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

Journal of Conference Abstracts
Volume 5(2), 977

An Integrated Biogeochemical Approach for Studying Bio-availability of Microbial Nutrients Sorbed on Mineral Surfaces

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Nutrient Bio-availability

The bio-availability of mineral surface associated contaminants has been extensively studied from both biotic and abiotic points of view; however, the influence of microbially mediated processes on nutrient availability has received less attention and has been infrequently studied at environmentally relevant nutrient concentrations. Furthermore, phenomenological mechanisms responsible for macroscopically observed nutrient bio-availability have invariably been speculative in nature, with little or no supporting experimental data available. Therefore, we have developed and adopted an integrated biogeochemical approach for studying microbial acquisition of nutrients from mineral surfaces (Figure 1). In this approach, microbially mediated processes responsible for macroscopically observable nutrient bio-availability are considered in terms of interactions at microbe-solution, mineral-solution, and microbe-mineral interfaces.

The cornerstone of our integrated approach is use of chemostat bioreactors to obtain microbial communities able to effectively utilize a growth-limiting nutrient which is strongly sorbed to a mineral surface. Unlike batch culture experiments, in which continuously changing environmental and progressively depleted nutrient conditions bring about a continuous succession of species distributions and phenotypic traits within a microbial community, the constant and unvarying selective pressure imposed by chemostat culture results in a steady-state microbial community which exhibits a unique set of physiological and metabolic traits (Veldkamp, 1977). Thus, chemostat culture offers possibilities for studying environmental influences on microbial community structure not available using batch culture techniques.

Chemostat experiments using orthophosphate (P_i) adsorbed on colloidal goethite as a model nutrient mineral-surface system and the microbial community from an acidic mineral soil have demonstrated the utility of our integrated biogeochemical approach. Under aerobic conditions with glucose as carbon source, a chemostat culture able to utilize 82% of goethite-adsorbed P_i was selectively enriched. The culture formed goethite-cell colloidal aggregates at an influent glucose level of 100 mg/L, but was completely dispersed at an influent glucose level of 500 mg/L. Soluble polysaccharide levels as high as 20 mg/L were noted under aggregate forming conditions. A low molecular weight organic acid (LMWOA), tentatively identified by HPIEC retention time as citric or gluconic acid, was observed at the higher carbon level. Soluble iron ranging between 1 and 3 times the solubility limit of amorphous $Fe(OH)_3S$ was also noted.

The culture was dominated by two gram-negative bacteria, with no yeast or filamentous fungi observed. Two distinct 16S rDNA bands were isolated by temperature gradient gel electrophoresis of PCR amplified chemostat effluent DNA extracts. Two amplification products of equivalent size were also isolated from direct DNA extracts of the parent soil, indicating that the continuously saturated environment required for chemostat operation did not limit growth of bacteria from an intermittently saturated surface soil environment. One of the bacteria has been identified as *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) based on both 16S rDNA and fatty acid methyl-ester analyses. This identification is consistent with previous observations that when exposed to P_i limitation a number of Gram-negative bacteria including *Klebsiella pneumoniae*, *Erwinia herbicola*, and *Pseudomonas cepacia* produce gluconic acid by direct oxidation of glucose in the periplasmic space (Tempest and Neijssel, 1992; Babu-Khan et al., 1995).

Microbial Response to Environmental Stimuli - Nutrient Limitation

Essentially all micro-organisms are capable of adapting their physiology/metabolism to some extent in response to environmental changes (Stock et al., 1990). Microbes respond to environmental stimuli such as nutrient limitation through alternative patterns of gene expression, which regulate cellular physiology and metabolism. These physiological responses may be grouped into two fundamental categories: passive responses in which the effects of modulated cellular physiology responsible for adaptation to low nutrient concentrations are confined to the cell itself, or active responses in which cell surface physiology or an exported metabolic product physico-chemically alters the surrounding environment to enhance bio-availability of