

# Bacteria mediated dissolution of pyromorphite $Pb_5(PO_4)_3Cl$ in presence of *Pseudomonas putida* bacteria - an effect on Pb remobilization in the environment

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## Introduction

Broad knowledge exists about the role of Pb-apatites in regulating the behavior and the bioavailability of Pb in soils and wastewater. In situ Pb immobilization is one of the methods now routinely applied for the reclamation of Pb-contaminated soils, including shallow unconfined aquifers. This method is based on the principle that aqueous phosphates added to soil pore solutions form a very stable (insoluble) mineral pyromorphite (Pb-apatite)  $Pb_5(PO_4)_3Cl$ . Bioavailability of aqueous Pb is thus minimized due to the very low solubility and the high thermodynamic stability of pyromorphite. Several reports have examined the ability of different bacterial species including *Pseudomonas* to solubilise insoluble inorganic phosphate compounds for example apatites. Various species of *Pseudomonas* genera are encountered as common inhabitants of soils and wastes in the industrial areas under strong pollution influence. To date, there has not been any published evidence of microbial dissolution of pyromorphite. The major objective of the project was to study *Pseudomonas putida* growth in the presence of Pb-apatite ( $Pb_5(PO_4)_3Cl$ ) as the sole source of phosphate. It was to test the hypothesis that in the phosphate deficient environment bacteria are able to actively scavenge for P from the Pb-apatite which results in remobilization of Pb in the environment.

## Mineral

Pyromorphite was synthesized by drop-wise mixing of the respective chemical reagents at room temperature. The precipitate was analyzed by:

- Powder X-ray diffraction** carried out on a Phillips PW 3020 X'Pert-APD powder autodiffractometer with Bragg-Brentano geometry using Ni-filtered  $CuK\alpha$  radiation (1500 W sealed tube with a Cu target at 45kV and 35mA).
- Scanning Electron Microscopy** carried out on a HITACHI S-4700 SEM operated at 20kV of accelerating current and high vacuum/variable pressure

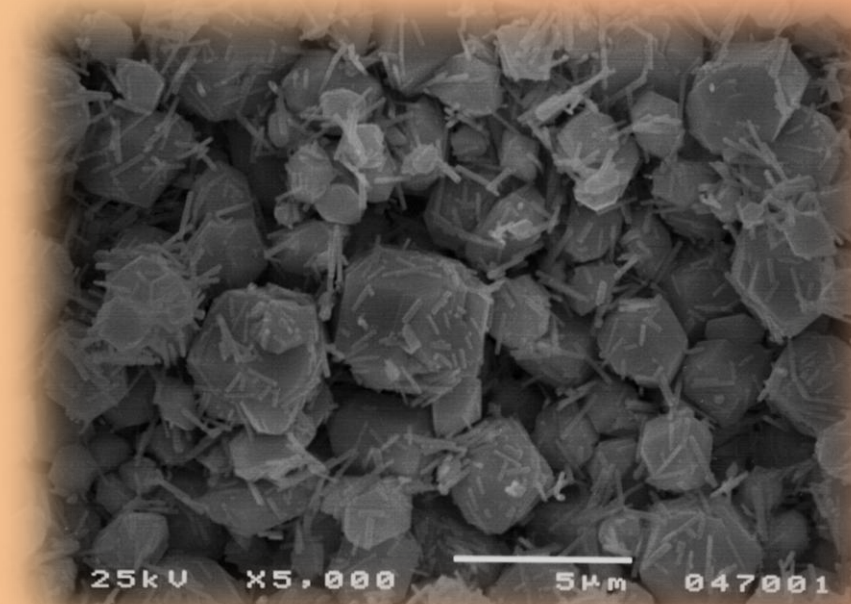
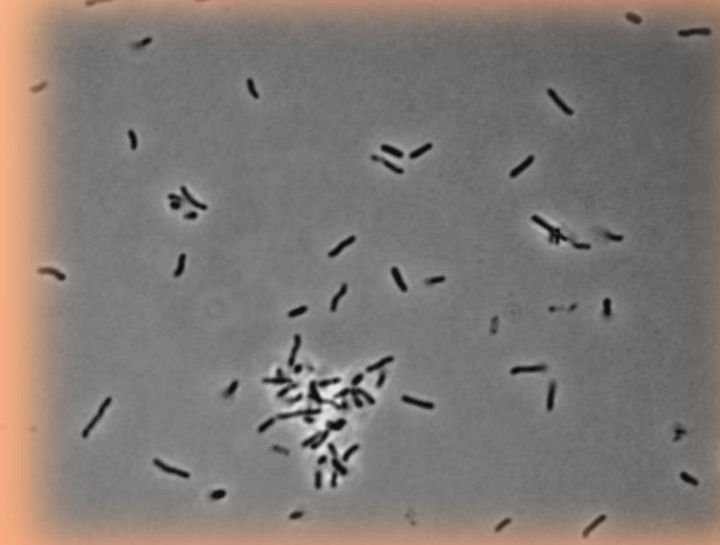


