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PROJECT UPDATE

MINOTAURUS: microorganism and enzyme immobilization: novel techniques and approaches for upgraded remediation of underground-, wastewater and soil

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Abstract The European project MINOTAURUS aims to deliver innovative bio-processes to eliminate emerging and classic organic pollutants. These bio-processes are all based on the concept of immobilization of biocatalysts (microorganisms and enzymes) and encompass bioaugmentation, enzyme technology, rhizoremediation with halophytes, and a bioelectrochemical remediation process. The immobilization-based technologies are applied to engineered ex situ and natural systems in situ for the bioremediation of groundwater, wastewater and soil. The selection and application of modern physico-chemical, biological and ecotoxicological monitoring tools combined with a rational understanding of engineering, enzymology and microbial physiology is a pertinent approach to open the black-box of the selected technologies. Reliable process-monitoring constitutes the basis for developing and refining biodegradation kinetics models, which in turn will improve the predictability of performances to be achieved with our technologies. A key strength of MINOTAURUS is the possibility of direct implementation of our technologies at five European reference sites that are confronted with pollutants (two technologies will be tested on-site starting from the first year). We will deliver not only a set of tools, techniques, and processes, which will enhance the ability of our communities to respond to the challenges of organic pollutants but also frameworks for structuring and making evidence-based decisions for the most sustainable and appropriate bioremediation measures. The MINOTAURUS consortium includes fifteen partners from eight European countries. Eight research & education institutions, five SMEs covering the whole chain of our bioremediation approaches (production, and monitoring of biocatalysts, bioremediation and engineering), one large end-user operating wastewater treatment plants and one environmental agency work together with the support of an advisory board mainly consisting of environmental decision-makers.

Keywords Environmental biotechnology · European project · Immobilization of biocatalysts · Bacteria · Fungi · Enzymes

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1 Introduction

The "chemical space" is the source of hundreds of thousands of compounds, which find novel industrial applications to improve our daily life (Daughton 2009). In the last decades a plethora of contaminations by organic pollutants and their associated effects on

human health and ecosystems have led to the definition of several classes of pollutants based on heterogeneous criteria, e.g. (eco)toxicological effects such as endocrine disrupting chemicals (EDCs) and uses such as the pharmaceuticals and personal care products (PPCPs) whereby some of these pollutants can, in some cases, belong to several classes.

Although emerging pollutants are attracting increasing attention of the scientific community and authorities in recent years, many problems are still related to "old classic pollutants". MINOTAURUS partners target also the degradation of such persistent organic pollutants (POPs), which are found in relevant concentrations and are also significantly represented in lists of harmful compounds, e.g. the Stockholm convention list and list of priority substances in the European Union.

Several scientists have pointed out the fact that micropollutants are not only found as individual chemicals in the environment, but rather as "cocktails" which can exert synergistic effects on living organisms (Pomati et al. 2008). In addition to possible cumulative adverse effects, mixtures of organic pollutants represent a challenge for environmental technology concerning their elimination. Even non-specific physico-chemical treatments such as ozonation and other advanced oxidation processes that in theory can degrade any micropollutant do not represent the ultimate solution for the elimination of micropollutants cocktails possessing various physico-chemical properties. For instance iodinated contrasting agents are not completely removed by ozonation treatment of wastewater (Ternes and Joss 2006).

2 Research and technology developments in MINOTAURUS project

As a research and technological development (RTD) initiative, the MINOTAURUS project aims to deliver an innovative set of novel environmental biotechnologies, which are all based on the concept of immobilization of biocatalysts, in order to eliminate emerging as well as classic organic pollutants. MINOTAURUS makes use of both ground-breaking new biocatalysts and well-established, tried and proven ones. The project deliberately addresses the elimination of compounds representative of several classes of pollutants and mixtures thereof, thus reflecting the real problem of contamination by organic pollutants. The proposed technologies apply to both engineered (ex-situ) and natural (in situ) systems for the bioremediation of groundwater, wastewater and soil. The technologies aim at an improved control and enhancement of degradation reactions by immobilized biocatalysts, i.e. microorganisms. Among these seven technologies, five are reactor-based technologies (ex situ) and two are in situ-based biotechnologies (Fig. 1).

