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

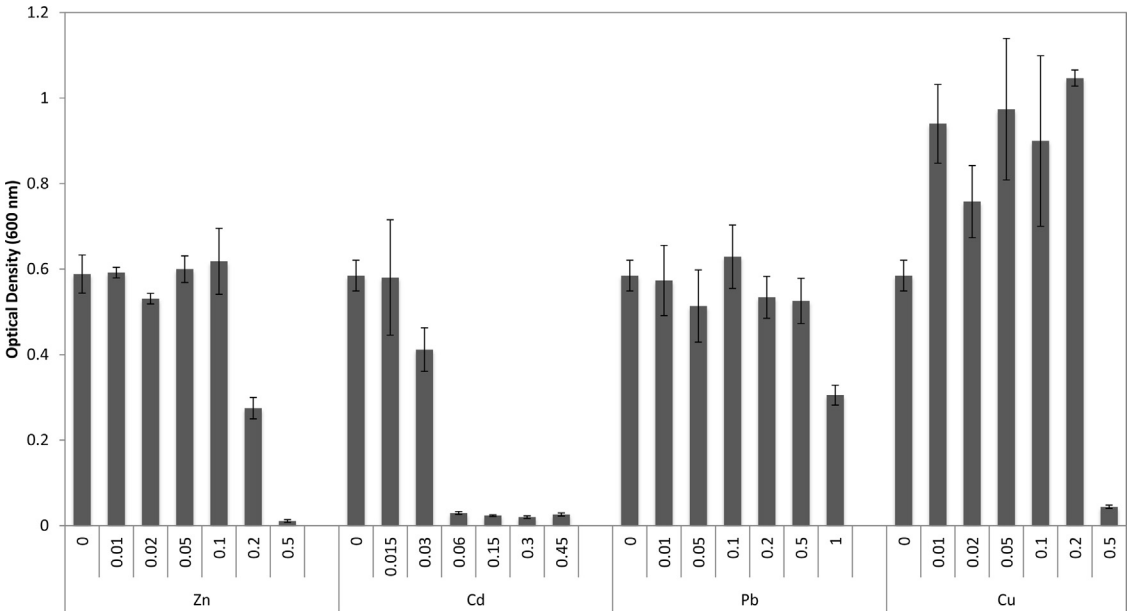

Fig. 1. Optical density (OD_{600}) of *S. pasteurii* culture at various metal concentrations (mM) after 3 days incubation at 30 °C, relative to control cultures (zero metal content). [Error bars ± 1 SE].

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.

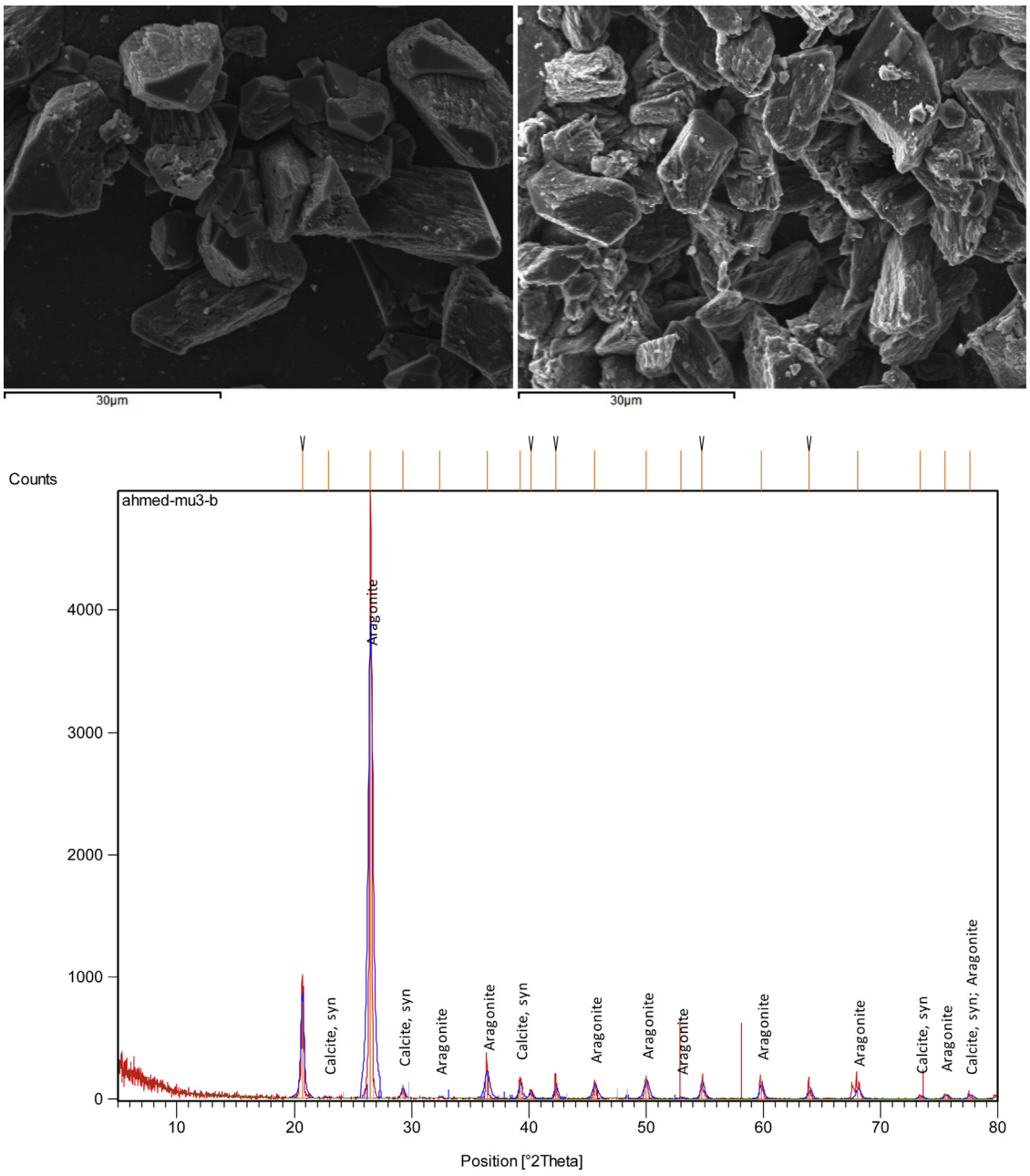

Fig. 2. Scanning electron microscopy images and X-ray diffraction spectra of calcium carbonate precipitation (from parallel experiments, in the presence of quartz sand grains).

