ORC TECHNICAL BULLETIN # 2.2.3.2

Oxygen Release Compound, ORC®

Does Competitive Inhibition Play a Role in MTBE Bioremediation?

Competitive inhibition is a term used to describe enzymatic activity in which two or more different substrates compete for the same enzyme. One indication that competitive inhibition may be occurring is when the degradation of one substrate is repressed in the presence of another substrate.

Field observations suggest that background hydrocarbons may repress MTBE degradation and vice versa. As presented in Figure 10, data from an ORC site in Michigan show MTBE degradation occuring *after* BTEX concentrations subside. This effect has been documented at several MTBE-impacted sites that have applied ORC.

Prompted by such field results, Regenesis conducted a series of laboratory experiments to test whether background hydrocarbons interfere with MTBE degradation. In an in-vitro experiment using aerobic bacteria (known to be capable of degrading MTBE and BTEX), results indicate that MTBE metabolism is inhibited by background hydrocarbons. MTBE degradation was measured in the presence of (1) MTBE only and (2) MTBE and xylene during a seven day period. Results indicated a 52% reduction of MTBE in the absence of xylene versus a 9% reduction of MTBE with xylene present.

Regenesis then funded independent research (to be conducted by Pelorus EnBiotech Corporation) to explore the hypothesis that MTBE biodegradation is 1) an aerobic co-oxidative process and 2) that competitive inhibition could exist between a primary substrate and MTBE. The most likely primary substrates involved in co-oxidation and competitive inhibition are compounds found at the aerobic fringe of a petroleum hydrocarbon plume. Initial studies, using resting cell transformation tests, demonstrated that substantial removal of MTBE was achieved with cultures that were acclimated to benzene, camphor, o-xylene and cyclohexanone. In those tests a specific benzene acclimated culture, designated PEL-B_{201}, was most efficient in degrading MTBE (58% removal). This established the possibility that a single organism could metabolize both MTBE and alternate substrates and therefore be under the influence of competitive inhibition dynamics. In fact, the study demonstrated both inhibition of benzene metabolism in the presence of high MTBE concentrations *and* the inhibition of MTBE metabolism with increasing benzene concentrations.

The benzene utilizing culture (Pel-B_{201}) used in the experiments was grown in basal salts media on benzene vapors. Growth and activity experiments were performed to determine optimum conditions for biomass production. MTBE biotransformation experiments were performed in 160 ml Wheaton serum bottles containing oxygen-saturated phosphate buffer supplemented with MTBE (3.35 mg/L). The bottles were sealed with Teflon-lined serum septa. To evaluate the effects of benzene on MTBE degradation, a stock solution of benzene in DMF was added to achieve final concentrations of 1.9 mmM and 3.8 mmM respectively. PEL-B_{201} acclimated cell suspensions were added to each test reactor to a cell density of approximately 2.0 x 10^8 cells/ml. Controls were innoculated to the same level with unacclimated PEL-B_{201} cells grown on succinate. Over a 48-hour test period, samples were removed from each reactor and placed in 2.0 ml GC vials. Headspace samples were analyzed for MTBE by gas chromatography (GC/PID).

Optimum growth conditions established for strain PEL-B_{201} were developed through growth curve and oxygen uptake studies on benzene. Optimum degradative activity and cell yield were achieved when optical densities reached a nominal value of approximately 1.10 (OD_{600}). Results of oxygen uptake (OU) tests are shown in Table 1. *These tests clearly indicate that MTBE inhibits oxygen uptake associated with benzene metabolism.*

Results of the biotransformation experiments are presented in Figure 11. Cell suspensions containing benzene degraded >99% of the added MTBE. Increasing the concentration of benzene (1.9 uM and 3.8 uM) resulted in significant reduction in the rate of MTBE degradation. No degradation of MTBE was observed with cells grown on the non-inducing substrate succinate. The lack of MTBE degradation by cells grown on succinate serves as a control demonstrating that MTBE metabolism in this experiment occured by means of an enzyme system associated with benzene metabolism and reaffirms our hypothesis that MTBE is metabolized by co-oxidative mechanisms.