28 September 2013	Het-Pand, Belgium,	2013	80	Europe

NO.	Type of activities	Main leader	Title	Date/Period	Place	Type of audience	Size of audience	Countries addressed
	Congress	UCL, UFZ	5th Congress of European Microbiologists (FEMS 2013),	July 21-25, 2013	Leipzig, Germany,	2013	400	International
	Symposium	VITO, UNIRM, UNIBO, FHNW, UCL, UFZ-EM	2nd European Symposium on Water Technologies & Management	20-21 November 2013,	Leuven, Belgium	2013	160	Europe
	Conference	FHNW	10. Aachen Conference Water and Membranes,	29-30 October 2013,	Aachen, Germany	2013	350	Germany, Benelux countries
	Conference	TUC, UNIBO	7th International Conference on Environmental Engineering and Management (ICEEM, ,	18- 21 September 2013	Vienna, Austria	2013	250	International
		UNIBO	Proceedings of Ecomondo 2013	November 2013	Rimini	2013		Italy and wider Europe
	Conference	UFZ-UBT	Annual Conference of the Association for General and Applied Microbiology (VAAM) in collaboration with the Royal Netherlands Society for Microbiology (KNVM)	10-13 March 2013	Bremen	2013	120	Germany
	Conference	TUC	10th IPS Conference,	1-4 October 2013,	Syracuse, NY, USA	2013	400	International
	Symposium	UCL	BSM Symposium on Microbial Diversity for Science and Industry,	26-27 November 2013	Brussels, Belgium	2013	100	Benelux, Europe
	Conference	FHNW	IWA Micropol & Ecohazard	16-20 June 2013	Zurich, CH	2013	600	International

6.2 Section B

This section should specify the exploitable foreground and provide the plans for exploitation. All these data can be public or confidential; the report must clearly mark non-publishable (confidential) parts that will be treated as such by the Commission. Information under Section B that is not marked as confidential will be made available in the public domain thus demonstrating the added-value and positive impact of the project on the European Union.

Section B (Confidential or public: confidential information to be marked clearly)

6.2.1 Part B1

The applications for patents, trademarks, registered designs, etc. shall be listed according to the template B1 provided hereafter.

The list should, specify at least one unique identifier e.g. European Patent application reference. For patent applications, only if applicable, contributions to standards should be specified. This table is cumulative, which means that it should always show all applications from the beginning until after the end of the project.

Table 9 Applications for patents

Type of IP Rights	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)
Patent	Yes		EP 14 150 780.6	bacterial strains and consortium comprising same for degrading MTBE, TBA and /or HCHO	VITO

6.2.2 Part B2

Table 10 Exploitable Foreground

Type of Exploitable Foreground	Description of exploitable foreground	Confidential, Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
General advancement of knowledge	Identification of Microbacaterium BR1as sulfonamide degrader	No		new bacterial strain description and cultivation	Scientific re-search and development	2014 ongoing	none	FHNW
General advancement of knowledge	Reactor design and operational concept	No		Attached-cell bioreactor	Water supply; sewerage; waste management and remediation activities	2015	none	UNIBO
Commercial exploitation of R&D results	Various FISH kits for the detection of specialised micro-organisms	No	31.12.2014	FISH kits	Scientific research and development, general microbiological diagnostics; industrial micro biology	2014	none	VER
General advancement of knowledge	qPCR method for the detection of various degraders Sphingomo-nas/ Microbacterium	No		analytical method, primers and procedures for qPCR	Scientific research and development,	2014-15	none	FHNW
Commercial exploitation of R&D results	Improved carrier materials selected for bioreactors	No		carrier material for biomass growth and application in PBR	Drinking water supply; water management; remediation activities	2014	no	VITO
Commercial exploitation of R&D results	Data on the behavior of the M- consortium and its three key-organisms during remediation processes & Data proving the robustness &	No		Reactor design for MTBE remediation based on this biotechnology	Drinking water supply; water management; remediation activities	2013-2014	EP14150780	VITO

Type of Exploitable Foreground	Description of exploitable foreground	Confidential, Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
	reliability of the inoculated bioreactor for treating MTBE contaminated water with the M-consortium as inoculum							
General advancement of knowledge	Bioelectrochemical reactor design and operational concept	No		Bioelectrochemical reactor design and operational concept	Remediation activities	2014-2016	no	UNIRM; IRSA-CNR
General advancement of knowledge	Optimization of 16S rRNA gene amplicon data analyses & Optimization of functional gene amplicon data analyses	No		New molecular-biological Tools for characterising soil and plant microoganisms	Scientific research and development	2014 ff	no	ICTP
General advancement of knowledge	Identification of bacteria of the genus Rhodanobacter as biphenyl-utilizers	No		newly identified str ain for further metabolic investigations	Scientific research and development	2014 ff	no	ICTP
General advancement of knowledge	Bio-inspired enzyme entrapment	No		improved method ology to immobilise Enzyme into particles	R&D of biocatalytic processes	2014 ff	no	UCL
General advancement of knowledge	Continuous reactor with magnetic retention principle	No		Insight into new Retention mechanism as Basis for react or design improvement	R&D of biocatalytic processes	2014 ff	no	UCL