Figure 1. SEM microphotograph of synthetic pyromorphite

## Bacteria

Commercially available *Pseudomonas putida* strain (IBPRS KKP 1136) was used throughout.

Figure 2. *Pseudomonas putida* (IBPRS KKP 1136) (Nicon eclipse E1000)



## Batch dissolution experiments:

The bacteria growth was investigated at 22°C in:

- a phosphate rich and a phosphate deficient medium,
- a presence of the Pb-apatite (pyromorphite) in a phosphate rich and a phosphate deficient medium

The experiment and its controls (abiotic dissolution of pyromorphite) were run in standard growth medium (UND laboratory receipt) for the period of bacterial growth cycle (10 days for phosphate-rich medium and 21 days for pyromorphite-rich, phosphate-deficient medium). The medium was incubated in polycarbonate flasks on a gyratory shaker rpm (100 rpm). Starting pH=6.5, no buffer was used. Samples were periodically analyzed for pH, bacterial density, Pb and P (UV-vis, ICP) (Figure 5).

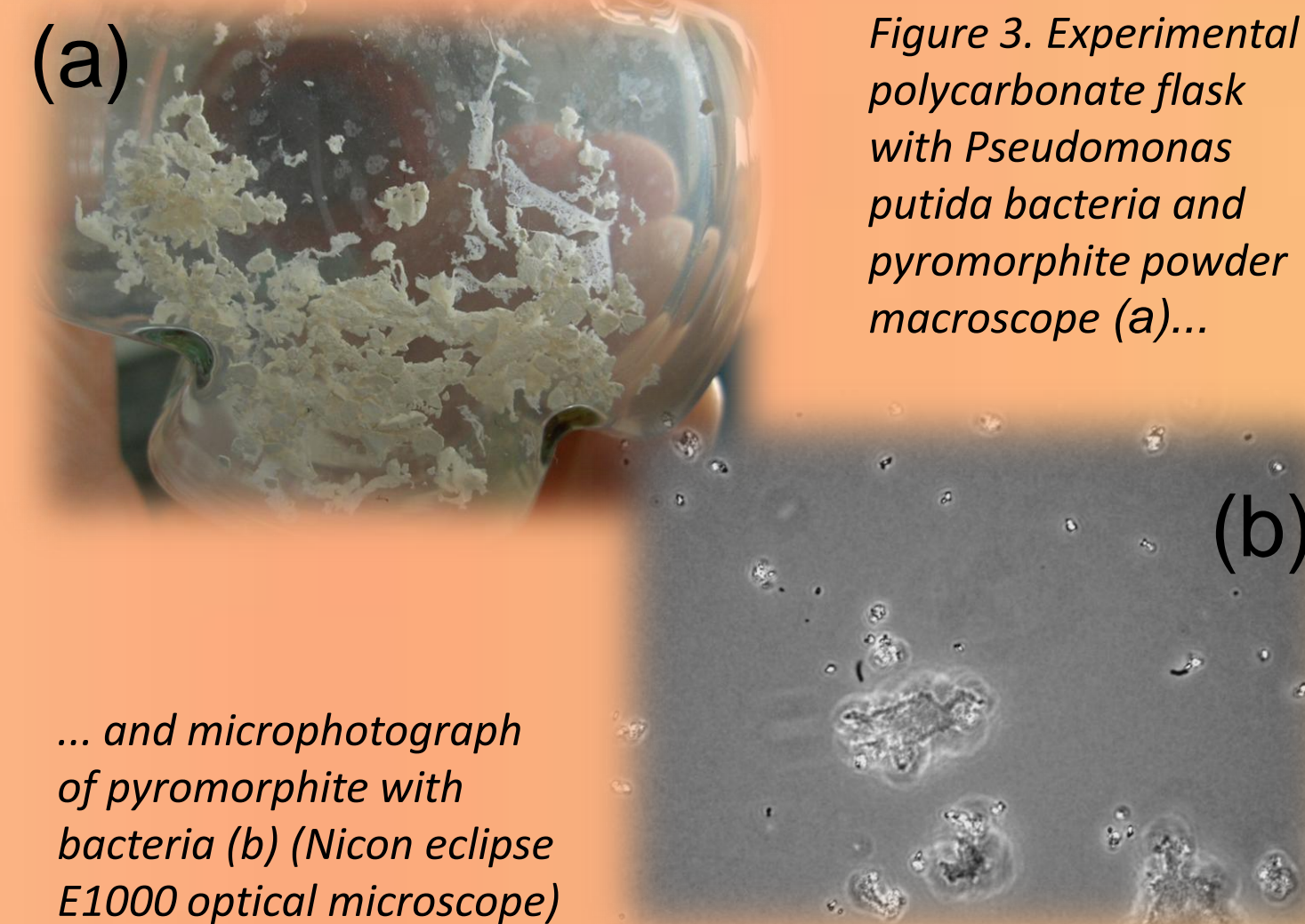


Figure 3. Experimental polycarbonate flask with *Pseudomonas putida* bacteria and pyromorphite powder macroscopically (a)...

... and microphotograph of pyromorphite with bacteria (b) (Nicon eclipse E1000 optical microscope)

## Colonization batch experiments:

In order to test the impact of bacteria colonization on pyromorphite dissolution natural crystals of pyromorphite, a few millimeters in size, were dissolved in a presence of *Pseudomonas putida* for a period of one week. The experimental conditions and medium were identical as for batch experiments. At the end of this experiment a reacted mineral was rinsed once with redistilled water and mounted on SEM stubs using carbon tape. Samples were examined using FEI Quanta field emission SEM.

