

HOW CAN A FINGERPRINT OF PRIMITIVE BACTERIA LOOK LIKE? A CARBON AND IRON STABLE ISOTOPIC STUDY OF AN IRON-OXIDIZING BACTERIAL COMMUNITY. D. Strapoc¹, B.L. Beard², J. Schieber¹, ¹Department of Geological Sciences, Indiana University, 1001E 10th St. Bloomington, IN ² Department of Geology and Geophysics, The University of Wisconsin, 1215 West Dayton St., Madison, WI.

Introduction

Judging from recent data, Martian surface waters of the past were rich in iron and sulfate [1], suggesting that metabolic pathways based on chemical reactions that utilize dissolved iron may have been employed by ancient life forms. On Earth, such pathways are abundantly exemplified by microbes that oxidize or reduce iron [2,3]. Microbial communities centered on these kinds of bacteria may be viable Mars analogs, and research on their biosignatures may help us to identify indications of past microbial life forms on Mars. Therefore we conducted a pilot study of carbon and iron isotopic characteristics of iron oxidizing bacteria in a small creek SE of Bloomington, Indiana (Fig. 1). We've chosen these two elements, because carbon is

major building block of life as we know it and iron provides a chemical energy for these organisms. The apparent exclusive occurrence of the mat biota in our study near a source of iron-rich (12.5 ppm Fe) waters, seems to suggest that iron availability confers a tangible benefit. This benefit could be (a) energy gain or alternatively a (b) reduction of ambient pO₂. The latter can be considered a benefit because these microbes are microaerophilic [4].

Carbon isotopes: *Leptothrix*, the predominant mat building organism, is a chemoheterotroph and requires an organic carbon source to flourish [5]. Because we observed that the mats recovered within about a week after strong rainfall events, regardless of season, we

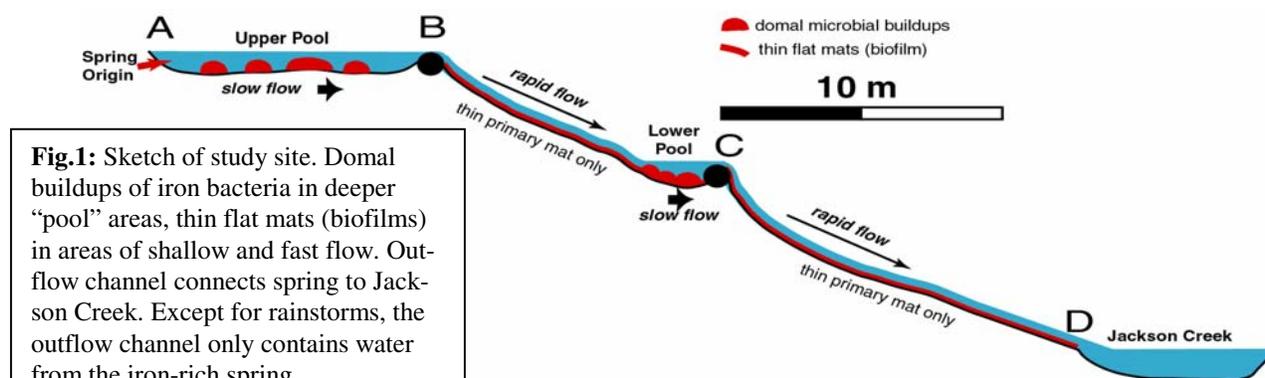
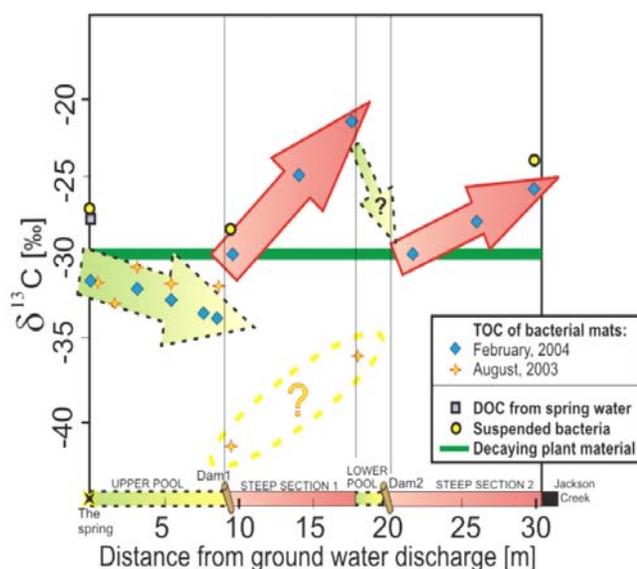


