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Phoenix, V.R. and Holmes, W.M. and Ramanan, B. (2008) *Magnetic resonance imaging (MRI) of heavy-metal transport and fate in an artificial biofilm.* Mineralogical Magazine, 72 (1). pp. 483-486. ISSN 0026-461X

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Deposited on: 08 January 2010

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Mineralogical Magazine, February 2008, Vol. 72(1), pp. 483–486

Magnetic resonance imaging (MRI) of heavy-metal transport and fate in an artificial biofilm

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ABSTRACT

Unlike planktonic systems, reaction rates in biofilms are often limited by mass transport, which controls the rate of supply of contaminants into the biofilm matrix. To help understand this phenomenon, we investigated the potential of magnetic resonance imaging (MRI) to spatially quantify copper transport and fate in biofilms. For this initial study we utilized an artificial biofilm composed of a 50:50 mix of bacteria and agar. MRI successfully mapped Cu^{2+} uptake into the artificial biofilm by mapping T_2 relaxation rates. A calibration protocol was used to convert T_2 values into actual copper concentrations. Immobilization rates in the artificial biofilm were slow compared to the rapid equilibration of planktonic systems. Even after 36 h, the copper front had migrated only 3 mm into the artificial biofilm and at this distance from the copper source, concentrations were very low. This slow equilibration is a result of (1) the time it takes copper to diffuse over such distances and (2) the adsorption of copper onto cell surfaces, which further impedes copper diffusion. The success of this trial run indicates MRI could be used to quantitatively map heavy metal transport and immobilization in natural biofilms.

Introduction

THE biofilm is an exceptionally common mode of life for bacteria. Significantly, these biofilms have tremendous ability to influence the cycling of heavy metals either via adsorption onto cell surface ligands, or via metabolic processes (e.g. Fein *et al*., 1997; Islam *et al*., 2004). In terms of reaction kinetics, biofilms are likely to behave differently to planktonic (free swimming) bacteria due to mass transport limitations. In short, the rate at which nutrients or contaminants diffuse through the biofilm controls the rate at which these components can be processed by the bacteria (Buffiere *et al*., 1995). Such mass transport limitations must also hold true for heavy metal immobilization by biofilms. Consequently, to fully understand metal immobilization in biofilms we must not only be able to measure the amounts of metals immobilized, but also the rates at which those metals move through the biofilm. Significantly, magnetic resonance imaging (MRI) has the potential to do just that. The effect that paramagnetic ions, such as Cu^{2+}, have upon the MRI signal enables us to quite literally watch the movement of these ions though a bioporous structure in real time. This technology works, not by imaging the paramagnetic ion itself, but by imaging its effect on the H nuclei immediately surrounding it (and there is an abundance of H nuclei in biofilms, notably from water). Paramagnetic ions such as Cu^{2+} cause dramatic reduction in a phenomenon known as the relaxation rate of H nuclei. Therefore, if one creates MR images based on this phenomenon, these images simply become maps of where the parmagnetic ion is. Moreover, because ^{1}H relaxation rate is proportional to the concentration of the paramagnetic ion (Bloembergen, 1957), it should be possible to calibrate these images to determine actual copper concentrations. A particular strength of MRI is that it is non-invasive, thus any biofilm analyzed would be alive, functioning and unharmed by analysis. Previous studies have utilized MRI to image heavy metal

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DOI: 10.1180/minmag.2008.072.1.483

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precipitation in bioreactors containing polyurethane immobilized biofilms (Nott *et al*., 2001). However, the mass transport and fate of heavy metals through individual biofilms has yet to be undertaken.

In this study we apply MRI and a calibration protocol to quantitatively map in real time heavy metal transport and immobilization in an artificial biofilm. This artificial biofilm is simply used here as an ideal test system before moving onto more complex, real biofilms.

Methods

The artificial biofilm was a 50:50 mix of the bacteria *Bacillus subtilis* and agar (1.5%). This represented cells suspended in an extracellular polymer matrix. Before the mix had set, it was poured into a flow cell with a glass rod running through the centre. Once set, the rod was removed leaving a hole through which the copper would flow (Fig. 1). The flow cell was then placed in the MRI and a 10 mg l^{-1} Cu^{2+} solution was pumped through the cell using a peristaltic pump. The uptake of copper in the artificial biofilm was imaged by acquisition of T_2 weighted images (T_2 is the transverse relaxation rate of ^{1}H). Images were collected at regular time intervals over 0 to 36.5 h) and obtained using a multi-slice, multi-spin echo 2-D imaging sequence using 64 consecutive spin echoes with echo time TE = 6.8 ms and recovery time TR = 7 s, providing, per slice, 64 T_2 weighted images at increasing echo time. T_2 weighted images were converted into T_2 parameter maps revealing the T_2 value in each image pixel at each time interval. From this, copper concentration could be determined using the following calibration (Bloembergen, 1957).