2.3. Metal bioprecipitation experiments

Microcosms were prepared using a similar method to that in Section 2.2. Following pelletisation and washing, cells were re-suspended in the urea-amended medium before 25 ml was placed aseptically in sterile 50 ml polypropylene centrifuge tubes and amended with heavy metals as in Section 2.2 to give a total final volume of 30 ml. Initial microbial numbers were approximately 5×10^6 cells/mL, based on OD_{600} data, and final metal concentrations were in the following ranges (mM): zinc – 0-10; cadmium – 0-1.5; lead – 0-5; copper – 0-5. Killed-cell controls were implemented by autoclaving the initial bacterial suspensions prior to incorporation in the urea-amended medium. Incubation was again at 30 °C. Samples (5 ml) were obtained after 0, 1, 3 and 7 days, filtered through 0.2 µm and sub-divided for analysis. The pH was measured in a 2.5 ml aliquot then calcium and heavy metal ion concentrations were determined by ICP-OES (Optima 2100 DV, PerkinElmer) from the remainder. The weight change of microcosm tubes over the experiment was recorded in order to determine the mass of precipitation seen adhering to the tube. Following completion of the experiment, tubes were emptied, carefully rinsed with deionised water and dried in an oven at 30 °C (to prevent heat damage to tubes) to constant weight before final weighing.

2.4. Speciation analysis

Visual MINTEQ [18], a Windows version of the USEPA code MINTEQA2, was used to simulate metal speciation in experiments. Visual MINTEQ cannot reproduce biological phenomena due to a lack of equilibrium in such processes, and therefore has been

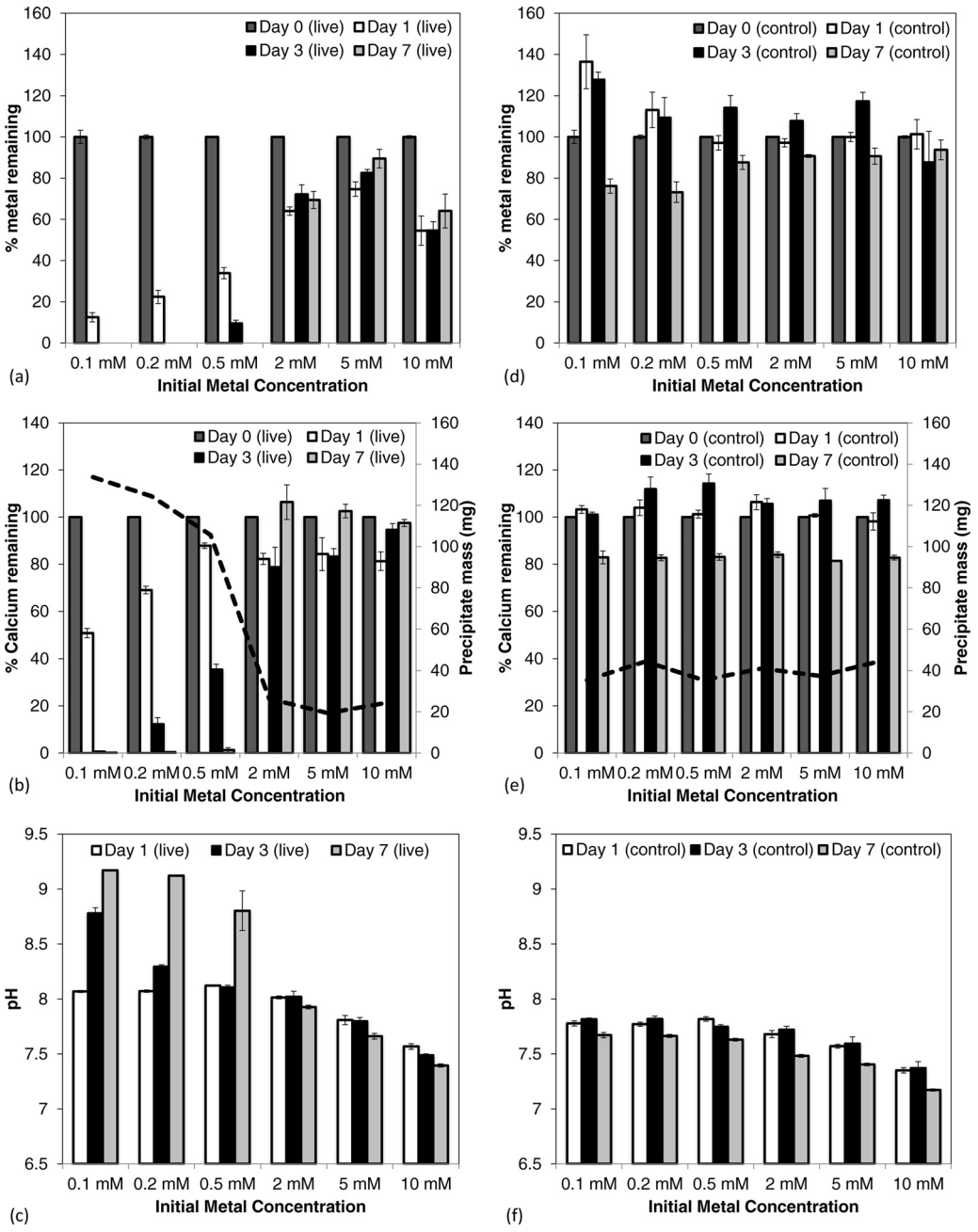

Fig. 3. Impact of *S. pasteurii* activity on zinc removal (a, d), calcium removal (b, e) and pH (c, f) at a range of zinc concentrations, with live (a-c) and killed (d-f) cells at 30 °C with urea growth medium. Dashed lines on (b) and (e) represent precipitate mass. [Error bars ± 1 SE].

used here to confirm whether metal removal is a predictable phenomenon based purely on chemical equilibria, or whether there is a dynamic, microbially-driven mechanism causing removal. It has been used to predict final metal speciation using knowledge of major ion concentrations present, ambient temperature and the final pH. For comparison with experimental data, models of live cell experiments adopted a 'best case' approach, assuming that all urea was hydrolysed and all resulting ionic species were available at the pH measured, based on the reactions described by DeJong et al. [19]. Conversely, models of killed cell controls adopted a worst case approach, assuming that no urea was hydrolysed. This simplified approach to modelling idealised final conditions is necessary as no information was available on ionic species present.

