Biodegradation of Cyclohexane under Different Reclamation Scenarios

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Background/Objectives. Current extraction technology used in bitumen recovery from surface mined oil sands in Alberta (Canada) has large environmental foot print. Due to the enormous volume of water utilized for bitumen extraction and subsequent generation of extensive amounts of waste fluid fine tailings (FFTs), an alternative process was developed that uses hydrocarbon solvents (e.g., cyclohexane) for non-aqueous extraction (NAE). The NAE process produces small amount of dry tailings with residual unrecoverable solvents. Therefore, NAE solid wastes could potentially impact the environment upon disposal. Hence, an in-depth study of NAE solvent's biodegradation is necessary under upland and wetland reclamation scenarios.

Approach/Activities. To better understand NAE solvents' biodegradation, small-scale microcosm experiments containing FFT amended with cyclohexane and cyclopentane (300 ppm each), and incubated under aerobic and anaerobic (sulfate-reducing, nitrate-reducing, iron-reducing and methanogenic conditions) conditions were conducted as FFT harbors a diverse hydrocarbon-degrading microbial community. The effect of nutrients (N and P) and supplementation of *n*-heptane on degradation of cyclohexane and cyclopentane was also evaluated under methanogenic degradation. Furthermore, microcosms containing oil sands process affected water (OSPW) were also conducted to evaluate co-metabolic degradation of cyclohexane under aerobic condition by monooxygenases produced during growth of ammonia-oxidizing or methane-oxidizing bacteria. DNA-Stable Isotope Probing (DNA-SIP) is being employed to characterize cyclohexane degrading communities both in aerobic and methanogenic conditions. To monitor cyclohexane degradation, residual headspace cyclohexane was monitored periodically using gas chromatograph-mass spectrometer (GC-MS), while CO_2 and CH_4 gas generation or consumption were analyzed by GC equipped with a flame ionization detector.

Results/Lessons Learned. From the data obtained, microcosms containing FFT (under aerobic conditions) showed cyclohexane degradation with associated CO_2 production. Once the microbial communities were established in the microcosms incubated under aerobic condition, complete cyclohexane degradation occurred within 4 months. However, no cyclohexane degradation was detected so far in microcosms of FFT under other electron accepting conditions (sulfate-reducing, nitrate-reducing, and iron-reducing conditions). Enrichment of aerobic ammonia oxidizing bacteria in microcosms inoculated with concentrated OSPW, led to the rapid degradation of cyclohexane (~ 99% of 300 ppm cyclohexane was degraded in 4 weeks) with concomitant oxidation of the added ammonia (4 mM) to nitrite. However, in control cultures without ammonia, 40-49% of cyclohexane was degraded. Similarly, co-metabolic degradation of cyclohexane was observed in OSPW inoculated microcosms supplied with methane and oxygen. In this set, cyclohexane degradation occurred in 4-6 weeks of incubation and was accompanied with methane consumption. Overall, our results show that cyclohexane could be degraded through the activities of aerobic heterotrophs and autotrophs in OSPW via co-metabolism through the action of ammonia monooxygenases and methane monooxygenases. In methanogenic incubations, cyclohexane and cyclopentane were cometabolically degraded in the presence of heptane with or without nutrient supplementation. These are the **novel** findings that will enable appropriate devising of reclamation strategies for NAE solids management.