Bacterial trajectories tracked with time-lapse video-microscopy reveal the impact of manganese biomineralization on bacterial sedimentation

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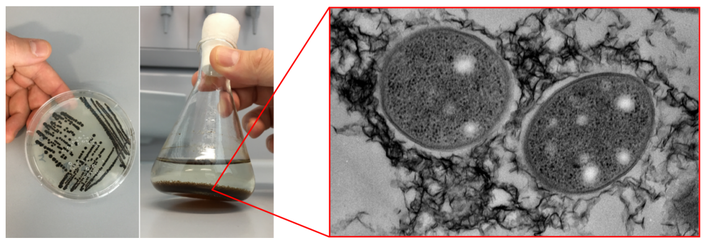
Context

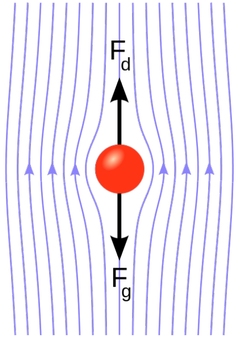
Biogeochemical processes like bio-mineralization have been observed at precise locations in soils and sediments where reaction rates are disproportionately high relative to the surroundings. Oxidized manganese in the form of Mn oxide minerals participates in a plethora of biogeochemical processes (e.g. contaminant adsorption, organic matter oxidation). Typically, Mn bio-mineralization proceeds through the enzymatic oxidation of aqueous Mn^{2+} into Mn^{4+} and precipitation of MnO_2(s) minerals in a biofilm matrix outside the bacterial cell. Here, we present a study of the impact of bio-mineralization on the sedimentation properties of bacteria at small scale (individual bacterium) under hydrostatic conditions.

Microscopic processes

We hypothesize that bacteria will sediment faster when bio-mineralization is active due to encrustation of the organisms by heavier mineral particles (ρ_{Mn} = 3.3 ρ_{H_2O}) and their aggregation in larger clusters. To test this hypothesis, we tracked the vertical motion of individual bacteria (*Pseudomonas putida GB-1*) using time-lapse video-microscopy.



Sedimentation process



The gravitational force

$$F_g = (\rho_f - \rho_s) g \frac{4\pi r_s^3}{3}$$

The frictional force (drag force, Stoke's law)

$$F_d = 6\pi\mu r_s V_s$$

By balancing the two we get the sedimentation

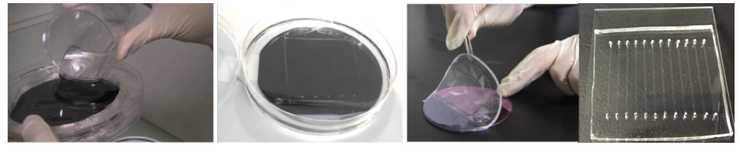
$$V_E = \frac{2}{9}\frac{\Delta\rho g R^2}{\mu}$$

Methods

Instead of using bulk methods (such as the ones based on turbidity measurement) **we track individuals bacteria** using microfluidic channels and video-microscopy. This novel approach allows us to:

- have in situ visualization
- resolve some aspects of the bio-mineralization dynamics
- measure sedimentation velocity (uncertainty $\sim$ 1%)
- measure the microbial heterogeneity (i.e. size or shape)

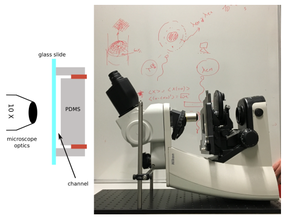
Microfluidics



Microfluidic devices are transparent microscale channels of PDMS (Polydimethylsiloxane) mixed with a curing agent, chemically bond to a glass slide.

Microscopy

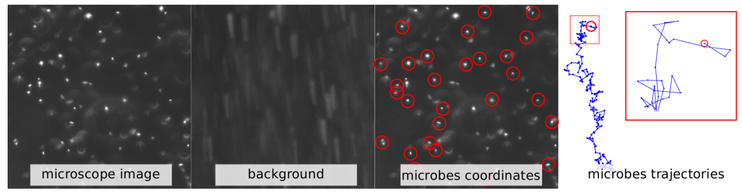
High magnification is required in order to visualize microbes. To resolve the vertical displacements, we place the microfluidics vertically and visualize sedimentation with a microscope turned by 90°.



Data acquisition and analysis

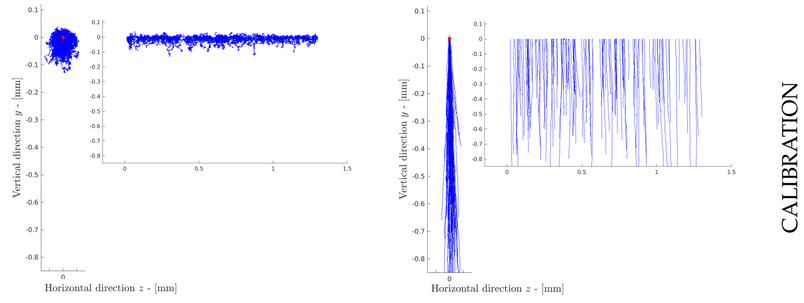
Every set of recorded images is analyzed as follows:

1. Removing background
2. Finding each particle coordinates
3. Linking particles between images (trajectories)
4. Measure the instantaneous and average sedimentation velocity

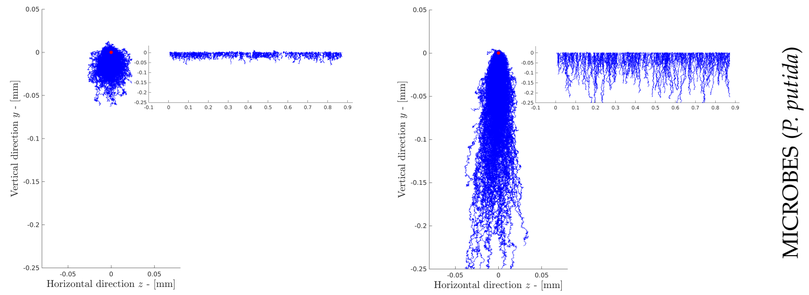


Results

Rescaled trajectories tracked **over about 1/2 hour** for 1μm (left) and **over about 5 minutes** for 10μm (right) polystyrene fluorescent microspheres.



Rescaled trajectories tracked **over about 1/2 hour** of *P. putida* with MnO_2(s) (left) and **over about 1/2 hour** of *P. putida* (right).



Results (error of 1%)	Calibration		Microbes	
	1 μm	**10 μm**	**with Mn**	**without Mn**
No. of objects	1061	293	39228	16109
Size [μm]	1	10	$\sim$ 1	$\sim$ 2
Expected velocity [μm/s]	0.0294	2.94	$\sim$ 0.03	$\sim$ 0.12
Measured velocity [μm/s]	0.0291	2.65	0.065	0.138

Perspectives

In the future, we are planning to explore the impact of manganese oxides on microbial growth, size and shape and the consequences for the sedimentation velocity distribution. Moreover, we are planning to investigate the impact of sedimentation on microbial transport in porous media under different flow conditions.

Acknowledgments

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