Joint US-EC Short Course on Environmental Biotechnology
Microbial Catalysts for the Environment

University of Lausanne, Switzerland
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A Workshop organized under the auspices of the EC-US Joint Task Force on Biotechnology Research

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Course Background

The Joint US-EC Short Course on Environmental Biotechnology is designed for several purposes. One of the central tenets is to bring together young scientists (at the late Ph.D. or early postdoctoral stages of their careers) in a forum that will set the groundwork for future overseas collaborative interactions. The course is also designed to give the scientists hands-on experience in modern, up-to-date biotechnological methods for the analysis of microbes and their activities pertinent to the remediation of pollutants in the environment. The University of Lausanne (UNIL), Switzerland has been the fourth organizer to host this short course. It has a strong microbiology faculty with expertise in many areas of environmental biotechnology. Furthermore, it is the coordinating member of the European 7th Framework project BACSIN, a project dedicated to environmental microbiology and targeted bioremediation (http://www.bacsin.org).

The 2011 course covered multiple theoretical and practical topics in environmental biotechnology. The practical part was centered around a full concise experiment to demonstrate the possibility for targeted remediation of contaminated soil. Eexperiments included chemical, microbiological, and molecular analyses of sediments and/or waters, contaminant bioavailability assessment, seeded bioremediation, gene probing, PCR amplification, microbial community analysis based on 16S rRNA gene diversity, and microarray analyses. Each of these topics is explained in more detail in the next section. The practical part of the course was complemented with two lectures per day, given by distinguished scientists from the US and from Europe, covering a research area related to what the students are doing in the course. Seminar speakers spent considerable time with the students to promote interaction and discussions.

Rationale

The 2011 Theoretical and Practical Course on *Microbial Catalysts for the Environment*, offered jointly by the US and the EC, focused on oil-derived compounds (alkanes, BTEX and polycyclic aromatic hydrocarbons) as model substrates for investigating and illustrating biodegradative processes and the potential for remediation intervention. This group of hydrocarbons was chosen as a model substrate for the following reasons: (1) there is a considerable knowledge base related to their biodegradation both in natural and in engineered systems; (2) contaminants typically occur as mixtures rather than single pure compounds; (3) oil-related contamination is one of the most commonly encountered pollutions; (4) the course instructors had extensive experience investigating oil biodegradation and bioavailability, both in the laboratory as well as in the field; (5) existing pure cultures were available to ensure the success of the course exercises and (6) the objectives and tasks outlined for this two week course could be accomplished using the more readily degradable and relative water soluble contaminants (BTEX and short chain alkanes) as models

In the laboratory, the students learnt through a combination of well-defined experimental designs and practical lectures, the most relevant issues at stake to intervene at a contaminated site with microbial catalysts in order to achieve targeted biodegradation.

Course Outline and Results

Field trip. The course started with a discussion of and field trip to a contaminated shallow aquifer site underlying a former gasification site in the city of Geneva. This site is contaminated with a number of organic solvents, including benzene, toluene, and xylenes (BTEX). This will be the location where students were instructed on various sampling procedures, real-life hydrological difficulties and treatment options for contaminated sediment and ground water. The students were introduced to a variety of site-related issues such as safety, site characterization, contaminant containment, sample transport, storage conditions, past history as well as the goals and objectives of the treatment. Samples from this site were then further used in the laboratory portion of the course.

Microcosm experiments: The main experimental design for the complete course consisted of laboratory-scale soil microcosms derived from the material of the contaminated site. The main focus of the practical part was to study the potential of preselected organic-compound degrading strains to accelerate contaminant biodegradation in the material. Hereto the students applied several strains alone or in combination, notable a *Pseudomonas veronii* (degrading BTEX), a *Sphingomonas wittichii* and a *Burkholderia sartisoli* (both PAH degraders). Microcosms with or without inoculated strains were operated for the entire two-weeks of the course and were

analyzed for (1) microbial community development: observing changes in the microbial community as a consequence of pollution, (2) catabolic gene profiling: determining the catabolic potential of the community in the beginning and its evolution over time of the contamination, (3) Catalyst survival: introduction of a specific microbial catalyst degrading one or more target compounds from the contamination, of which the survival was monitored over time in the microcosms, (4) catabolic gene activity: the expression of the catabolic genes in the microbial catalyst over time, (5) pollution bioavailability: the changes in pollution availability over time in the microcosms measured using bioreporter assays.

Microbial community profiling: DNA was successfully extracted from the all microcosms on two occasions (wk 1 and wk 2), and then PCR amplified with standard "T-RFLP" (e.g. terminal restriction fragment length polymorphism) primers designed to amplify partial 16S rRNA gene sequences of bacteria. T-RFLP results clearly demonstrated the presence of the inoculated strains and their different development over time as compared to non-inoculated controls.

Catabolic gene profiling: The same purified DNA samples were then labeled in a specific procedure and analyzed on a catabolic gene chip. This chip contains a large number of oligonucleotides representing twelve important catabolic enzyme families, such as Rieske type non-heme iron oxygenases, ferredoxins, extradiol dioxygenases, benzyl succinate synthases or muconate cycloisomerases, and, as a control gene fragments representing a wide diversity of 16S rRNAs. Unfortunately, not all of the slides were of excellent quality, but the student groups managed to interpret a number of them, which confirmed the T-RFLP results for the presence of the inoculated strains. In addition, they could see the large repertoire of genes that is present in the contaminated material itself, from bacteria which have slowly accumulated over time.