3. Results and discussion

3.1. Metal toxicity

Cadmium exhibited the greatest toxicity with a minimum inhibitory concentration of 0.03–0.06 mM, whilst zinc and copper were similar, with an MIC of 0.2–0.5 mM (Fig. 1). Resistance to lead was highest, with growth unhindered below 1 mM. Resistance of *S. pasteurii* to heavy metals does not appear to have been investigated previously. MIC values for these metals are available for some other species, for example Radford et al. [20] reported *Bacillus subtilis* strains (*S. pasteurii* was formerly classified as *Bacillus pasteurii*) having a copper MIC of 0.2–1 mM, and Majzlik et al. [21]

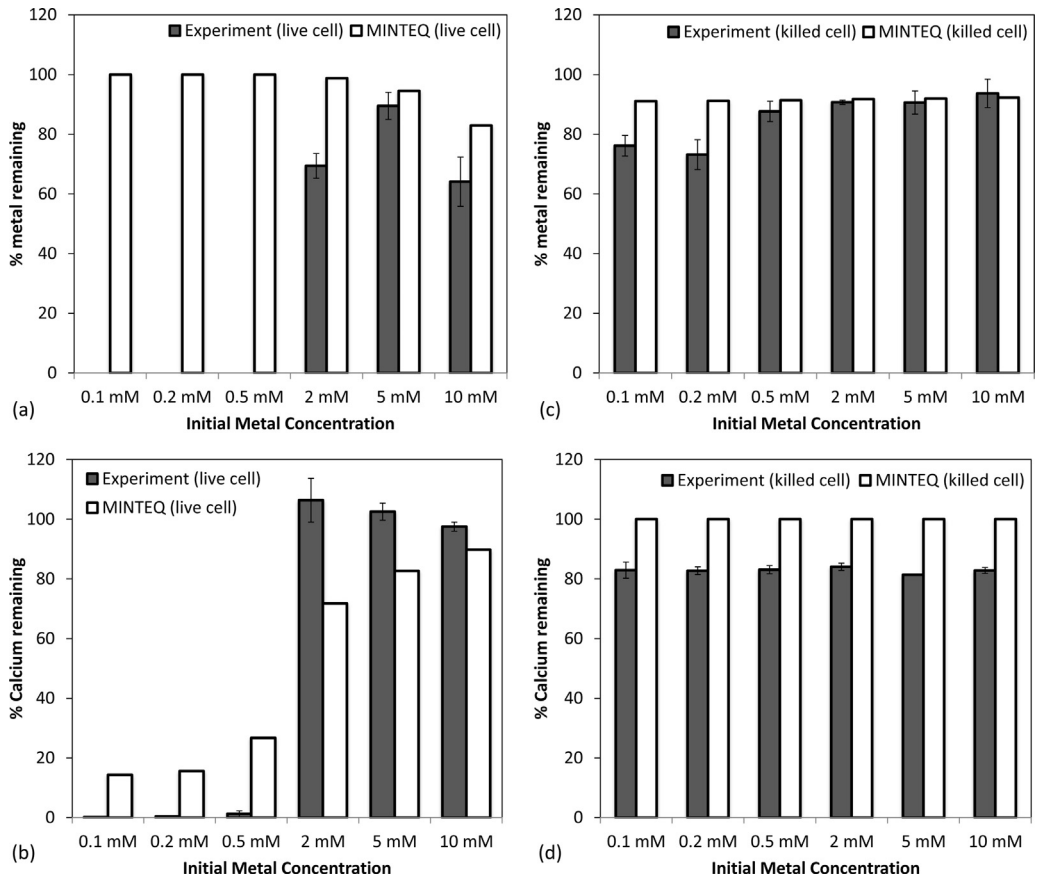

Fig. 4. Comparison of actual and predicted (Visual MINTEQ) dissolved zinc and calcium concentrations after 7 days for live cell (A, B) and control (C, D) experiments. [Error bars ± 1 SE].

identified MICs for *Streptomyces* sp. of 0.5–0.8 mM for copper and 0.45–0.6 mM for zinc. These are comparable to the values found in the present study. Many inhibition studies concern species isolated from various metal-containing environments and so have a degree of metal resistance. As a result, MIC values are higher than those seen here. For example, Altimira et al. [22] found MICs for copper (3.1–4.7 mM), zinc (<0.8–17 mM) and cadmium (<0.4–2.5 mM). Yamina et al. [23] found elevated resistance for various species, including *Bacillus* sp. with MICs of 5.8 mM for lead, 2.7 mM for cadmium and 3.8 mM for zinc.

It is unclear why microbial growth appeared to increase above that seen in the metal-free solution with a range of copper concentrations. It is known that copper is a micronutrient, playing a role in various protein structures (*e.g.* Arguello et al. [24]). A stimulatory effect on growth has been identified previously [25] although this was at low concentrations of 10 μM and with very different organisms (anaerobic methane oxidisers).

3.2. Metal bioprecipitation

The ability of *S. pasteurii* to cause precipitation of the metallic contaminants *via* urease hydrolysis is demonstrated in the following sections. Normalised metal and calcium concentrations for live and killed-cell experiments are presented relative to initial values, indicating the extent of removal over time at the concentrations tested. In addition, pH and precipitate mass data are presented. The conjunction of calcium removal and pH data provides an indication of whether calcium carbonate precipitation was occurring.

Unfortunately the mass of precipitate produced in bioprecipitation experiments was small and so X-ray diffraction was not possible on individual specimens. However, XRD analysis performed in parallel experiments using the same organism and media (albeit in the presence of quartz sand) suggest the presence of calcite with some aragonite, with SEM analysis identifying rhombohedral crystals akin to those seen previously with calcite [26] (Fig. 2).

