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Characterization of anaerobic microbial community degrading DiButyl-Phthalate

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Introduction. Phthalic acid esters are a class of refractory organic compounds widely used as plasticizers. DiButyl Phthalic acid (DBP) is commonly used as industrial chemical additive for plastic synthesis and is suspected to present toxic properties. Due to its high hydrophobic properties, DBP is easily adsorbed into sewage sludge during wastewater treatment and DBP concentrations can increase by several orders of magnitude between influent and sewage sludge outlet. Since disposal of sewage sludge is an increasing problem in Europe due to increasing quantities, recycling of sludge by spreading on agricultural land represents a potential environmental way for sludge disposal. However, behaviour of organic pollutants such as DBP still needs to be determined to reduce the impact on quality of human life. For this, anaerobic digestion of contaminated sludge is a common and energetically highly beneficial method of treatment. DBP biodegradation under anaerobic conditions has been studied for the last five years but nothing is yet known about involved microbes (Angelidaki *et al.*, 2000; Gavala *et al.*, 2003; Kleerebezem *et al.*,1999). The purpose of this study is the characterization of the anaerobic microbial community degrading DBP in order to provide a new understanding of the anaerobic DBP biodegradation process. In the present study, it is proposed an original approach based on a dynamic point of view of the microbial community evolution correlated to the kinetic xenobiotic degradation. For this, molecular tools were diverted from their traditional use of microbial population description to a dynamic approach of the microbial community changes.

Material & Methods. Sewage sludge presenting significant DBP biodegradation ability was used as inoculum of enrichment cultures. Anaerobic cultures were enriched with a specific anaerobic medium amended with yeast extract and DBP as sole carbon sources (Angelidaki et al.,2000). Four enrichment cultures were done under anaerobic methanogenic conditions in continuous reactors (without DBP, DBP 10 mg/l, DBP 200 mg/l and an abiotic control with 2% formaldehyde). DBP was measured by GC-MS according to Angelidaki *et al.* (2000). DBP biodegradation was determined using xenobiotic-optimized mass balance model (Traby *et al.*, 2004). Anaerobic microbial community of each sample was characterized by 16S rDNA extraction/amplification and analyzed by Single Strand Conformation Polymorphism (SSCP). Involved micro-organisms were identified by cloning step and probes were designed for further *in situ* characterization by Fluorescent *In Situ* Hybridization (FISH).

Results & Discussion. The blank reactor (without DBP) presented low methanogenic activity due to yeast extract consumption. A gradual inhibition of the methanogenic activity was shown according to the increasing DBP concentrations from 10 to 200 mg/l, in accordance with previous results presented by Angelidaki *et al.* (2000). Control reactor presented 20% of abiotic losses versus 85% in 10 mg/l DBP reactor and 97 % in 200 mg/l reactor. Thus DBP

biodegradation significantly occurred and kinetics were established during experimentation time.

In addition, the basic microbial growth on synthetic medium was stated in the blank enrichment culture (without DBP). Using a dynamic characterization of the microbial community, it has been shown a strong correlation between growth of three species and DBP degradation kinetics, demonstrating clearly the involvement of these species in DBP biodegradation. Thus, it has been proposed to use traditional descriptive ecological tools such as SSCP for dynamic microbial monitoring of the reactors. Clones VY1 to VY3 were selected and identified to be especially involved in DBP biodegradation. Sequencing of these clones is in progress and specie determination should be completed soon (end of June 04). Bacterial specie/group determination will provide new information on microbial interactions.

In the same way, the archaeal community evolution was determined and a strong inhibition of a group of archaeal species has been shown. Inhibition of the VZ clone group was gradual according to the DBP concentration (from 0 to 200 mg/l) and the increasing inhibition of methanogenic activity. This result clearly demonstrated the specific inhibition of this group of probable methanogenic archaeal species (specie determination in progress).

According to the clone sequences, design and test of FISH probes is planned to be done in order to confirm a probable establishment of spatial microbial interactions during enrichment. This part of the study should be completed before end of July 2004.

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