Fig. 1: Sketch of study site. Domal buildups of iron bacteria in deeper "pool" areas, thin flat mats (biofilms) in areas of shallow and fast flow. Outflow channel connects spring to Jackson Creek. Except for rainstorms, the outflow channel only contains water from the iron-rich spring.



assumed that the critical carbon source was not decaying plant in the outflow channel, but rather dissolved organic carbon (DOC) in the springwater [6] (2 mg/l). The DOC consists probably of simple organic molecules like acetate.

When we began analyzing mat samples for carbon stable isotopes, our initial expectation was that mat microbes would cause a shift towards more negative $\delta^{13}\text{C}$ values. Indeed, our first measurements on samples from August 2003 seemed to support this hypothesis (Fig. 2). However, when we resampled in February 2004, our analyses showed a curious see-saw pattern of $\delta^{13}\text{C}$ values (Fig. 2).

Fig. 2: Changes in $\delta^{13}\text{C}$ values of microbial mat carbon along the outflow channel (Fig. 1). See-saw pattern of $\delta^{13}\text{C}$ values correlates with pools (decreasing $\delta^{13}\text{C}$) and stretches of rapid flow (increasing $\delta^{13}\text{C}$).

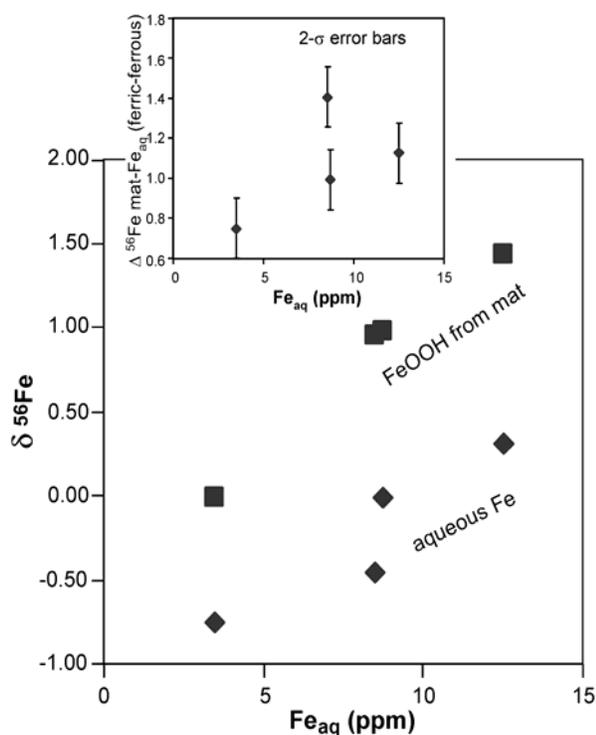


Fig. 3: Plot of $\delta^{56}\text{Fe}$ measured for aqueous Fe and ferrihydrite encrusted bacterial mats from the spring vent (high Fe concentration) to points down the flow path (lower Fe concentration). Error bars smaller than symbol size. Inset shows the variability in the fractionation factor between ferrihydrite encrusted bacterial mats and aqueous Fe(II) solutions as a function of aqueous Fe(II) concentration.