3.2.1. Zinc

Complete removal of zinc from solution in 7 days occurred at concentrations up to 0.5 mM with live cells present (Fig. 3), indicating improved resistance to metal toxicity over that seen earlier (Fig. 1 – MIC of 0.2–0.5 mM). The pH increase and removal of calcium and zinc from solution correlate well; at 2 mM or above the pH in live cell experiments is consistent with killed cell controls and little zinc or calcium removal was observed. The mass of precipitate recovered correlates reasonably well with removal of calcium from solution.

Fig. 4 presents Visual MINTEQ predictions of the proportion of soluble zinc and calcium present under the prevailing conditions at the end of the experiments, compared to experimental data. These agree reasonably well in terms of calcium removal, and also for zinc in control specimens. This suggests that in these cases the changes are driven by chemical equilibrium at the pH found. However, there is considerable disparity between predicted and actual zinc concentrations in live cell experiments, with Visual MINTEQ suggesting the majority should remain in solution but experimental data finding this only at higher zinc concentrations (Fig. 4a). The correlation with removal of calcium from solution suggests that sorption, co-precipitation and possible formation of a solid solution of zinc in calcium carbonate is occurring as the latter is laid down. These are

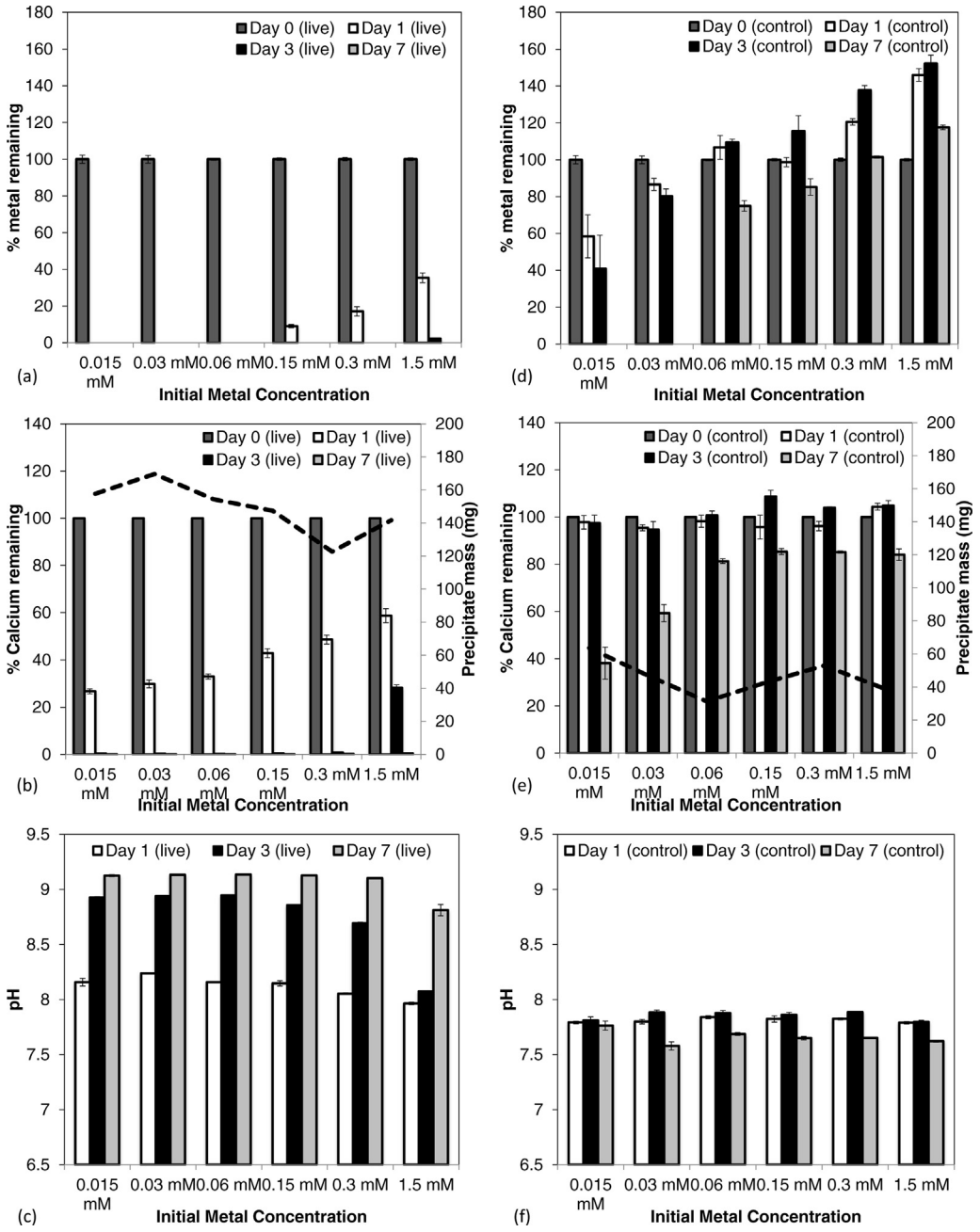

Fig. 5. Impact of *S. pasteurii* activity on cadmium removal (a, d), calcium removal (b, e) and pH (c, f) at a range of cadmium concentrations, with live (a–c) and killed (d–f) cells at 30 °C with urea growth medium. Dashed lines on (b) and (e) represent precipitate mass. [Error bars ± 1 SE].

dynamic processes that would not be modelled by the equilibrium-driven Visual MINTEQ model. At higher zinc concentrations (2 mM or greater), microbial urea hydrolysis is limited by its toxicity and so the increase in pH necessary to facilitate both calcium carbonate formation and zinc coprecipitation do not arise. The predicted and experimental data then increasingly agree.

Zinc sorbs strongly to calcium carbonate minerals, with sufficient sorption capacity on calcite to fully sorb zinc at all but the largest concentration tested here [27,28] though is readily desorbable and only slowly absorbed into solid solution unless recrystallization occurs. Here, however, calcium carbonate crystals are being formed concurrently and so sorption and subsequent encapsulation as a Ca-Zn-CO_3 solid solution (noted by Buekers et al. [11]) is likely, offering more durable sequestration.

