MILESTONE REPORT

TASK 3.4

MILESTONE 3.4. “SCREENING FOR SUITABLE MICROBES FOR BIOACCUMULATION / BIOSORPTION / BIOMINERALIZATION”

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1 EXECUTIVE SUMMARY

This milestone report presents results regarding screening experiments in order to select suitable microbes to achieve metal bioaccumulation, biosorption, or biomineralization from geothermal fluids. The experiments considered both actively growing biomass and dead biomass. Active biomass was used to induce Li biomineralization as Li_2CO_3 via two metabolic activities: oxalic acid production in fungi (*Aspergillus niger* and *Meyerozyma* sp.) and oxalate oxidation by bacteria (*Pandoraea* sp.). In another experiment, biomass from three Gram-positive bacterial strains was used to foster Li accumulation/biosorption within their cell wall. Finally, dead and processed biomass of a *Penicillium citrinum* strain isolated from a geothermal fluid was used to assess Pb biosorption under pressure, temperature, and salinity conditions corresponding to the conditions of actual geothermal energy systems.

2 INTRODUCTION

In this report we present results comparing three different microbiological processes to change the solubility of target elements initially present in a geothermal fluid. We compared biomineralization (microbially induced precipitation of a mineral phase) and biosorption (elemental adsorption on biological surfaces), while bioaccumulation (elemental accumulation within cells) was not considered directly. In terms of elements, we focused on Li as an economical valuable element and on Pb as a problematic element that can trigger scaling in some geothermal power plants. Finally, we also considered model strains, already known from the literature for their metal-related capabilities, along to strains originating from geothermal environments, thereby assuming that they would be already adapted to the extremophilic conditions of geothermal energy systems.

3 BIOMINERALIZATION

Li biomineralization was explored by combining two microbial metabolisms: fungal oxalic acid production and bacterial degradation of oxalic acid/oxalate (Palmieri et al. 2019). First, we assessed oxalic acid production in two oxalogenic fungi (Fig. 1A), *Aspergillus niger* (a well-known oxalogenic filamentous fungus) and *Meyerozyma* sp. (a yeast-fungus isolated from a geothermal fluid during the REFLECT project; Bregnard 2023). Then, using an actual geothermal fluid provided by CLL, we applied an elemental filtration step by mixing oxalic acid to the fluid to trigger precipitation of metal-oxalates for most elements (in particular Ca that is highly concentrated in this context). In this filtration step, elements such as Li, Na, and K, are expected to remain in solution (Verma et al. 2019; Fig. 1B). Further, this “pre-treated” fluid can then be used, supplemented with small amounts of oxalic acid, to feed the oxalotrophic bacterium *Pandoraea* sp. (UniNE own isolate) which will then increase the pH (Fig. 1C), thereby changing the solubility of selected salts, among which Li-containing salts. Using this scheme, we have been able to achieve Li biomineralization as Li-phosphates (Fig. 1D). The latter also acted as surfaces for the co-precipitation of NaCl, indicating that NaCl concentrations might be an issue to selectively obtain Li-only biominerals. Further steps stemming from these results will consist in: 1) optimizing oxalic acid production by the two fungal strains for the filtration step, 2) triggering Li-carbonate precipitation instead of Li-phosphate, 3) assessing whether NaCl affect Li recovery from Li-carbonate phases.