2.1 Ex-situ technologies

1. Covalently linked enzyme aggregates (CLEAs) and magnetic nanoparticle conjugates of laccase are applied to a basket perfusion reactor and magnetic separation reactor, respectively, for the degradation of endocrine disrupting chemicals (EDCs, i.e. nonylphenols (NPs) and bisphenol A (BPA)) as well as PPCPs, i.e. sulfamethoxazole (SMX) and carbamazepine (CBZ)) in wastewater.
2. Immobilization of laccase on nanostructured silica for the removal of EDCs and PPCPs in a fixed-bed/membrane reactor treating municipal wastewater.
3. Immobilization of other relevant enzymes on membranes for the removal of methyl tert butyl ether (MTBE) and its degradation product tert-butyl alcohol (TBA) in a membrane bioreactor (MBR) treating contaminated groundwater.
4. Bioaugmentation of an MBR using isolated strains of bacteria and fungi as well as microbial consortia immobilized on natural and cheap material (e.g. clay) for the degradation of EDCs and PPCPs in wastewater.
5. Bioaugmentation of packed-bed bioreactors for the increased degradation of (i) MTBE, TBA and benzene toluene ethylbenzene xylene (BTEX) by immobilized cells of an enriched microbial consortium in groundwater; (ii) low chlorinated aliphatic hydrocarbons (CAHs) via co-metabolic degradation by immobilized cells of pure strains and microbial consortia in groundwater.

2.2 In situ technologies

6. Intensified biodegradation of highly chlorinated CAH by microorganisms immobilized on polarized solid state electrodes (cathodes and anodes) in aquifers.

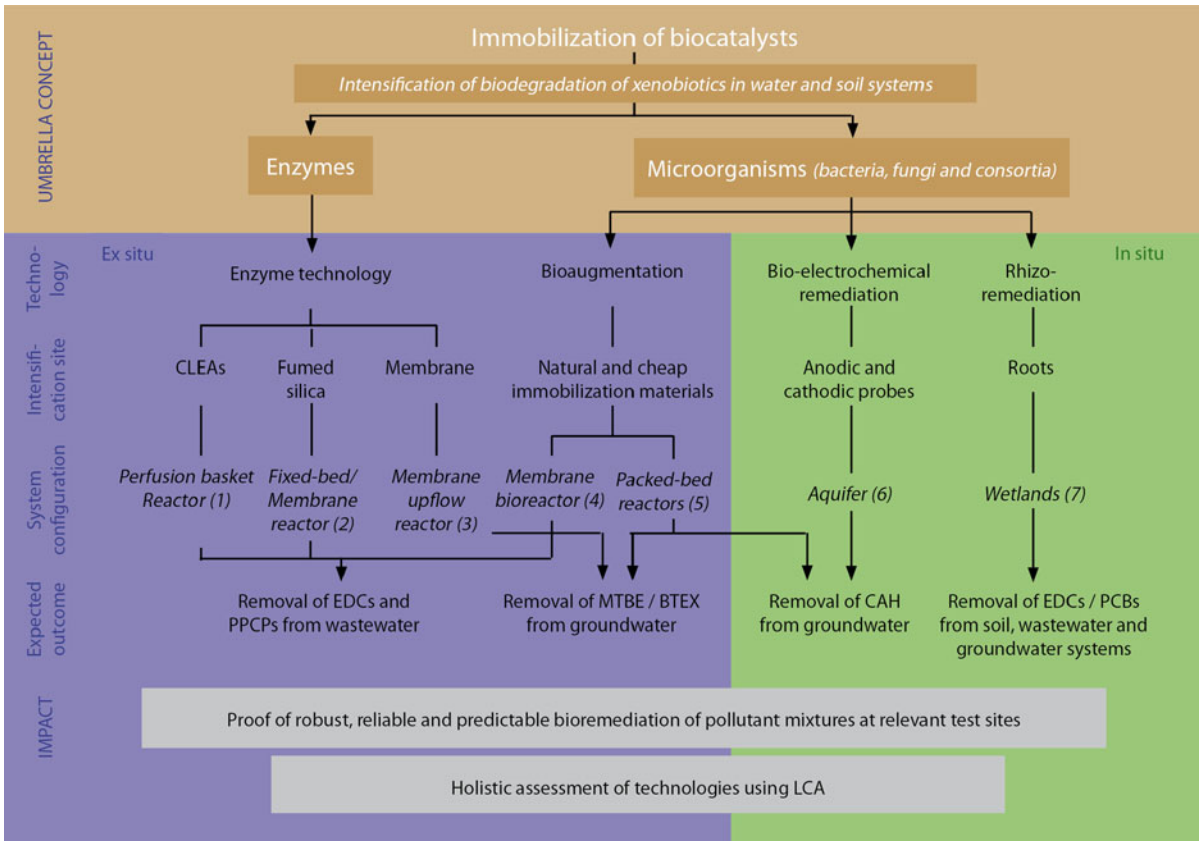

Fig. 1 Conceptual overview of the project MINOTAURUS. Numbers in parentheses indicate the technology number