3.2.2. Cadmium

Removal of cadmium again correlates well with pH change and calcium removal (Fig. 5). Almost all cadmium was removed by day 3 at all concentrations tested, although more slowly at higher concentrations. Apparent resistance of *S. pasteurii* to cadmium is substantially improved, with the organism able to remove the metal from solution at concentrations at least 25 times that at which growth was impaired when urea was not present (at least 1.5 mM, compared to an MIC of at most 0.06 mM). In controls, despite no observed changes in pH there was some removal of calcium and cadmium at low cadmium concentrations. This may indicate that a similar mechanism of calcium precipitation driving cadmium removal also occurred here but only very slowly. *S. pasteurii* is nominally a spore-forming organism and so spores may survive autoclaving and subsequently regenerate, although

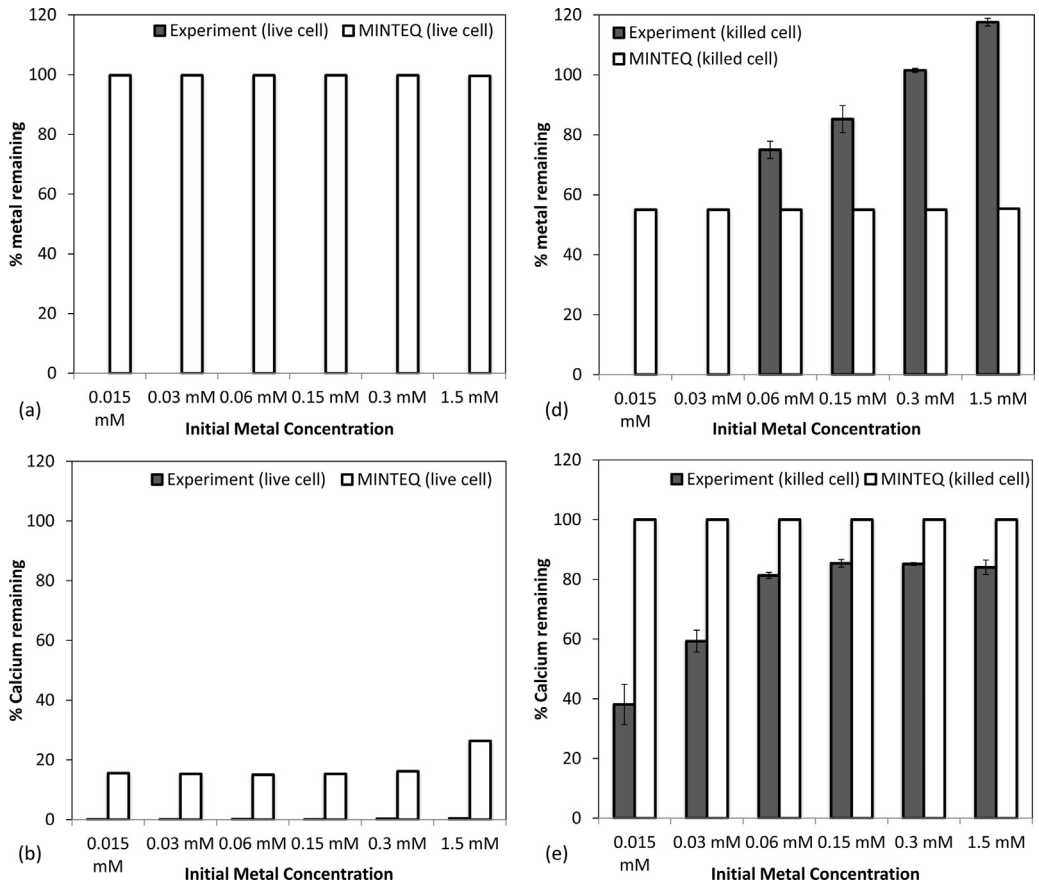

Fig. 6. Comparison of actual and predicted (Visual MINTEQ) dissolved cadmium and calcium concentrations after 7 days for live cell (A, B) and control (C, D) experiments. [Error bars ± 1 SE].

sporulation with this strain was not detected using Schaeffer-Fulton staining. No significant pH increase was seen, although it may be that a small change in pH was sufficient to cause a degree of precipitation but that this was then buffered by the mineralisation process. The excess recovery of cadmium at intermediate time points in controls cannot be explained with available information. Again, precipitate mass broadly correlated with calcium removal.

As with zinc, there is a degree of commonality between Visual MINTEQ and experimental data for removal of calcium from solution (Fig. 6), the unexpected calcium and cadmium removal at low cadmium concentrations (discussed above) notwithstanding. In addition, increasingly large proportions of cadmium were found in solution in killed cell controls, despite predictions that around 45% of the metal should precipitate as cadmium chloride at all concentrations. The most significant disparity arises again with the heavy metal concentration in live cell experiments. Although Visual MINTEQ predicts that all cadmium should remain in solution, none was found. Again, it is thought that sorption and co-precipitation during calcium carbonate formation are responsible.

Cadmium is similar in atomic radius to calcium and forms a similar octahedral carbonate structure [29]. It therefore can readily substitute for calcium in carbonate minerals. On existing surfaces of calcite, rapid sorption can form an epitaxial layer, preventing further sorption of the metal and limiting dissolution of the mineral [30,31]. Sorption to other, more openly structured forms of calcium carbonate such as aragonite is not affected as an epitaxial layer does not form [32] although these minerals are relatively soluble and over time may recrystallize as calcite, incorporating the metal as a solid solution. As with zinc, the continuing growth of carbonate minerals may prevent formation of a coherent epitaxial layer due to continuous deposition of new material. Again, it is unclear whether sorption or co-precipitation prevails, as both are linked to increasing amounts of calcium carbonate. However, Garcia-Sanchez and Alvarez-Ayuso [28] quote a sorption capacity for cadmium on calcite of 10 mg/g, which is more than enough to sorb all the cadmium present in these experiments given the amounts of precipitate produced.

