Journal of Hazardous Materials 314 (2016) 237–248

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Toxicity effects on metal sequestration by microbially-induced carbonate precipitation

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HIGHLIGHTS

- Minimum inhibitory concentrations (MIC) are determined for *S. pasteurii* with a range of metals.
- Zinc & cadmium bioprecipitation is strongly linked to microbial carbonate generation.
- Lead & copper carbonate bioprecipitation is limited & abiotic processes may be significant.
- Bioprecipitation allows survival at & remediation of higher metal concentrations than expected.

ARTICLE INFO

Article history:
Received 5 January 2016
Received in revised form 14 April 2016
Accepted 16 April 2016
Available online 19 April 2016

Keywords:
Bioprecipitation
Sporosarcina pasteurii
Urea hydrolysis
Heavy metals
Bioremediation

ABSTRACT

Biological precipitation of metallic contaminants has been explored as a remedial technology for contaminated groundwater systems. However, metal toxicity and availability limit the activity and remedial potential of bacteria. We report the ability of a bacterium, *Sporosarcina pasteurii*, to remove metals in aerobic aqueous systems through carbonate formation. Its ability to survive and grow in increasingly concentrated aqueous solutions of zinc, cadmium, lead and copper is explored, with and without a metal precipitation mechanism. In the presence of metal ions alone, bacterial growth was inhibited at a range of concentrations depending on the metal. Microbial activity in a urea-amended medium caused carbonate ion generation and pH elevation, providing conditions suitable for calcium carbonate bioprecipitation, and consequent removal of metal ions. Elevation of pH and calcium precipitation are shown to be strongly linked to removal of zinc and cadmium, but only partially linked to removal of lead and copper. The dependence of these effects on interactions between the respective metal and precipitated calcium carbonate are discussed. Finally, it is shown that the bacterium operates at higher metal concentrations in the presence of the urea-amended medium, suggesting that the metal removal mechanism offers a defence against metal toxicity.

1. Introduction

The behaviour of metal and radionuclide contamination in the subsurface environment is controlled by soil and groundwater chemistry, particularly redox potential and pH, which strongly affect species solubility, precipitation and sorption to the solid phase [1]. Microbial methods of groundwater chemistry control offer a highly localised approach to contaminant removal, and biological production of certain chemical species can directly reduce mobility and bioavailability of contamination. The contaminants themselves, however, will negatively impact upon the activity and viability of sub-surface organisms, and above certain limits will prevent any microbial activity [2].

Microbial action can stimulate remediation of metallic contamination through various means, including alteration of groundwater chemistry (*e.g.* pH), or through organisms or their exudates acting as nucleation sites to which cations are attracted [3,4]. Bioprecipitation, or biomineralisation, is the removal of mobile contaminant ions from solution through biological production of precipitating chemical species, to form a range of minerals such as sulphates, phosphates, silicates and oxides [5–7].

Production of calcium carbonate (often in the form of calcite) as a stable mineral phase offers long-term sequestration of contamination. Divalent metallic ions are then co-precipitated within the carbonate phase by substituting for calcium ions during production, allowing sequestration for long periods [8]. Removal of a number of

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http://dx.doi.org/10.1016/j.jhazmat.2016.04.039

