Goldschmidt 2000
September 3rd–8th, 2000
Oxford, UK.

Journal of Conference Abstracts
Volume 5(2), 349

Biodegradation of Aromatic Hydrocarbons: Microbial and Isotopic Studies

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The extensive occurrence of aromatic hydrocarbons through accidental spills and leakage of underground storage tanks has caused tremendous contamination of surface and groundwater environments. Low molecular weight hydrocarbons such as the monoaromatic BTEX compounds have become the focus of most environmental studies because of their toxic and carcinogenic potential.

Aerobic degradation catalysed by inherent microbial populations is one of the mechanisms that could aid in the complete removal of aromatic hydrocarbons in the environment. Several indicators have been utilized to evaluate this process but their measurement (e.g. of hydrocarbon concentration, bacterial count, metabolites) may be affected by other chemical and physical processes (NRC, 1993). Stable carbon isotope analysis is one technique that has been previously used to trace sources of organic pollutants (O'Malley et al., 1994). Compounds have characteristic carbon isotopic compositions that can be used to pinpoint their origins. Any process in which the compounds are involved with may likewise impart significant isotopic fractionation. It was shown that abiotic processes affect the $^{13}C/^{12}C$ ratio but biological transformation appears to produce the largest fractionation (Abrajano and Sherwood Lollar, 1999).

The purpose of this study is to determine the magnitude and direction of transformation of stable carbon isotopes (^{12}C, ^{13}C) during microbial degradation of selected low molecular weight hydrocarbon compounds such as toluene, ethylbenzene, naphthalene, methanol and hexadecane. Coupled with this objective is the identification of the various species that make up the consortium used in the study and the metabolic pathways by which these organisms degrade the compound. The overarching goal is to examine if the isotopic fractionation associated with such pathways can be employed for monitoring *in situ* bioremediation.

Replicate microbial biodegradation experiments modified from an earlier protocol (Stehmeier et al., 1999) were done using microbial cultures grown aerobically at room temperature (22 C + 1 C) in 274ml side-arm flasks equipped with Mininert valves for ease of sampling. Each flask contained 35ml of a hydrocarbon degrader medium, augmented with 2µl of a particular hydrocarbon culture and inoculated with 5ml of microbial culture, and shaken at about 150rpm with a Gyratory shaker. To establish microbial growth, optical density measurements were undertaken at 600nm at the beginning, at hourly intervals and at the end of the experiments. Hydrocarbon isotope analysis was also done by periodically removing a specific headspace concentration from the culture flask and analysing it by gas chromatography continuous flow isotope ratio mass spectrometry (GC-IRMS).

Laboratory biodegradation studies on toluene showed that microbial growth exhibited an overall increasing trend as indicated by increase in optical density. A corresponding decrease in hydrocarbon concentration with no significant changes in the $\delta^{13}C$ values was also noted. Similar observations were obtained using higher substrate concentration (10µl of toluene). Experiments conducted on ethylbenzene as the substrate likewise demonstrated the same effects on microbial growth as well as in the concentration of residual hydrocarbon. Isotopic compositions also remained considerably constant.

Identification of the microcosm gave various species that make the different hydrocarbon-specific cultures. These cultures were composed of Gram negative as well as Gram positive bacterial strains. Gram negatives included strains from the genera of *Pseudomonas*, *Stenotrophomonas*, *Oligella*, and *Acidovorax* while Gram positives belonged to *Micrococcus*, *Staphylococcus*, *Dermacoccus* and *Kokuria* (or *Erythromyxa*). The present study revealed that no isotopic fractionation accompanied microbial degradation of toluene. Recent study employing two different competitive microcosms likewise exhibited the same outcome (Sherwood Lollar *et al*., 1999). In contrast, another published work obtained a substantial fractionation associated with biodegradation of the same compound (Meckenstock *et al*., 1999). These contrasting results indicate that the occurrence of isotopic fractionation depends on the degradative pathways utilized by the respective microbial consortia. Specifically, the nature of the initial metabolic step (e.g., attack on methyl group versus scission of aromatic ring) could control the extent of carbon isotope fractionation.

Based on the results of the present study, application of stable carbon isotope analysis in aerobic degradation of aromatic hydrocarbons particularly the BTEX compounds do not appear promising for assessment of natural or engineered *in situ* bioremediation. Future studies should look more closely into the different degradative pathways and enzyme systems used by individual micro-organisms as well as mixed populations and

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their effects on the magnitude of isotopic fractionation. Site-specific studies are also necessary to determine the inherent presence of (these) microbial consortia and quantify the associated biological isotope fractionation.

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