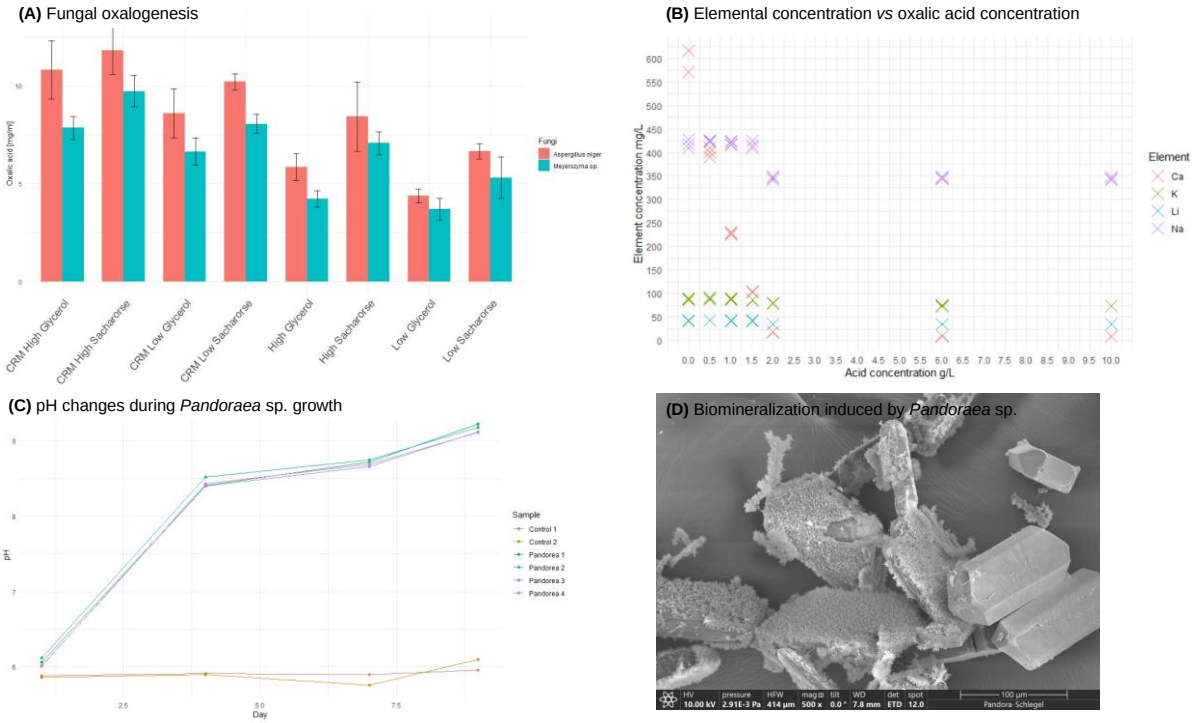

Figure 1: (A) oxalic acid concentrations produced by two fungal strains, *Aspergillus niger* and *Meyerozyma* sp., when grown in: water or a CLL raw fluid, supplemented with either Glycerol or Saccharose as a carbon source, both at either a high (30 g/L) or a low concentration (10 g/L). **(B)** Evolution of the soluble concentrations of Calcium (Ca), Potassium (K), Lithium (Li), and Sodium (Na) within the CRM fluid with the addition of oxalic acid. Measured with an ICP-OES. n=3. Al, Fe, and P were not detected. **(C)** Evolution of the pH over 9 days induced by the metabolic activity of *Pandoraea* sp. when grown in a medium with Li-oxalate as a C-source. **(D)** Scanning Electron Microscopy (SEM) image showing precipitates of Li-phosphate covered with NaCl retrieved from the Li-oxalate medium inoculated with *Pandorea* sp.

4 BIOSORPTION

Li biosorption/accumulation was assessed using the procedure described in Tsuruta (2005), where the aim is to trigger Li complexation on the cell wall of Gram-positive bacteria (chelation via teichoic acids). The following strains were assessed: *Bacillus subtilis* DSM347, *Priestia megaterium* DSM3228, *Glutamicibacter mysorens* DSM20123. Each experiment compared active biomass at 60 mg or 150 mg per 100 mL fluid. Li concentration in biomass was assessed with an ICP-OES (670.778 nm, axial, iFR) by retrieving biomass on 0.22 µm membrane filters and digesting the filters with Aqua regia. Li was effectively transferred from the fluid to the cells with *B. subtilis* showing a slightly better performance. In addition to this, we identified that the ratio of cells:liquid also influenced the complexation efficacy. As a result, the removal of Li from fluid is technically feasible with the help of microorganisms.

Li-Removal in culture

	Li Raw Data (mg/L)			Li-Removal				
Culture	0 h	1 h	Removal	µg/100 mL	SOLL/IST[2]	µg/mg cells	µg/10^{10} cells	"Tsurata"
Sterile medium (100 mL)[1]	3.332	3.315	0.017	1.700	n/a	n/a	n/a	about 10 to 20 times more (ca. 3.8 µg Li/mg cells)
Bacillus 60 mg in 100 mL	3.251	3.064	0.170	17.00	1:2,5/1:2,2	0.283	4.05	
Bacillus 150 mg in 100 mL	3.232	2.846	0.369	36.90		0.246	3.51	
Glutamicibacter 60 mg in 100 mL	3.257	3.112	0.128	12.80	1:2,5/1:1,6	0.213	3.75	
Glutamicibacter 150 mg in 100 mL	3.217	2.996	0.204	20.40		0.136	2.39	
Priesta 60 mg in 100 mL	3.211	3.070	0.124	12.40	1:2,5/1:1,7	0.207	47.33	
Priesta 150 mg in 100 mL	3.215	2.985	0.213	21.30		0.142	32.52	

[1] Offset, with which the following measurement series were set to zero

[2] Target/actual balancing

Li-Recovery from cells after filtering and aqua regia digestion

Filter	Li Raw Data	Filtered culture		Li-Recovery				
	mg/L	mL	Li total (µg)	µg/100 mL	SOLL/IST[2]	µg/mg cells	µg/10^{10} cells	Recovery (%
Sterile filter in 10 mL[1]	0.01	80	0.120	0.150	n/a	n/a	n/a	n/a
Bacillus 60 mg in 10 mL	1.56	80	15.48	19.35	1:2,5/1:1,8	0.323	4.61	114
Bacillus 150 mg in 10 mL	2.85	80	28.38	35.48		0.237	3.38	96
Glutamicibacter 60 mg in 10 mL	1.22	80	12.08	15.10	1:2,5/1:1,2	0.252	4.43	118
Glutamicibacter 150 mg in 10 mL	1.52	80	15.08	18.85		0.126	2.21	92
Priesta 60 mg in 10 mL	1.18	80	11.68	14.60	1:2,5/1:1,5	0.243	55.73	118
Priesta 150 mg in 10 mL	1.10	50	10.86	21.72		0.145	33.16	102

[1] Offset, with which the following measurement series were set to zero

[2] Target/actual balancing

Finally, we also assessed the biosorption potential of a fungal strain originating from a geothermal fluid (Bregnard 2023). There the aim was to explore whether an already adapted strain would be effective in biosorbing a target element under *in situ* conditions, i.e. at pressure, temperature, and salinity of a geothermal fluid before it enters heat exchange as a mean of avoiding scaling, which is a common issue decreasing the performance of geothermal plants. Such an approach would allow the immobilization of some elements (i.e. those with low economical value) and keep other elements that are more valuable in order to optimize geothermal energy generation. In this experiment, we have been using biomass of the fungus *Penicillium citrinum* (isolated from a geothermal fluid within the REFLECT project) and have exposed dead biomass to a Pb-rich synthetic fluid under high P and T. This has allowed identifying that P and T are not an issue for biosorption but that the main obstacle lies in NaCl concentrations. As a result, in order to prevent scaling via (bio)sorption in both an economic and an environmental-friendly ways, one has to deal first with Na and Cl before considering other elements (Leins et al. in prep).

5 REFERENCES

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