$$[C] = \frac{1}{R}\left\{\frac{1}{T_{2i}} - \frac{1}{T_{20}}\right\} \quad (1)$$

Where T_{20} is the relaxation time in the absence of ions, T_{2i} is the relaxation in the presence of ions, [C] denotes the concentration of the ions and R is the relaxivity constant of ions. In the current study, T_{2i} and T_{20} are known variables as they are taken directly from T_2 parameter maps. R, however, is unknown and must be determined separately to enable calculation of [C]. R for copper varies with the solids content of the media. Thus, we constructed a series of 'gels' (using water, bacterial pellets, agar, bacteria-agar composites) each with different solids content (determined by comparing mass before and after drying), and each containing a known amount of

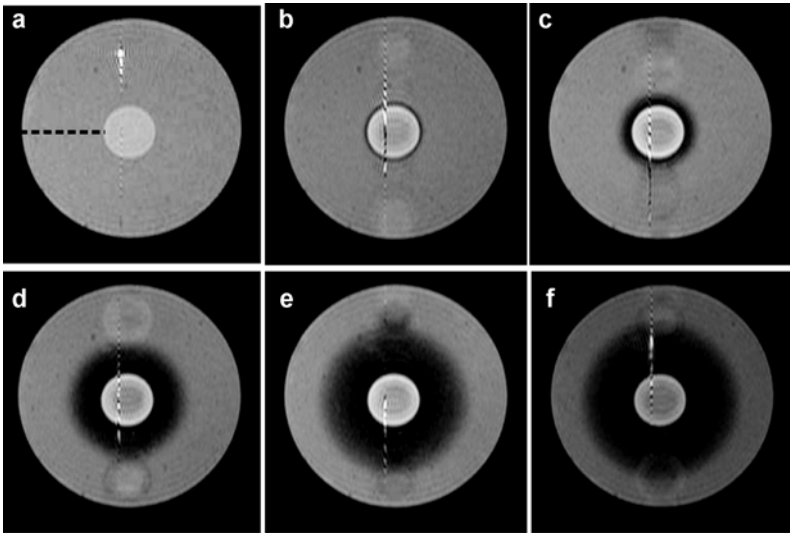

FIG. 1. T_2 weighted images of cross sections through artificial biofilm after 0, 1.5, 6.5, 19.5, 32.5 and 36.5 h reaction with copper (*a* to *f* respectively). Darker regions are regions with lower T_2 values. The brighter circle in the middle is the conduit through which the copper solution flows. The material surrounding this is the artificial biofilm. Note the gradual expansion over time of a dark halo around the central conduit. Scale: the artificial biofilm (including conduit) is 14 mm in diameter. The dashed black line in (*a*) shows the line of section for Figs 2 and 3.

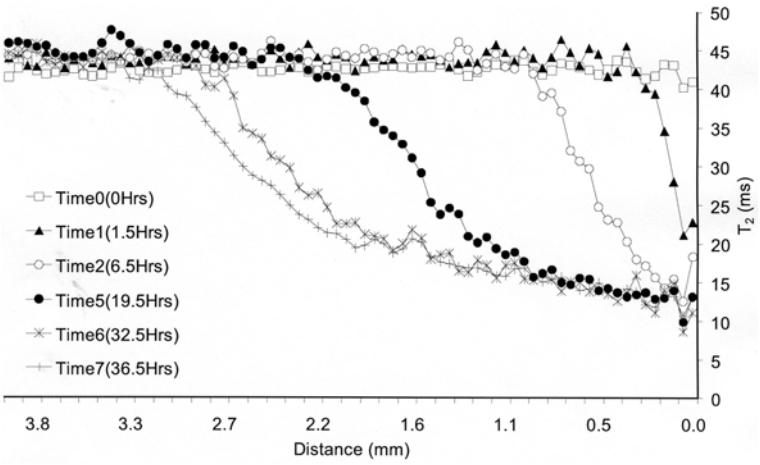

FIG. 2. Raw T_2 values at different time intervals along line of section indicated by dashed line in Fig. 1*a*.

copper. From this, the dependence of R on solids content was determined. The solids content of the artificial biofilm was then determined by weighing before and after drying at 60°C enabling the appropriate value of R to be input into equation 1 above.

