FACTORS INFLUENCING MICROBIAL DEGRADATION OF DIOXINS IN THE HOUSTON SHIP CHANNEL AND GALVESTON BAY, TEXAS

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Introduction

The factors influencing microbial-mediated degradation of dioxin-like compounds in Houston Ship Channel (HSC) and upper Galveston Bay (GB) were evaluated. Galveston Bay is the largest estuary in Texas and influenced by industrial and municipal effluents and runoff, as well as from atmospheric wet and dry deposition. High levels of dioxin contamination in sediments extends from downtown Houston, throughout the 50 nautical mile HSC and its side bays, and is widespread in GB. Physical removal and/or chemical treatment of ship channel and bay sediments are not economically practical or ecologically sound.

Materials and Methods

A total of seven coring stations were identified, and samples were collected over two field excursions in September, 2006 and April, 2007. Sediment cores were collected using PVC sleeves, physically driven to refusal (3-5 ft). Two sets of shorter cores were also collected to facilitate sediment pore water sampling (~ 60 cm) and DNA/RNA analysis (~30 cm). Sediment core pore water samples were analyzed at the Laboratory for Oceanographic and Environmental Research (LOER), Department of Marine Science, Texas A&M University at Galveston (TAMUG); the Center for Trace Analysis (CETA), Department of Marine Science, the University of Southern Mississippi; and the Department of Oceanography, Texas A&M University (TAMU). Pore water variables including chlorinity, nitrite, nitrate, ammonium, hydrogen sulfide, sulfate, iron, manganese, orthophosphate and silicate were determined for all stations. Dissolved organic carbon (DOC) was analyzed according to Guo et al. [1, 2] and Guo and Santschi [3]. Sample aliquots for dioxin determination were deposited into pre-cleaned amber glass jars with a Teflon seal and preserved at 4 °C prior to analysis. Dioxins were quantified by HRGC/HRMS using EPA Method 1613B at a commercial laboratory (Uni-Prove Technology, Inc.). Using sterile technique, sample aliquots were collected from cores every 1 cm and up to 80 cm. DNA was extracted using a protocol previously described by Zhou et al. [4] and the Power Soil Total RNA Isolation Kit (MoBio) was used to extract RNA. 16S ribosomal RNA clone libraries were constructed using primers specific for all bacteria (8f and 1492r) and a second primer pair (DET730f and 1492r) that targeted *Dehalococcoides* spp. bacteria [5]. Clones were de-replicated using ARDRA, and 2-3 clones with unique DNA fingerprints were sent to the DNA Analysis Facility on Science Hill at Yale University. Phylogenetic analysis of sequences was conducted with ARB software. Anaerobic microcosms consisting of 5 to 10 ml anaerobic sediment slurries sealed in 20 ml glass vials were used for dioxin degradation experiments. Slurries were prepared from equal volumes of wet sediment from a core re-sampled at station 11193 near the paper mill sludge waste pit and artificial, sulfate-free, autoclaved seawater. 13C-labeled 2,3,7,8 TCDD was added to the slurries. Microcosms were amended under anaerobic conditions to evaluate the effects of added sulfate, and carbon (mixture of pyruvate, acetate, and glycerol) on degradation of 2,3,7,8 TCDD. Slurries were incubated at 20ºC. Autoclaved microcosms served as controls for each experimental condition.

Results and Discussion

Dioxin TEQs in sediments ranged from 2.85 to 11,255 ng/kg dry wt with the highest contamination at the site of a former paper mill sludge waste pit (at the confluence of the HSC and the San Jacinto River) revealed by the Texas Commission on Environmental Quality during the course of our study. Molecular characterization of 16S rRNA

genes in HSC and GB sediments found that anaerobic deltaproteobacteria (i.e. sulfate reducing bacteria) and several members of *Chloroflexi* including *Dehalococcoides ethenogenes* 195 / spp. strain CBDB1 and several novel *Dehalococcoides* strains were among the most active fraction of the bacterial community. *Dehalococcoides* use chlorinated compounds as growth supporting electron acceptors and are capable of directly degrading TCDDs and TCDFs making these compounds more susceptible to degradation by other bacterial groups such as sulfate reducers and methanogens. *Dehalococcoides* were detectable in sediment cores (our 7 plus 8 more from a previous study) and grab samples throughout the ship channel and bay when at least 3 TEQ ng/kg dry wt were present and were not detected at our control site with <1 TEQ ng/kg dry wt making them excellent bioindicators of highly chlorinated compounds. Multi-year mesocosm experiments using ship channel sediments amended to compare electron donor and carbon sources determined that those favoring sulfate reduction produced the most significant degradation of dioxins (more than 50% in 2 years). Sediment core pore water analysis of redox constituents (nitrate, phosphate, silicate, ammonium, nitrite, chloride, sulfate, hydrogen sulfide, iron and manganese) and total organic carbon (30 to 330 ppm) determined conditions highly favorable for microbial degradation of dioxins below 1 cm. These results as well as our mesocosm experiments indicate that sulfidogenic electron donor and carbon augmentation of HSC and GB sediments could accelerate dioxin degradation by the in situ microbial population.

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