3.2.3. Lead

In the presence of lead up to 5 mM, live cell experiments exhibited large pH increases, with corresponding removal of calcium ions from solution and almost complete removal of lead ions (Fig. 7). However, with killed cell controls similar, albeit slower, lead removal was observed, despite limited pH change and calcium concentration decrease. Whilst the latter may be explained by spore regeneration at low lead concentrations (although no sporulation was observed), the removal of lead is not correlated with this and increases at higher lead concentrations; an abiotic mechanism is therefore suspected. Such a mechanism could allow greater microbial activity at higher lead concentrations with live cells. It is unclear, therefore, whether pH increase and calcium removal were the cause of lead removal, or facilitated by it. However, bacterial activity occurred at higher concentrations (previous MIC of 0.5–1 mM), and live cell experiments did have an improved response compared to killed cell controls, suggesting that urea hydrolysis did play a role in at least part of the removal of lead from solution. Once more, precipitate mass broadly agrees with the degree of calcium removal, although the mass produced in live cell specimens was greater than that in controls even when calcium removal was comparable.

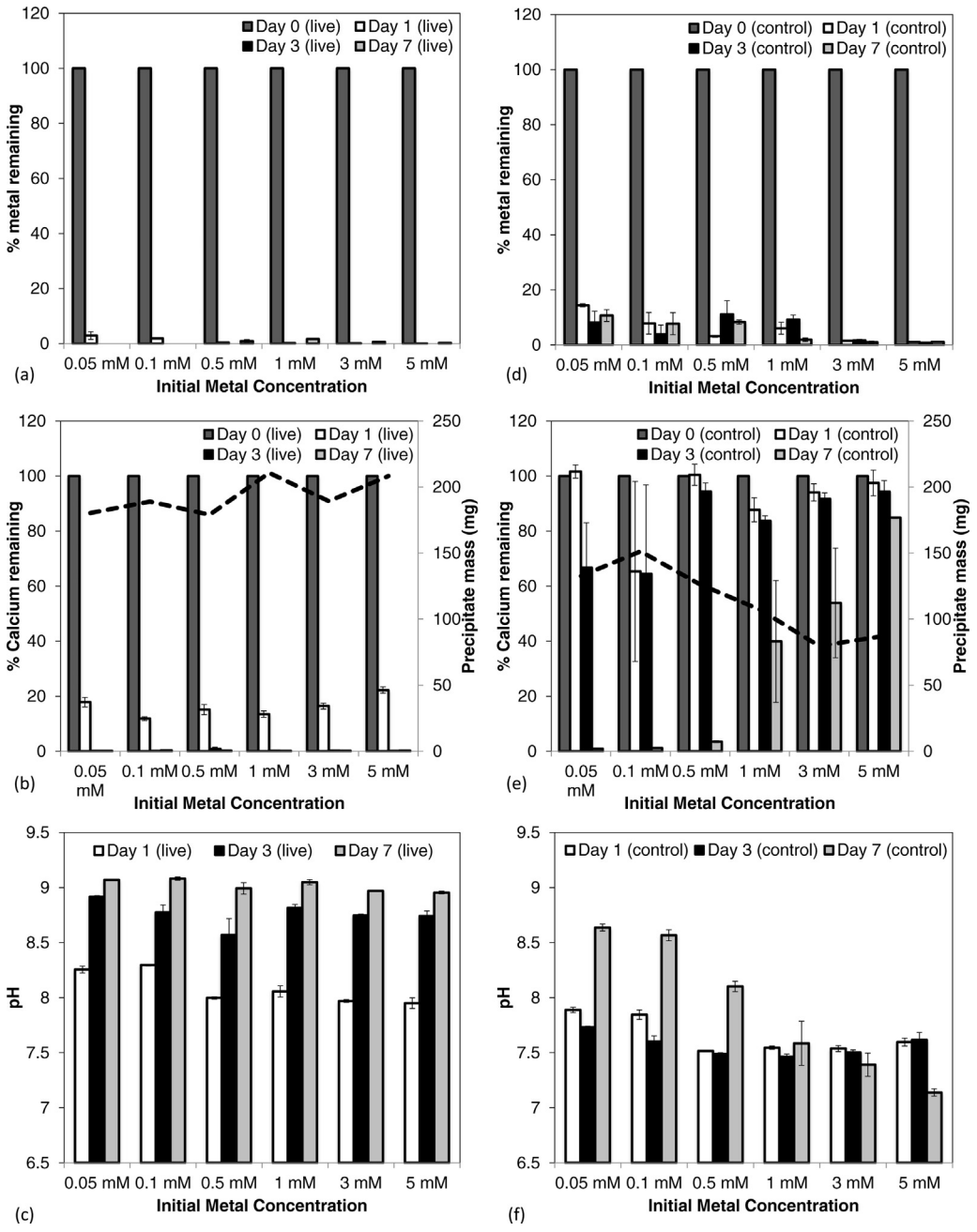

Fig. 7. Impact of *S. pasteurii* activity on lead removal (a, d), calcium removal (b, e) and pH (c, f) at a range of lead concentrations, with live (a–c) and killed (d–f) cells at 30 °C with urea growth medium. Dashed lines on (b) and (e) represent precipitate mass. [Error bars ± 1 SE].

Visual MINTEQ predictions of calcium behaviour in live cell experiments are similar to actual data in that the majority of calcium is removed from solution (Fig. 8). Killed cell control predictions of calcium are higher than those observed, likely to be due to the unexpected pH increase in these specimens. Only a small amount of insoluble lead was predicted with live cells, whereas complete removal was observed experimentally. With killed cells, a similar outcome arose, although decreasing lead solubility with lead concentration is predicted by Visual MINTEQ due to formation of insoluble lead carbonate. Such a trend is observed in experimental data, but the magnitude of removal is far greater.

Lead ions sorb strongly to calcium carbonate, particularly calcite, and can form a solid solution in this mineral, albeit with a distorted lattice [29,30,33]. However, the experimental and model data suggest an abiotic mechanism is at least partially responsible for the observed results although a proportion of metal removal is thought to be due to microbial carbonate precipitation.

