MOLECULAR TOOLS FOR DETECTION OF BACTERIA AND ENZYMES - TOOLS

RIVER BASIN MANAGEMENT ISSUE

Water Quality						Water Quantity		Alterations		Others
1	2	3	4	5	6	7	8	9	10	
C, M, (T,R)		C, M, (T,R)	C, M, (T,R)							C, M, (T,R)

(1) Diffuse pollution by agriculture
(2) Salinisation
(3) Contaminated sediment and floodplain soils
(4) Large scale pollution due to past mining / industries activities
(5) Pollution by organic matter
(6) Emerging compounds
(7) Water scarcity
(8) Floods and low flow
(9) Hydromorphological alterations
(10) Soil erosion

C = System Characterisation
M = System Monitoring
T = System Trend
R = System Remediation, Mitigation

RIVER BASIN

Danube	Ebro	Meuse	Elbe	Brévilles	Others
✓	✓		✓	✓	Not river basin specific

Spec. : Results specific to selected River Basin

KEY FINDING TYPE

Laboratory based	Field based	Modelling
✓		

BENEFITS TO END-USERS

Technical		Management	Policy	
WFD Implementation	Research	River Basin	Compliance	Policy making
	✓			

INTRODUCTION

BGC 4 deals with analyses at a molecular level (DNA and RNA). It aims at detecting general or specific bacteria, depending on the process of interest, and type of comounds they degrade. Different types of organisms are present at the site depending on the site conditions (especially redox conditions) and type of contamination. BGC4 develops methods to quantify the amount of bacteria present in groundwater, soil, and sediment samples and to quantify the activity of these bacteria, expressed as the amount of enzymes present. The enzyme quantity reflects (or corresponds to) the microbial activity of the bacteria. A large quantity of enzymes means that the bacteria are active and therefore that the degradation takes place. Especially when comparing the results of non-contaminated and contaminated areas within a site, conclusions about the role of the bacteria can be made. Using the amount of bacteria to assess biodegradation is used more and more over the past years. **Quantifying the bacterial activity for various degradation processes (by quantifying the responsible enzymes) to assess biodegradation for the selected contaminants is an innovative process developed by BGC4, e.g quantitative Polymerize Chain Reaction (PCR) or even multiplex quantitative PCR.**

TOOL SUMMARY

Molecular tools for the detection of bacteria and enzymes give site-specific information, and give more specific information about the degradation capacity for specific contaminants. The characteristics of the tools depend on the type of compounds and therefore the type of bacteria present. The tools can both be very

general but also specific for a pollutant or redox condition. Tools have been developed for bacteria associated with Dichloroethane (DCA) degradation and chlorinated benzene degradation. The methodology used is similar for both compounds and is lab-based. This methodology is high tech and relatively expensive in comparison with measurement of other standard biodegradation indicators (>500 euros per analysis). However, when compared to using lab tests for the degradation of contaminants (over a longer period of time), this method becomes competitive.

Molecular fingerprinting of microbial communities by DGGE

Denaturing Gradient Gel Electrophoresis (DGGE) fingerprinting provides a powerful tool for assessing field microbial communities with a broad perspective in terms of their richness or evaluation of changes due to environmental disturbance in location and time. DGGE, first applied by Muyzer et al. (1993), allows the separation of small fragments of DNA (polymerase chain reaction (PCR) products) as a function of their different G+C content and distribution. Thus, the fingerprinting pattern is built according to the melting behaviour of the sequences. The DGGE techniques were applied using 16S rRNA gene fragments to the analysis of bacterial communities in numerous habitats such as soil and rhizosphere and aquatic environments. The sensitivity of DGGE analysis can be refined with the targeting of precise and even non-dominant taxonomic groups, by using specific PCR primers or by using of 16S rRNA as a target to highlight metabolically active populations only. DGGE findings are analysed with special statistics software, which allows the characterization of banding patterns. As a result of this analysis it is possible to draw conclusions about similarities and differences between different samples.

Quantitative Polymerise Chain Reaction

Quantitative Polymerise Chain Reaction (Q-PCR) provides an accurate method for determination of levels of specific DNA and RNA sequences in the samples. Using a special PCR device (e.g. BioRad iCycler), amplification-associated fluorescence is detected at each cycle during PCR amplification. Computer based analysis of the cycle-fluorescence is made in a time course. There are many advantages to quantifying microbial communities with this technology, foremost being sensitive and precise. This precision exists because quantification of the DNA and RNA target molecules is determined by the C_t (threshold cycle), which is calculated during the exponential phase of the reaction