The observation that these mats of iron bacteria produce carbon isotope fractionation relative to the likely source materials in both a negative and positive direction may reflect the combined effects of flow regime and availability of organic carbon. In pools with a slow flow regime, more negative $\delta^{13}\text{C}$ values in downstream direction may indicate greater availability of DOC (therefore more selective uptake) and probably increase in recycling of previously grown and decayed mat material [7]. In contrast, in the shallow fast flowing sections, where the DOC is much less available, the microbes gradually enrich the remaining downstream flowing DOC in ^{13}C , causing an upwards shift in $\delta^{13}\text{C}$ values in mats downstream (Fig. 2).

Iron isotopes: A preliminary set of Fe isotope data on aqueous Fe solutions and ferrihydrite-encrusted bacterial mats from our study site have been analyzed for their Fe isotope compositions (Fig. 3). The ferric phase (Fe^{3+} from mat samples) has higher $\delta^{56}\text{Fe}$ values than the corresponding ferrous phase (aqueous Fe^{2+}) as

would be expected based on the general observation that ferric iron tends to have higher $^{56}\text{Fe}/^{54}\text{Fe}$ ratios as compared to ferrous iron [8]. However, the difference in Fe isotope composition between ferrous and ferric iron is variable. At low Fe_{aq} concentration $\Delta^{56}\text{Fe}_{\text{ferric-ferrous}}$ (the ferric-ferrous fractionation factor) is smaller as compared to high Fe_{aq} concentration (Fig. 3). It may suggest that in the latter conditions, the precipitation of ferric iron is partially catalyzed by iron-oxidizing bacteria, as it is easier to precipitate $^{56}\text{Fe}^{2+}$ iron, because the heavy isotopes preferentially tend to be at higher oxidation state. At low Fe^{2+} concentration $\Delta^{56}\text{Fe}_{\text{ferric-ferrous}}$ value reaches its typical abiogenic level, because bacteria can't be selective anymore and iron precipitation reaches prevalingly a spontaneous fassion. The one outlier of this trend on figure 3 was sampled in a deep standing water in the upper pool where the available amount of Fe^{2+} was more accessible for bacterial selectiveness than from the fast flowing channel passing by the pool, although they both had the same Fe^{2+} concentrations.

Conclusions: The data indicate that our site, while a simple system, displays sufficient complexity to serve as a natural laboratory for the study of factors that determine geochemical characteristics of iron oxidizing microbial communities and their potential manifestation in the rock record. The closely associated mix of positive and negative, sometimes erratic looking, excursions of $\delta^{13}\text{C}$ values and relatively large $\Delta^{56}\text{Fe}_{\text{ferric-ferrous}}$ values (1.1 – 1.4‰) are of interest in the context of using isotopic data as a biosignature to identify microbial involvement in ancient sedimentary rocks [9]. Because these organisms rely for their survival on compounds that were in abundant supply on early Earth and probably also on early Mars, comparable life forms might be found in Martian sedimentary rocks in the not too distant future.

References: [1] JPL MER Press Release, 3/2/2004. [2] Emerson, D. 2000. p. 31-52. In D. R. Lovley (ed.), ASM Press, Washington, D.C. [3] Lovley, D. R. 1997, FEMS Microbiol Rev 20. [4] Nealson, K.H., 1982, Microbiological oxidation and reduction of iron. In: Mineral Deposits and the Evolution of Biosphere, H.D. Holland and M. Schidlowski (eds.), Springer, Berlin, p. 51-66. [5] Spring, S. 2004, In M. Dworkin (ed.), 3rd ed, 2004. Springer-Verlag, New York. [6] Amon, R. M. W. and Benner, R. 1996. Limnology and Oceanography. Vol. 41. pp. 41-51. [7] Hellings, L., Dehairs, F., Tackx, M., Keppens, E. and Baeyens, W. 1999. Biogeochemistry. Vol. 47. pp. 167-186. [8] Beard BL and Johnson CM (2004a), Reviews in Min. and Geochem v. 55, 319-357. [9] Des Marais et al., 2003, The NASA Astrobiology Roadmap. Astrobiology, v. 3, p. 219-235.