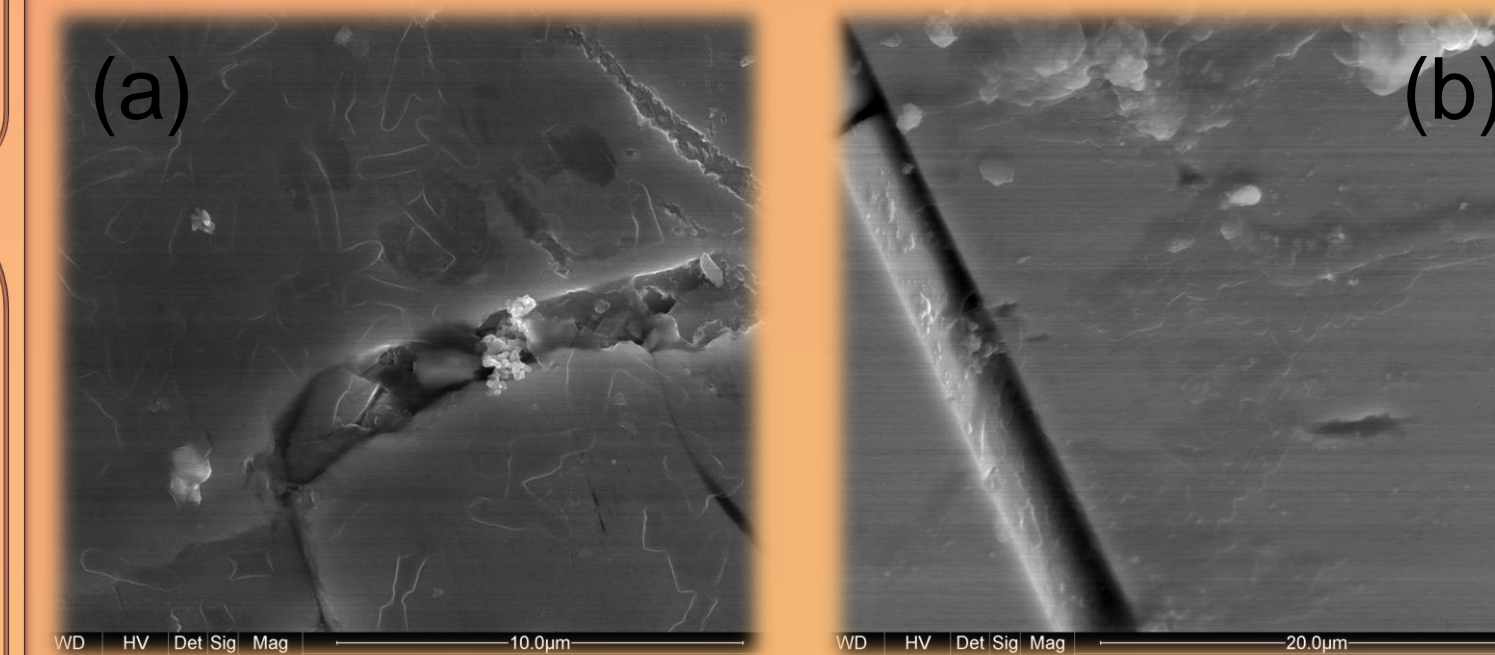
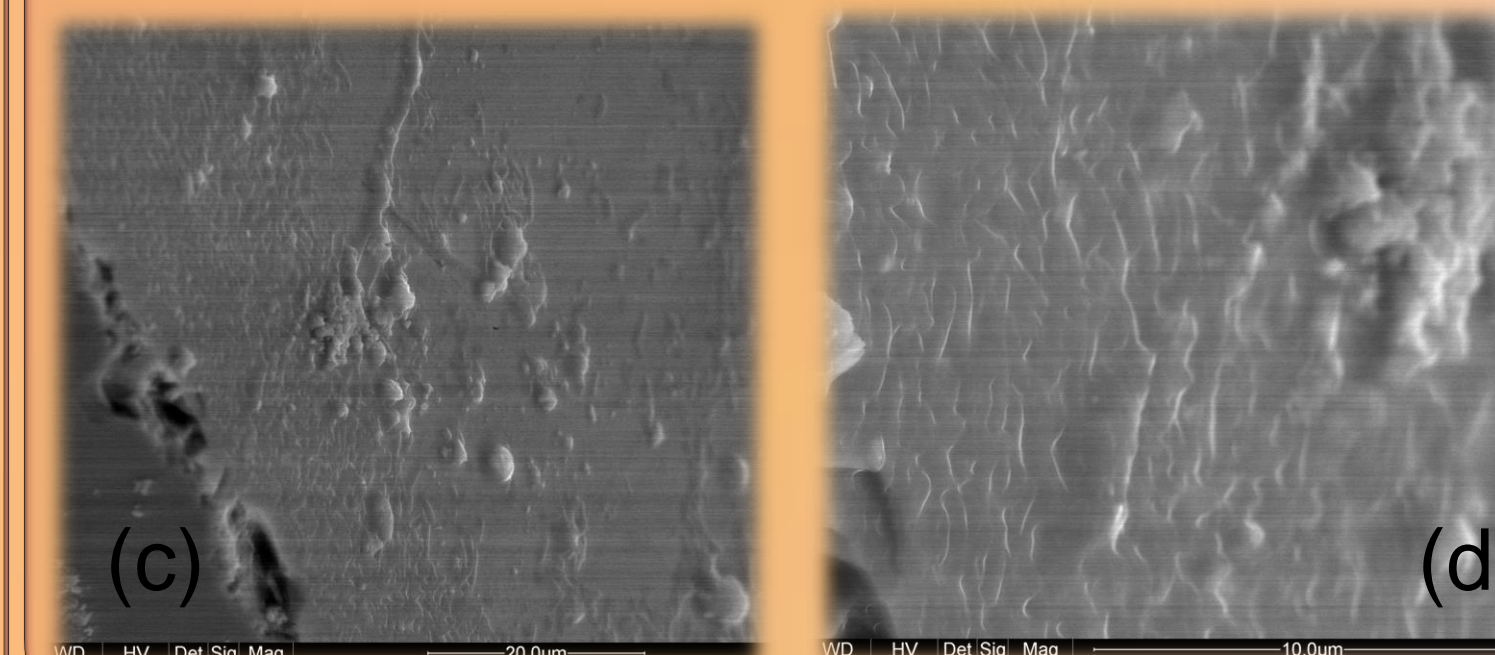
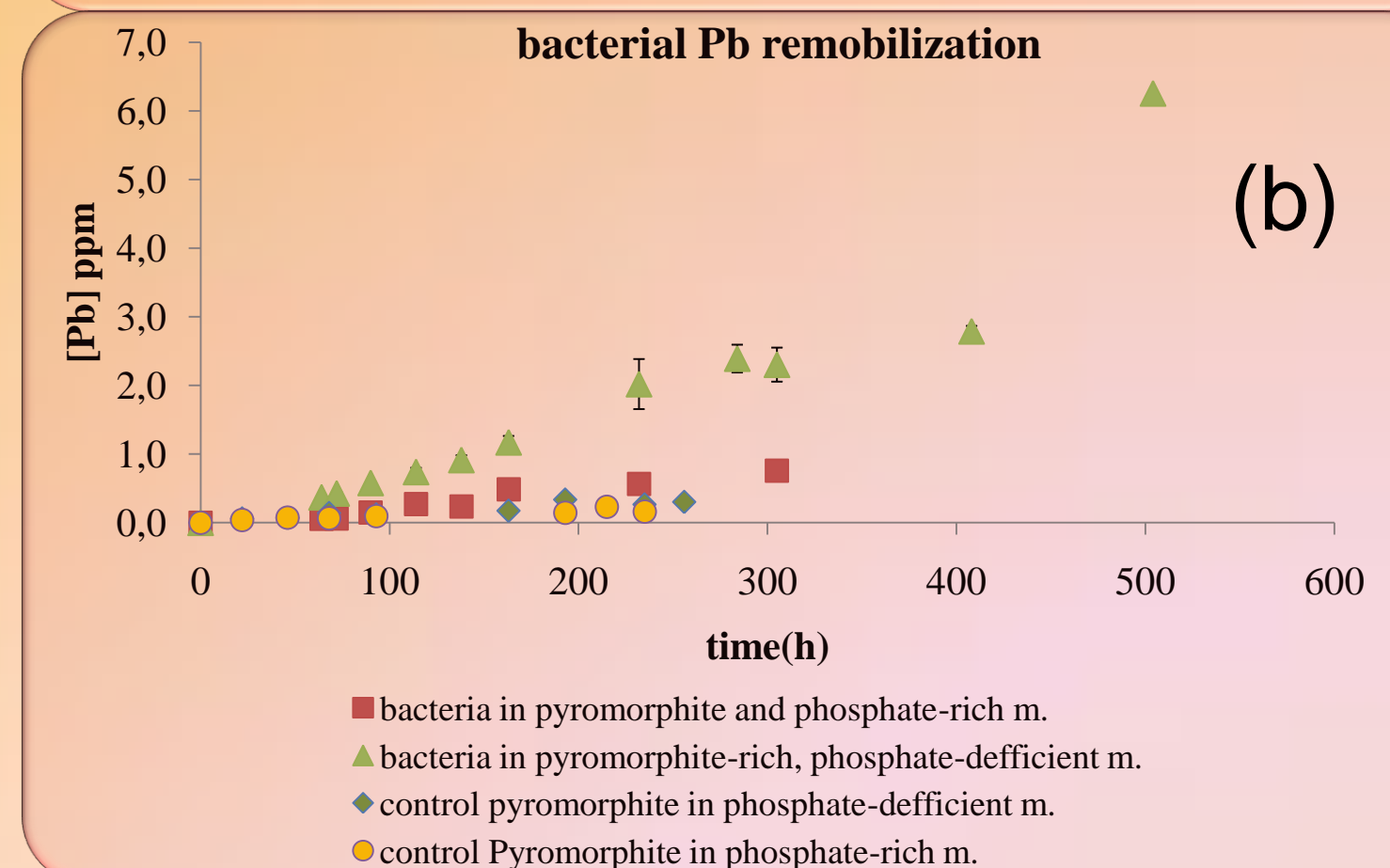
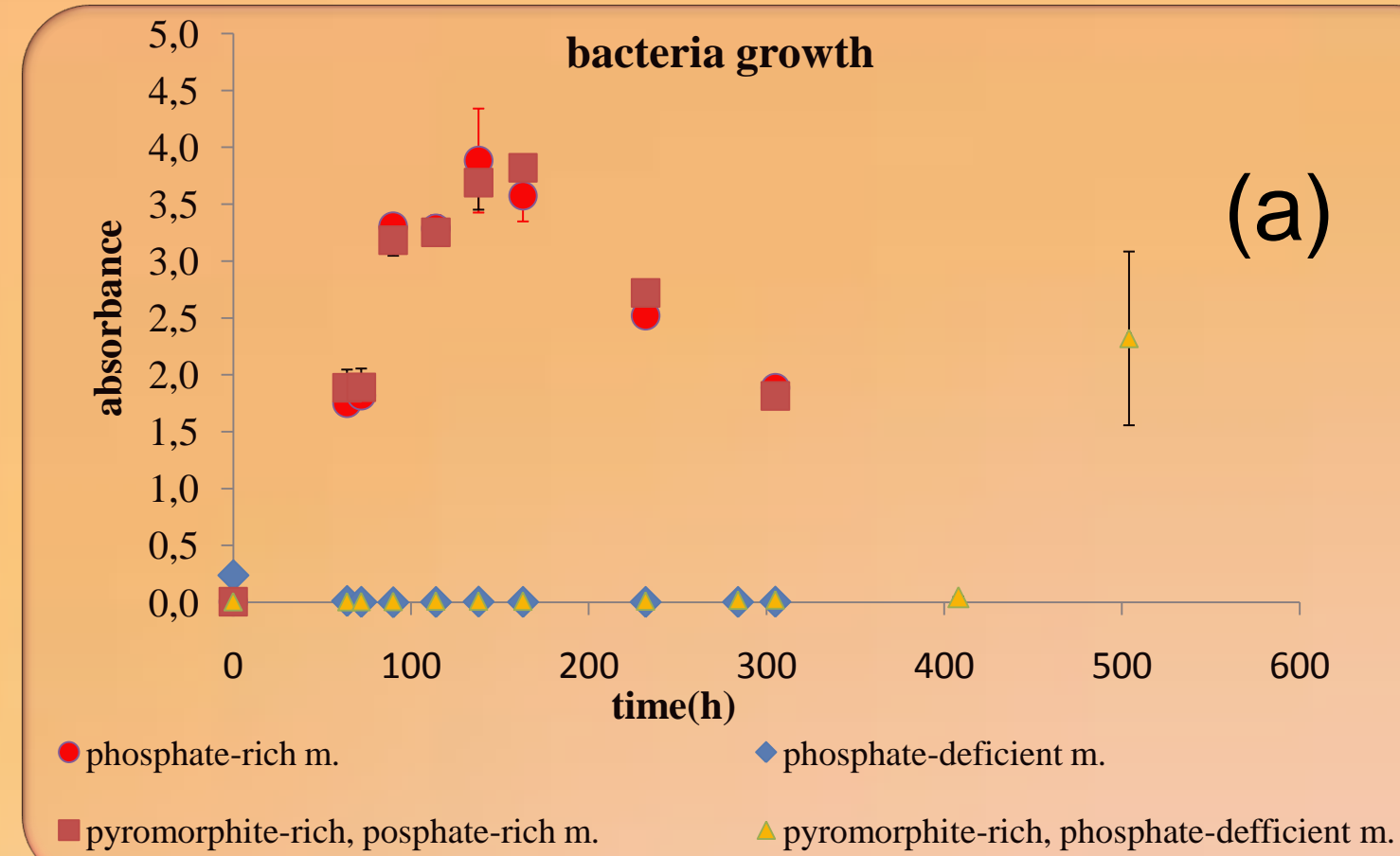


Figure 4 (a, b, c, d). SEM images of natural crystals of pyromorphite colonized by *Pseudomonas putida* bacteria. Biofilm on the crystals can be observed.



## Acknowledgements

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## Conclusions

- Pseudomonas putida* bacteria can successfully grow on pyromorphite as a sole source of phosphates, though the lag phase of bacteria growth cycle is prolonged in comparison to phosphate-rich environment.
- Pb may be mobilized from pyromorphite by microbial activity, both in phosphorous-rich and phosphorous-deficient environment. The effect of bacteria colonization of mineral surface can be observed.
- The presence of bacteria enhances the dissolution of pyromorphite, resulting in up to 20 times increase of  $Pb_{aq}$ .
- The observed effects of microorganisms on Pb remobilization need to be considered in Pb remediation strategies that rely on pyromorphite formation.