7. Intensified biodegradation of polychlorinated biphenyls (PCBs) and BPA (as a degradation product of e.g. polybrominated flame retardants) by naturally occurring microorganisms and exogenous ones immobilized on the roots of halophytes in wetland systems depolluting soil/waste- and groundwater systems.

3 Concept of the MINOTAURUS project

To ensure the optimal development of the technologies, each bioremediation process will be monitored and assessed using a set of technology-tailored tools. To open the "black-box" of the proposed environmental bio-processes a variety of cutting-edge physico-chemical and biological methods (e.g. optrodes, metagenomics and isotope fractionation) is adapted. The reliability of the analytical tools is proofed through a set of tests for the chemical, biological and ecotoxicological monitoring. The acquisition of reliable measurements will constitute a solid basis to develop and refine our biodegradation kinetics models, which will be the means to improve the predictability of performances to be achieved with the investigated biotechnologies. To facilitate the transfer and upscaling of the technologies, on-site testing is performed for a selected number of the developed and improved technologies, the so called "process champions".

The application of bioremediation technologies under real conditions will be achieved with the active participation of institutions and companies (SMEs and a large end-user Aquafin), which are in charge of the operation and decontamination of contaminated sites and wastewater treatment operations, respectively. The participation of stakeholders represents a checkpoint for the technological and economical relevance of the solutions proposed.

Table 1 Partners in the MINOTAURUS project

Partner	Expertise
University of Applied Sciences and Arts Northwestern Switzerland, School of Life Sciences, Institute for Ecopreneurship, Switzerland	Project coordination, production and application of laccase-fumed-silica conjugates, MBR bioaugmentation for the degradation of EDCs and PPCPs
Helmholtz Centre for Environmental Research – UFZ, Germany	Characterization of laccases and producing strains, stable isotope probing and compound specific stable isotope analysis
Université Catholique de Louvain, Belgium	Production of CLEAs and magnetic nanoparticle-laccase conjugates
University of Bologna, Italy	Development of CAH aerobic and anaerobic dechlorination processes
Technical University of Crete, Greece	Rhizoremediation approaches
University of Rome La Sapienza, Italy	Bio-electrochemical approaches for the elimination of CAH
Wetlands Incubator sprl, Belgium	Large-scale production of laccase
Institute of Chemical Technology Prague, Czech Republic	Molecular biology approaches for the characterization of biocoenosis in the rhizosphere, ecotoxicity testing
Flemish Institute for Technological Research (VITO)., Belgium	PBR process for the remediation of MTBE contaminated groundwater
Hefer Systems & Controls Ltd., Israel	Design and construction of MBR and application of bioaugmented MBR to hospital wastewater
Environment Agency Austria, Austria	Assessment of technological and economic relevance of the developed biotechnologies
Vermicon AG, Germany	Design of microbial detection kits (FISH)
Madep SA, Switzerland	Production of microorganisms and operation of processes on-site
Creative Research Solutions BVBA, Belgium	Design and construction of optrode-based systems
Aquafin N.V., Belgium	On-site operation of bioaugmented MBR process

An advisory board with national and European representatives of decision-making institutions in the area of environmental policy and legislation will constitute a solid warrant for the technology implementation processes.

Through its collaborative programme and intense dissemination activities MINOTAURUS will deliver not only a set of tools, techniques and processes which will enhance the ability of our communities to respond to the challenges of organic pollutants but also frameworks for structuring and making evidence-based decisions for the most sustainable and appropriate bioremediation measures.

4 The MINOTAURUS project consortium

MINOTAURUS is an EU-funded research project, which has been initiated in the frame of the KBBE. 2010.3.5-01 theme "Biotechnology for the environment—Soil and water treatment and bioremediation—Call: FP7-KBBE-2010-4". The 36-months project started in January 2011 and includes 15 partners: six universities, two research centres, five SMEs, one large end-user, and one environmental agency representing in total eight countries (Table 1). Further information is available at: http://www.minotaurus-project.eu.

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