Catalyst survival: The survival of the introduced catalyst bacteria was followed by two independent methods (in addition to the indirect methods mentioned above): regular selective plating and through the constitutive gfp label that all strains had been marked with. Despite antibiotic resistance markers and selective media, not all students interpreted the types of colonies appearing on plates in the same manner. Therefore, the variability in plating results was high. Washed cell dilutions from the samples were also analyzed by flow cytometry for the presence of the gfp label. Both techniques showed that the cells survived during the first week but then declines in population size in the second week. Perhaps this was due to the toxic nature of the sample material.

Catabolic gene activity. Further parallel inoculated microcosms served to isolate community RNA. This purified RNA preparation was then subjected to reverse transcriptase reaction and subsequently to quantitative PCR, in order to quantify the mRNA copy number for key catabolic

genes of the inoculated microbial catalysts. As a control the students used the constitutively expressed gfp gene. Because of the complexity of this method, only two time points could be analyzed. Results were not dramatically good (high variability), but confirmed the trend seen with the plating and flow cytometry. Catabolic gene expression was thus detected in the first week but strongly decreased in the second week.

Pollutant bioavailability. In this final experimental technique, the students used bacterial (luciferase) bioreporter assays to quantify the bioavailability of alkanes, PAHs, BTEX and heavy metals in an aqueous extract of the contaminated material. All students could produce excellent calibration curves of the reporter signal as a function of known concentrations of target compound. In contrast, they could not detect changes in available compound fractions over time of the incubation, which suggests that the inoculated strains did not function well.

Data analysis and presentation: Because the microcosm experiments all concentrated around one experimental design, the students spent considerable time to combine all their results together. They presented the different parts of the experiments in oral form on the last day.

Speakers

Graeme Paton, University of Aberdeen (UK)
Dietmar Pieper, Helmholtz Institute for Infectious Research, Braunschweig (Germany)
Teresa Lettieri, European Joint Research Centre, Ispra (Italy)
Carrie Harwood, University of Washington (US)
Jerry Schnoor, University of Iowa (US)
Vladimir Sentchilo, University of Lausanne (Switzerland)

Larry Halverson, Iowa State University (US)
Hermann Heipieper, Helmholtz Institute for Environmental Research, Leipzig (Germany)
Diana Northup, University of New Mexico (US)
Joe Suflita, University of Oklahoma (US)
Wilfred Roling, University of Amsterdam (NL)
Becky Parales, University of California at Davis (US)
Terry McGenity, University of Essex (UK)
Jennifer Pett-Ridge, Oak Ridge National Labs (US)
Willy Verstraete, University of Ghent (Belgium)
Hauke Smidt, Wageningen University Research (NL)
David Johnson, Swiss Federal Institute for Aquatic Research (CH)
Victor de Lorenzo, Centro Nacional de Biotecnologia, Madrid (E)
Hauke Harms, Helmholtz Institute for Environmental Research, Leipzig (Germany)

Participating UNIL and BACSIN Faculty

Course Directors

Jan Roelof van der Meer, Professor of Environmental and Evolutionary Microbiology, Coordinator of the BACSIN project, University of Lausanne, Switzerland.

Course Instructors

Ramiro Vilchez Vargas, Senior Postdoc, Helmholtz Institute for Infectious Diseases, Braunschweig, Germany
Siham Beggah, Vladimir Sentchilo, Senior postdocs, University of Lausanne.
Paula Martinez, Senior postdoc, Helmholtz Institute for Environmental Research, Leipzif, Germany
Silvia Moreno, Edith Coronado, Davide Merulla, Artur Reimer, Kamila Czechowska, Felix Goldschmidt, Assistants, University of Lausanne.

Student participation

The course involved 24 students, with 12 from each side of the Atlantic. Participants were from a number of different backgrounds, including microbial ecology, molecular microbiology, and environmental science, and both at PhD and postdoc level. The participants were selected on basis of an application, motivation and recommendation letters. Course advertizing was done in *Science* and *Nature* and through a mass electronic mailing to appropriate people in the field; and through the internet with an interactive web site and postings to the appropriate newsgroups. Students worked in groups of two, and care was taken to obtain a mixture of backgrounds in each group. Students also brought posters on their own work which were on display during the whole period. Participants were housed in University Apartments specifically constructed (in 2007) for this purpose.

Student evaluation

All students were unanimously extremely enthusiastic about the course, despite its long daily working hours and the intensive program. Some citations from the 'overall evaluation'

"Beyond my expectations regarding the content and organization"

"I was very happy with the course. It was a very busy two weeks but I wouldn't change that because I wouldn't have wanted to cut anything out."

"I already expected that this course would be very useful for my own research and I can continue (?) this fact at the end of this two weeks. I am very grateful for this opportunity."

"I would highly recommend the course. It was my exact research interest for the theme. I really liked the packed and intense atmosphere so we could learn so much. I was humbled to be in association with most of the people whom I met."

"Very good! Very intense, very interesting and very well-prepared and very flexible organizers!"

"This was an incredible experience. I learned a lot, talked a lot, laughed a lot and got a lot of positive energy."

"Fantastic! I was exposed to so many new concepts and protocols. I loved interacting with people from such different backgrounds."

"The course was very well organized and in my opinion the overall objectives were achieved. I just would like to have more time to go into all the techniques and results. But I also know how difficult it is to achieve it in only two weeks."

"Very intense, ambitious, complete, well organized, a good experience, networking."

"Over the expectations: I think that many factors influenced the success of this course. One, the possibility to interact with many people from all the work, exchanging ideas with them and comparing all the personal experiences. Second, the opportunity to have really interesting scientific discussions with the lectures and at least the opportunity to learn and know other techniques."

"This course was extremely valuable to my future as a scientific researcher. Not only did I learn many new practical techniques, but I also learned so much from the breadth of speakers. Perhaps most important, it has made me think more outside the US for research opportunities."