3.2.4. Copper

Experiments with copper again indicate that microbial pH amendment leads to calcium removal (Fig. 9). In this case, activity was seen up to a concentration of 1 mM copper, larger than the <0.5 mM noted earlier (Fig. 1). This increased activity is despite a lack of complete removal of copper ions from solution apart from at the lowest concentration. Despite the apparent formation of calcium carbonate, and the elevated pH, a significant proportion of copper ions remain in solution. However, the amount of copper removed may be sufficient in each case (other than at 1.5 mM) to reduce the concentration to below the MIC, allowing improved survivability of the bacteria. As with lead, a limited pH increase and

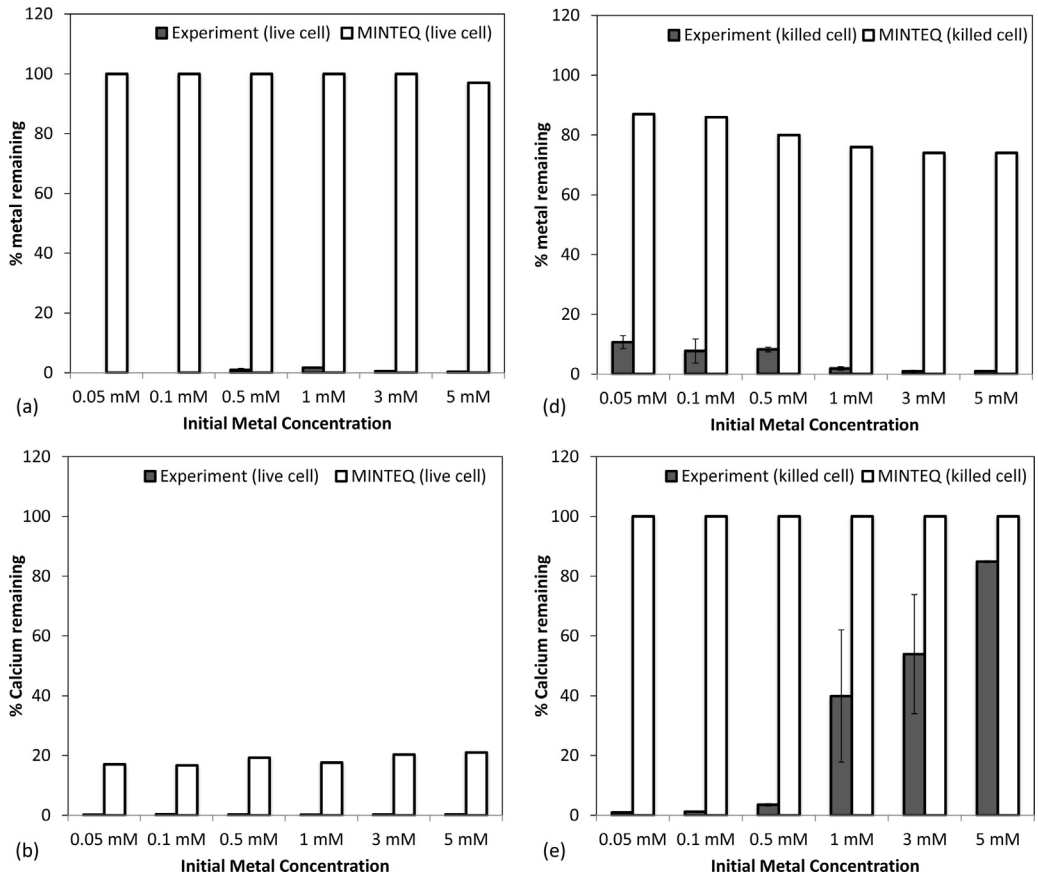

Fig. 8. Comparison of actual and predicted (Visual MINTEQ) dissolved lead and calcium concentrations after 7 days for live cell (A, B) and control (C, D) experiments. [Error bars ± 1 SE].

corresponding calcium concentration decrease was seen in killed cell controls. Trends in production of precipitate were very similar to those observed with lead: broad agreement with calcium removal but greater mass observed in live cell specimens.

Calcium removal from solution with increasing copper concentration gradually slowed in live cell experiments (Fig. 9b) though was complete after 7 days apart from at the highest concentration. Modelling showed a qualitatively similar trend of decreasing microbial action with increasing copper, although predicted a more gradual increase in the amount of soluble calcium with increasing copper concentration (Fig. 10). All or most copper was predicted to remain in solution in all cases with both live and killed cells. This is different to the experimental data (Fig. 9a & d), which has total removal at the lowest concentration, substantial removal (~30–60%) at intermediate concentrations and almost no removal at 5 mM.

Copper is reported to impact upon both microbial activity in mineralising carbonates as well as the formation of carbonate crystals themselves. Warren et al. [7] found that 0.75 mM copper hindered its microbially mediated removal. This compares well to the upper limit of observed microbial effects here, at concentrations of around 1 mM. Calcium removal in the presence of copper was not notably hindered apart from at the highest concentration of 5 mM, but the amount of copper concurrently removed was less than observed with other metals. Schosseler et al. [34] found that copper carbonate complexes on the surfaces of calcite and vaterite can hinder further sorption as well as growth or dissolution of the mineral, and at high concentrations an epitaxial layer may form. This may account for the limited removal of copper in these experiments even when carbonate minerals are expected to be present.

4. General discussion

Available metal concentration is the primary determinant of toxicity, and data from Visual MINTEQ suggest that all metals are near fully or fully available under initial conditions in all experiments. Therefore, initial metal toxicity in the bioprecipitation experiments would be expected to be similar to that in the metal toxicity experiments at the same concentration. Microbial activity in the presence of urea-amended medium has been shown to occur at metal concentrations higher than the minimum inhibitory concentrations (MIC) determined for each of the metals considered. It is suggested that at metal concentrations above the MIC there may be a low level of microbial activity that allows a diminished rate of urea hydrolysis, leading to a small amount of carbonate, and metal, precipitation. This in turn reduces the overall toxicity, allowing enhanced microbial activity and therefore further biomineralisation; once this process commences, provided sufficient nutrients and feed are provided, similar remedial outcomes to those seen at lower concentrations might be expected. Therefore, as long as the conditions permit the initial survival of a viable population of cells, remediation may continue even at relatively high metal concentrations.

