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Quantitative NMR microscopy of iron transport in methanogenic aggregates

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Abstract

Transport of micronutrients (iron, cobalt, nickel, etc.) within biofilms matrixes such as methanogenic granules is of high importance, because these are either essential or toxic for the microorganisms living inside the biofilm. The present study demonstrates quantitative measurements of metal transport inside these biofilms using T_1 weighted 3D RARE. It is shown that iron(II)-EDTA diffusion within the granule is independent of direction or the inner structure of the granules. Assuming position dependence of the spin-lattice relaxivity, Fick's law for diffusion in a sphere can be applied to simulate the diffusion within the methanogenic granules under investigation. A relatively low diffusion coefficient of $2.5*10^{-11}$ $m^2 \cdot s^{-1}$ was obtained for iron diffusion within the methanogenic granule.

Keywords

Methanogenic biofilms; Metal transport quantification; MRI microscopy

1. Introduction

Up-flow Anaerobic Sludge Bed (UASB) reactors are the most widely applied type of anaerobic waste water treatment reactors. The actual purification process takes place in methanogenic granular sludge that consist of rigid, well settling microbial aggregates that develop by the mutual attachment of bacterial cells in the absence of a carrier material. Heavy metal transport into these inhomogeneous, spherical biofilms is an important process both from the point of view of toxicity and bioavailability. Detailed experimental data on metal transport and biosorption within biofilm matrices is lacking due to the absence of adequate,

non-invasive analytical tools, hindering refined modeling of these complex processes. In this study previous work on methanogenic granular sludge by Lens *et al.* [1] is extended to quantitative MRI microscopy of metal transport.

2. Materials and methods

Methanogenic granular sludge was obtained from a full-scale UASB reactor operated at 30°C treating alcohol distillery wastewater at Nedalco (Bergen op Zoom, The Netherlands). The total suspended solids and volatile suspended solids concentration of the wet sludge were 6.93±0.02% and 6.47±0.03%, respectively.

The experimental set-up consisted of a glass measuring tube (4 mm i.d.), in which one single granule was fixed by glass wool. The iron(II)-EDTA solution circulated through this tube with a flow rate of 0.96 mL·min^{-1}. The entire set-up was flushed with argon to avoid iron(II) oxidation. The ratio between iron(II) and EDTA was 1.3:1 to avoid changes in the granular matrix properties due to complexation and wash out of calcium from the biofilm matrix structure [2].

All MRI measurements were done at 30±2 °C on a 0.7 T NMR imager. A custom build solenoid RF probe was used with an i.d. of 5 mm, which was inductively coupled to avoid continuous retuning and matching due to the change in loading by the iron solution.

Each experiment consisted of a series of T_1 weighted 3D RARE measurements using short repetitions times till signal intensity inside the granule reached a new equilibrium, after applying the iron(II)-EDTA solution. Each series was preceded and followed by T_2 mapping using 3D MSME and T_1 mapping using 3D IR-RARE. All images and maps acquired consisted of a matrix of 128×40×20 voxels with a spatial resolution of 0.109×0.109×0.218 mm^3. The repetition time T_R for the T_1 weighted 3D RARE measurement was 200 ms with an effective echo time T_E of 4.53 m*s*. The time demands of the T_1, T_2 and T_1 weighted 3D RARE measurements were 160, 80 and 11 minutes, respectively. For further experimental details see [2].

The experimental data are presented as averaged profiles that were obtained by segmenting the granule in layers of one voxel thickness followed by averaging the T_1 weighted signal intensity per layer.

The actual iron(II) concentration based on the T_1 weighted RARE signal intensity was calculated using:

$$[\mathrm{Fe(II)}] = -\frac{1}{T_R \cdot R_{1,\mathrm{Fe(II)}}} \cdot \left[\ln\left(1 - \frac{S}{A_0}\right) - (1/T_1)_{\mathrm{Bulk}} \right] \tag{1}$$

with T_R the repetition time, $R_{1,\mathrm{Fe(II)}}$ the spin-lattice relaxivity for iron(II)-EDTA, and S the signal intensity from the T_1 weighted 3D RARE measurements, A_0 proton density from 3D T_2 mapping and T_1 from 3D T_1 mapping, both prior to iron(II)-EDTA addition. In this equation the effect of T_2 on the signal intensity S is neglected, because T_E is much shorted than the shortest T_2 present. This assumption is justified in Fig. 1A where no short T_2's are visible. Fractions with very short T_2 could be present but are invisible to the experiment and therefore do not affect the results.