Results and discussion

The diffusion of copper into the artificial biofilm was recorded by T_2 weighted images as shown in Fig. 1. The presence of Cu^{2+} reduces T_2 values, hence monitoring T_2 reveals the location of copper. In Fig. 1, the diffusion of copper into the artificial biofilm is shown by the expansion over time of the dark halo around central conduit (note, darker areas in Fig. 1 represent lower T_2 values).

The raw T_2 values across the line of section (shown in Fig.1*a*) are shown in Fig. 2. By applying the calibration protocol described above, these raw T_2 values were converted into copper concentrations as shown in Fig. 3 which illustrates well the gradual build up of copper in the artificial biofilm. As is expected, the build up of copper is most rapid immediately adjacent to

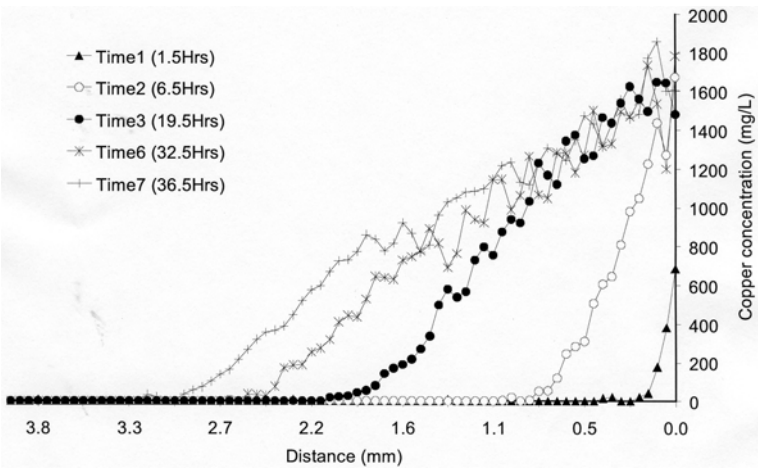

FIG. 3. Copper concentrations at different time intervals along the line of section shown in Fig. 1*a*. The copper concentrations were calculated using the calibration protocol and the raw T_2 values shown in Fig. 2.

the supply conduit, but much slower deeper into the biofilm.

The impact of mass transport limitation is well illustrated here. Even after 36 h, the copper front had migrated only 3 mm into the artificial biofilm and at this distance from the source conduit, copper concentrations were very small (Fig. 3). This slow accumulation, however, is not just due to the length of time it takes copper to diffuse through the artificial biofilm. As copper ions enter the artificial biofilm, many ions are immobilized by adsorption onto cell surface reactive ligands. This process slows the transport of copper into the system even further. Examination of the copper concentrations shown in Fig. 3 reveals a very high adsorption capacity of close to 2000 mg of copper per litre of biofilm. This is three orders of magnitude greater than the concentration (10 mg l^{-1}) of the source solution and such a high adsorption capacity will significantly reduce the amount of free Cu^{2+} available to migrate further into the biofilm. Notably, adsorption processes in planktonic systems take <<1 h to reach equilibrium (Fein *et al*., 1997). Evidently, metal adsorption in biofilms must take considerably longer to reach equilibrium due to the relatively slow migration of the metal through the biofilm matrix.

Although only one slice through the artificial biofilm was shown here, multiple adjacent 2D slices or 3D images can be collected. MRI is certainly a versatile tool. At its simplest, it is an excellent tool for imaging. Thus, in a real biofilm, the impact of any variations in biofilm structure on metal transport and fate can be correlated. Morover, biofilm density at different locations can be estimated from A_0, the initial amplitude of the MRI signal immediately after excitation (Wieland *et al*., 2001). As biofilm density is a key player in mediating diffusion rates, the impact of this parameter on heavy-metal uptake can be correlated. Finally, MRI-based Pulsed Field Gradient analysis (PFG) can be used to map water diffusion coefficients, which can be used to estimate diffusion coefficients for other low-molecular-mass species, such as Cu^{2+} (when not subject to adsorption). Such data can help us to understand heavy-metal transport and fate by input into reaction-transport models, such as the Bartlett and Gardner (1996) model, which describes combined adsorption-diffusion in thin films.

Conclusions

The task of this study was to test the ability of MRI to quantify heavy-metal transport and fate in an artificial biofilm. The success of this trial run illustrates that this technique could be used to quantify heavy-metal transport and fate in natural biofilms *in vivo*, *in situ* and in real time. In this study we only examined Cu^{2+}. Many other metals, however, are sufficiently paramagnetic to be detected by MRI, including Ni^{2+}, Cr^{3+}, Co^{2+}, Ti^{2+}, Nd^{3+}, Th^{4+}, Fe^{3+}, Fe^{2+} and Mn^{2+}. Consequently, magnetic resonance imaging has potential to examine transport and fate of a broad range of metals in biofilms.

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