Type of Exploitable Foreground	Description of exploitable foreground	Confidential, Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
General advancement of knowledge	Identification of Phoma sp. UHH 5-1-03 as a versatile biocatalyst degrading diclofenac, triclosan, 17#-ethiny lestradiol, bisphenol A, nonylphenol, and sulfamethoxazole in mixture	No		thorough description of Phoma Degradation capacities	Scientific research and development	2014 ff	no	UFZ-EM, FHNW, AQF
General advancement of knowledge	In situ identification of organic pollutant degrading microbes using stable isotope probing linked to microcosms	No		BACTRAP (Product development started prior MINO-TUARUS)	Scientific research and development	2014 ff	no	UFZ-UBT
General advancement of knowledge	In situ identification of organic pollutant degrading microbes using radio isotope probing & for assessment of contaminated sites for natural attenuation	No		Assessment of contaminated sites for natural attenuation	Scientific research and development	2014 ff	no	UFZ-UBT
General advancement of knowledge	Knowledge about pollutant fate in constructed wetland and conclusion of design parameters	No		The halophyte-based CW can be exploited for tertie ry treat-ment of munici-pal wastewa ter for the removal of emerging contaminants (like bisphenol-A)	Sewage treat-ment; waste management; reuse	2014 ff	no	TUC

Table 11 Explanation of Exploitable Foreground

Exploitable Foreground	Explanation
Identification of Microba-caterium BR1as sul-fonamide degrader	Identification of Microbacaterium BR1as sulfonamide degrader, knowledge for further utilisation of its capacity in wastewater treatment processes
Reactor design and operational concept	Reactor design and operational concept for the removal of chlorinated solvents from groundwater, Mid-term goal (2015): development of on-site processes for groundwater bioremediation in the framework of pump and treat pro-cesses
Various FISH kits for the detection of speciali-sed microor-ganisms	Molecular-biological method for tracking and analysis of specific microorganisms in wastewater
qPCR method for the detection of various degraders Sphingomo-nas/ Microbacterium	Molecular-biological method for tracking and analysis of specific microorganisms in wastewater
Improved carrier materials selected for bioreactors	Basic requirement for improved effectiveness of technology
Data on the behavior of the M- consortium and its three key-organi sms during re-mediation proces ses??Data proving the robustness & reliability of the inoculated biore actor for treating MTBE contam inated water with the M-consor tium as inoculum	Better characterisation of processes and utilisation of this knowledge for reactor operation
Bioelectrochemical reactor design a nd operational concept	Effective, sustainable groundwater remediation (cholrinated solvents) whose most important advantage is no need for subsurface injection of any chemi cals.?Possibe ex-tension to other contami-nats is being considered?
Optimization of 16S rRNA gene ampli-con data analyses??Optim ization of functional gene amplicon data analyses	Improved analytical procedures to characterise complex microbial communities and assess their Degradation potential for specific contaminants
Identification of bacteria of the g enus Rhodanobacter as biphenyl -utilizers	potential for use in bioaugmentation and intensification of PCB Degradation in contaminated soils
Bio-inspired enzyme entrapment	Enzyme en-trapment un-der mild conditions, The method can be exploited for the entrapment of many kinds of enzymes by R&D bioprocess engineers
Continuous reactor with magnetic retention principle	Continuous processes catalyzed by magnetid (bio)catalysts, The reactor can be exploited for any kind of process cataly-zed by magne-tid (bio)catalysts
Identification of Phoma sp. UHH 5-1-03 as a versatile biocatalyst degrading diclofenac, triclosan, 17#-ethinylestradiol, bisphenol A, nonylphenol, and sulfamethoxazole in mixture	Thorough knowledge about degrading capacities as to support application in wastewater treatment
In situ identification of organic p ollutant degrading microbes using s table isotope probing linked to mic rocosms	BACTRAP (Product deve-lopment started prior MINO-TUARUS)?Assessment of contaminated sites for natural attenuation

In situ identification oforganic po llutant degrading microbes using ra dio isotope probing??for asses sment of contaminated sites for nat u-ral attenuation	Assessment of contaminated sites for natural attenuation
Knowledge about pollutant fate in c onstructed wetland and conclusion of design parameters	Rhizodegradation combined with phytodegradation, The halophyte-based CW can be exploited for tertiery treatment of munici-pal wastewater for the removal of emerging contaminants (like BPA)