3. Results

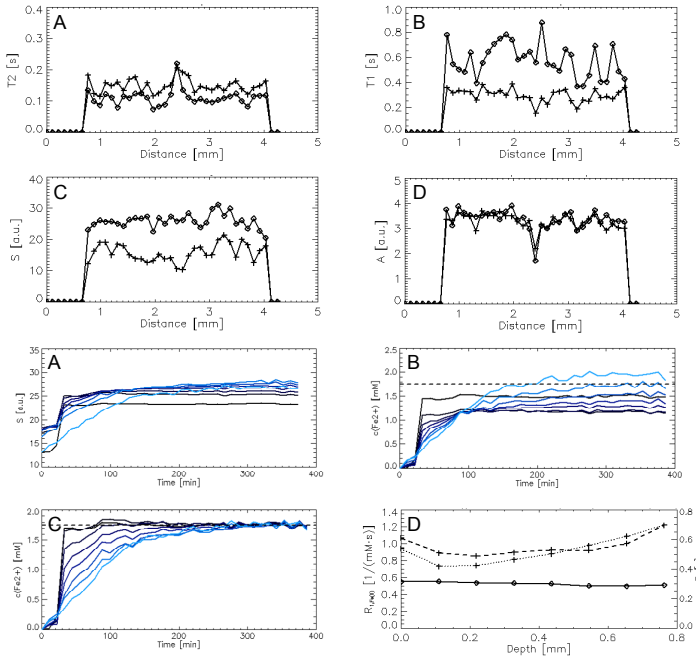

Fig. 1: Cross section of the methanogenic granule prior (+) and after (□) 15h exposure to a 1.75 mM iron(II)-EDTA solution. (A) Effect on T_2, (B) T_1, and (D) proton density A_0. (C) The signal intensity S of a T_1 weighted 3D RARE measurement with a repetition time of 200 ms shows a clear effect of the presence of iron(II)-EDTA.

Fig. 2: Effect of iron(II)-EDTA penetration into a single methanogenic granule. (A) Averaged T_1 weighted signal intensity per layer of the granule. (B) [iron(II)-EDTA] calculated using Eq. (1). (C) [iron(II)-EDTA] calculated under the assumption that the spin-latice relaxivity $R_{1,Fe(II)}$ is position dependent. (D) Average T_1 profile prior (--+--) and upon (–◊–) the exposure of the granule to the 1.75 mM iron(II)-EDTA solution and the distribution of the spin-latice relaxivity $R_{1,Fe(II)}$ (•••+•••).

4. Discussion

This study demonstrates a method for quantitative investigation of iron diffusion within an anaerobic granule. A complete methodology was developed including the experimental set-up, an optimized MRI sequence (3D RARE), image analysis and calculation of the increase of the iron concentration during time. The basic concepts were adopted from pioneering work by Nestle *et al.* [3].

It is shown that iron(II)-EDTA diffusion within the granule is independent of direction or the inner structure of the granules [2]. The final iron concentration after equilibration with the bulk solution, as measured by MRI, was not distributed equally over the granule, but ranged from 50 up to 115% of the concentration in the bulk solution (1.75 mM iron(II)-EDTA) (see Fig. 2B). One major assumption is that the spin-lattice relaxivity of iron(II)-EDTA in solution is representative for the relaxivity inside the granule matrix. An alternative approach is to use the T_1 maps before and after exposure to the iron(II)-EDTA solution to calculate spin-lattice relaxivity assuming that everywhere inside the granule the final iron(II)-EDTA concentration equals 1.75 mM (see Fig. 2C). This results in a position dependence of the spin-lattice relaxivity (see Fig. 2D).

One indication that this assumption is reasonable is given by simulating iron transport into a sphere using Fick's law for diffusion [4]. The results are well described by a diffusion coefficient of $2.5*10^{-11}$ $m^2 \cdot s^{-1}$ (see Fig. 3).

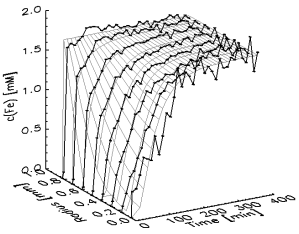

Fig. 3: Comparison of the measured iron concentration (black lines with points) and the theoretical values obtained applying the Fick's law for diffusion into a sphere (gray wire net).

5. Conclusions

MRI microscopy is feasible to quantify metal transport in biofilms.

References

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