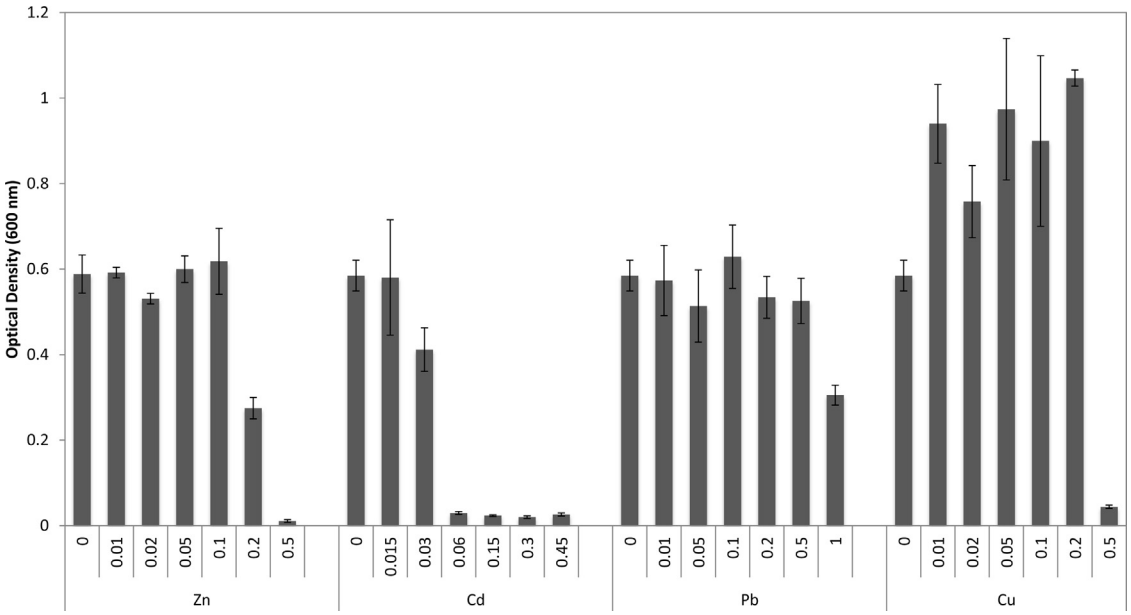

Fig. 1. Optical density (OD_{600}) of *S. pasteurii* culture at various metal concentrations (mM) after 3 days incubation at 30 °C, relative to control cultures (zero metal content). [Error bars ± 1 SE].

contaminants of interest through coprecipitation with or sorption to calcite has been reported, in natural and engineered situations and through abiotic and also biotic processes, including arsenic [9], cadmium [10], zinc and possibly nickel [11], copper [12], lead [13] and a range of others, including radionuclides, such as strontium, cobalt and uranium [14–16].

Commonly, generation of the necessary conditions for calcium carbonate biomineralisation is through urea hydrolysis, which generates both carbonate and ammonium ions and an elevated pH [8]. A wide range of organisms is capable of catalysing this reaction *via* urease enzymes, and the capability for carbonate biomineralisation is present in many environments [7,14]. Microbial cells act as focal points for the formation of calcium carbonate, due to their control of the immediate environmental chemical conditions and negative zeta potential attracting calcium ions.

Microbially-induced calcite precipitation for removal of heavy metal pollution from aqueous solution has previously been considered in only a limited fashion. The work reported here consists of two stages. First, we investigate how toxicity of a range of heavy metals (zinc, cadmium, lead and copper; chosen to represent common metallic contaminants susceptible to abiotic removal with carbonates) affects growth of a urease-positive bacterium, *Sporosarcina pasteurii*. We then demonstrate the extent to which these metals can be removed from solution *via* bioprecipitation with calcium carbonate and explore variations in performance with different metals. The impact of the metal removal mechanism at previously inhibitory concentrations of the different metals explores for the first time the effect of this mechanism on allowing microbial survival and remediation at higher concentrations than would otherwise be expected based on toxicity alone.

2. Methodology

2.1. Strains and culture media

Sporosarcina pasteurii, a gram-positive, urease-positive endospore-forming bacterium [17] was obtained from NCIMB (Aberdeen, UK; NCIMB8221/ATCC6453. It was grown at 30 °C for 24 h in autoclaved Oxoid CM0001 nutrient broth (13 g/L) amended with 0.2 μm filter-sterilised urea (20 g/L). Bacterial pellets were harvested by centrifuging at 1450 RCF for 20 min then resuspending in phosphate-buffered saline (PBS: Na_2HPO_4 [8.3 mM], NaH_2PO_4 [1.6 mM], NaCl [145 mM], pH 7.2). The centrifugation process was repeated before cells were re-suspended in working media.

For metal toxicity experiments, Oxoid CM0001 nutrient broth (13 g/L) amended with the relevant metal salt was used, corrected to pH 6.5 with hydrochloric acid. Metal bioprecipitation experiments employed a urea-amended medium, as follows: Oxoid CM0001 nutrient broth (3 g/L, autoclaved), urea (20 g/L), NH_4Cl (10 g/L), sodium bicarbonate (2.12 g/L), $CaCl_2{\cdot}2H_2O$ (50 mM) and a range of metal salt concentrations (all 0.2 μm filter-sterilised).

2.2. Metal toxicity experiments

Microcosms were prepared by resuspending washed bacterial pellets in nutrient broth and placing 6 mL in sterile 10 mL screw cap glass centrifuge tubes, before amendment with metal salt solutions to give a final total volume of 8 ml. The final optical density at 600 nm wavelength incident light (OD_{600}) was 0.064 (equivalent to approximately 2×10^6 cells/mL). Final metal salt concentrations were as follows (in mM): zinc chloride (0–0.5), cadmium sulphate (0–0.45), lead chloride (0–1) and copper chloride dihydrate (0–0.5). Concentrations were chosen to demonstrate the limiting concentrations where growth was inhibited for each metal (minimum inhibitory concentration [MIC]; defined as a 70% reduction in growth measured by optical density [2]). Each metal concentration was tested in triplicate.

Microcosms were incubated at 30 °C for 72 hours, after which the OD_{600} was measured to determine effect of metals on growth, as a measure of toxicity to the organism (as in Ruggiero et al. [2]). These values were corrected by subtracting the OD_{600} of the solutions only. This experiment only explored the absolute toxicity of various metal concentrations (similar to Ruggiero et al. [2]), and did not explore temporal issues such as increased lag, although the 72 hour period was sufficient to ensure that this did not impact upon the results.