

QUANTITATIVE ANALYSIS OF EXPERIMENTAL MICROBE-MINERAL INTERACTIONS USING VERTICAL SCANNING WHITE LIGHT INTERFEROMETRY (VSWLI). R. E. Blake¹ and A. Lüttge²,

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Interest in the interactions between microorganisms and mineral surfaces has greatly intensified in recent years. Although many studies have appeared showing high-resolution images of bacteria and other microorganisms in association with mineral surfaces using optical, electron, confocal, atomic force and other microscopic techniques, few of these studies have reported direct quantitative measurements of reacted mineral surfaces, or of the kinetics of specific microbially-mediated processes. Reactions involving microorganisms at the relatively low-temperatures of most microbial habitats, also involve the action of microbial enzymes and, therefore, are almost certainly dominated by kinetic rather than thermodynamic and equilibrium effects. Thus, precise and accurate determination of the kinetics of microbially mediated reactions of mineral surfaces is crucial to the interpretation and modeling of the contribution of microbial metabolism to geochemical processes.

Where microbially-mediated reactions of minerals have been quantified, for example, during some mineral dissolution and metal oxidation studies (e.g., [1], [2], [3]), reaction rates have usually been based on analyses of bulk solution chemistry and normalization to the total surface area of reacting minerals as measured by gas adsorption (BET). As with abiotic dissolution kinetics experiments, the proper handling of surface area is problematic. A large degree of uncertainty is associated with measurements of bulk BET surface area, and the contribution of actual reactive surface area is not known. Determination of the actual reactive surface area and specific reactive sites on mineral surfaces becomes even more important for microbially-mediated processes where reaction is often localized in microenvironments between bacterial cell walls and mineral surfaces at the site of cell attachment, or may involve a combination of enzymatic and abiotic/inorganic mechanisms with variable effects on the mineral surface depending on solution chemistry, bacterial strain, and growth conditions (e.g., Fe²⁺ oxidation).

The new analytical technique of vertical scanning white light interferometry (VSWLI) [4], applied herein, complements existing surface analytical techniques by allowing quantitative analysis of surface dynamics during mineral dissolution and growth, and direct determination of reaction rates without the need for measurement of surface area. Reaction rates are determined from the retreat or advance of the actual mineral surface over time, and not from changes in bulk solution chemistry. Importantly, this allows accurate determination of mineral dissolution rates under conditions that result in very low concentrations of dissolved products, or where dissolved components are incorporated into bacterial biomass or trapped via complexation with or-

ganic polymers (e.g., glycocalyx). By introducing an internal reference onto the mineral surface, absolute rates may be determined in addition to measurements of the relative changes in surface features. In addition to quantification of mineral surface reactions, VSWLI offers several advantages that are especially critical to the study of microbe-mineral interactions, specifically, a large areal field of view (up to 730 X 580 microns) and a large vertical scan range (up to ~1mm), with near atomic-scale vertical resolution (~2 nm) for surface features up to 100 microns high. These features complement other surface imaging and analytical techniques such as AFM, which offers higher resolution for detailed atomic-scale measurements, but a relatively small area of view.

We are using VSWLI in quantitative experimental investigations of reaction rates and interactions between bacterial cells and the surfaces of various minerals which may serve as important sources of nutrients, or in energy transfer (electron donors/acceptors), during heterotrophic and lithotrophic growth of microorganisms in nature including phosphate, Fe-oxide, and sulfide minerals. Results of initial culture experiments employing mineral phosphate as a sole P source for bacterial growth show highly localized and preferential colonization/reaction at only certain types of surface features such as fractures and microcracks by *Acinetobacter* ADP1. Microbes may be attracted to these features due to their higher surface energy. Specific coating and treatment of mineral surfaces allows resolution of single cells of *Acinetobacter* as well as associated etch pits using VSWLI, and the evolution of individual, microbially induced features such as pits may be followed over extended periods of time. Investigations of single cells and reaction rates of individual features are especially important in quantifying the spatially heterogeneous processes of microbial cell attachment and interaction with mineral surfaces. Results from additional microbial growth experiments will be presented and rates obtained from microbial and abiotic control systems will be compared.

References: [1] Ullman W.J. et al. (1996) *Chem. Geol.*, 132, 11–17. [2] Edwards K. J. et al. (1998) *Amer. Mineral.*, 83, 1444–1453. [3] Nordstrom D. K. and Southam G. (1997) *Rew. in Mineral.*, 35, 361–390. [4] Lüttge A. et al. (in press) *Amer. J. Sci.*