Of the few studies related to the work reported, Li et al. [35] tested biomineralisation of all the metals reported here with ureolysis-induced carbonate formation. There were, however, substantial differences in the experimental design – in Li et al.'s work no calcium was supplied and no abiotic controls were included, indicating that precipitates were pure metal carbonate and that abiotic mechanisms could not be ruled out. A strain of *S. pasteurii* was used, producing removal of >90% for all metals at concentrations often much higher than those observed in the experiments

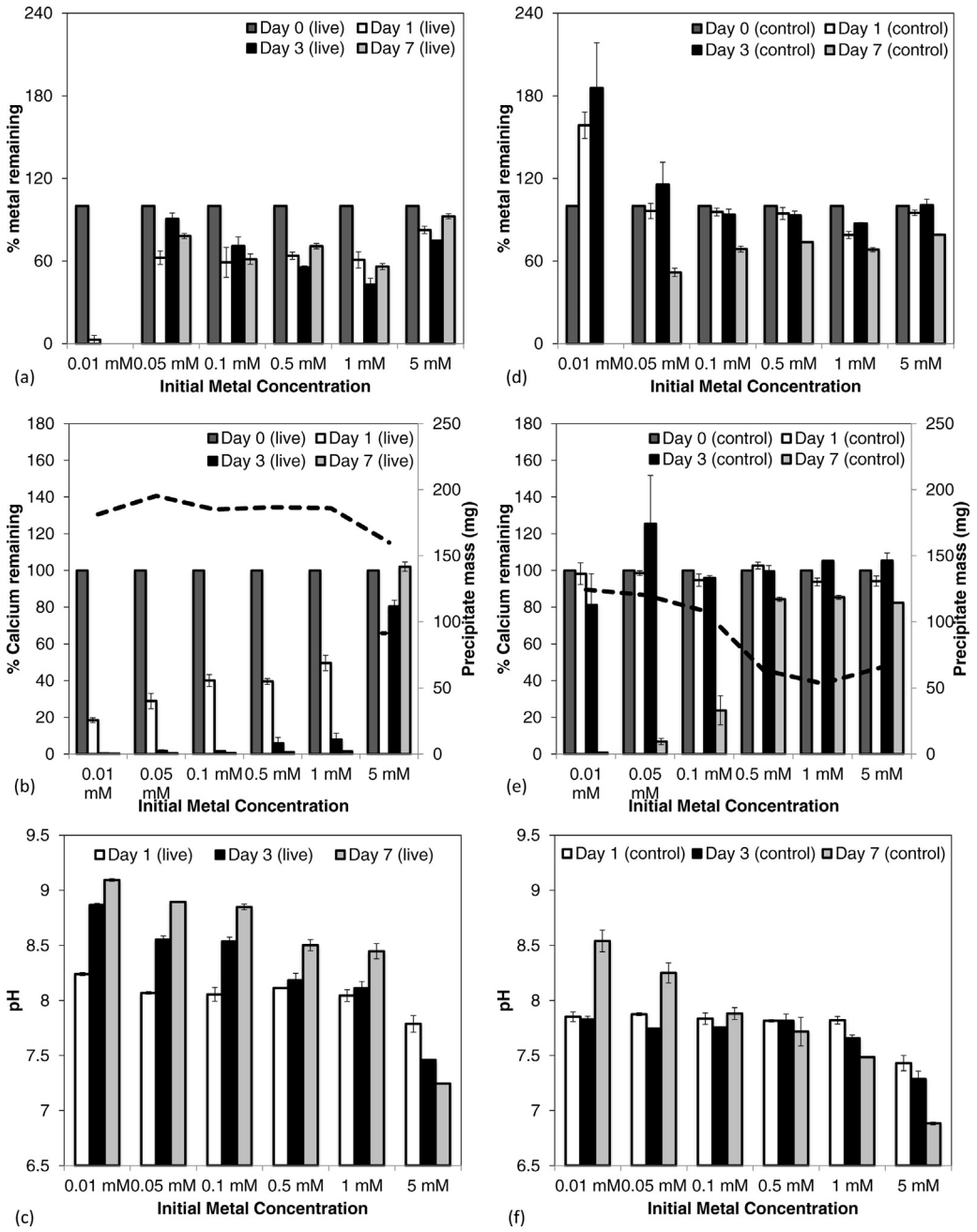

Fig. 9. Impact of *S. pasteurii* activity on copper removal (a, d), calcium removal (b, e) and pH (c, f) at a range of copper concentrations, with live (a–c) and killed (d–f) cells at 30 °C with urea growth medium. Dashed lines on (b) and (e) represent precipitate mass. [Error bars ± 1 SE].

reported here. Effects were noted surprisingly quickly, with pH increasing to 9 within 20 minutes (compared to 7 days here) and the majority of metal removal occurring within two hours (compared to within one day here). Removal of lead was, at 100%, higher than that of other metals, although it may be that an abiotic mechanism contributed similar to that seen here. Kang et al. [10] reported complete removal of cadmium under similar conditions.

The mechanism of removal here is likely to be a combination of a form of sorption or co-precipitation of the metal ions on or within calcium carbonate crystals. As noted by Tesoriero and Pankow [36], a range of divalent metal ions can form a solid solution with calcium carbonate. Results from basic Visual MINTEQ models of the experiments suggest that the heavy metals would be expected to be largely in soluble form at the end of each experiment if purely chemical equilibrium were considered. The removal process is therefore thought to be microbially driven through biomineralisation of calcium carbonate, leading either to co-precipitation as heavy metal ions associate with a growing calcium carbonate lattice or through sorption to calcium carbonate surfaces, possibly followed by encapsulation through continued mineralisation. In the latter case, the continued generation of calcium carbonate precipitate would continue to provide new surface area for sorption, preventing formation of a coherent epitaxial layer and increasing the potential for removal from solution. Unlike many previous studies (*e.g.* [27]) where interaction of heavy metals and calcium carbonate surfaces were explored under equilibrium conditions, the dynamic conditions experienced here with prolonged removal of metals alongside precipitate generation are more similar to conditions found by Schosseler et al. [34], where dissolution

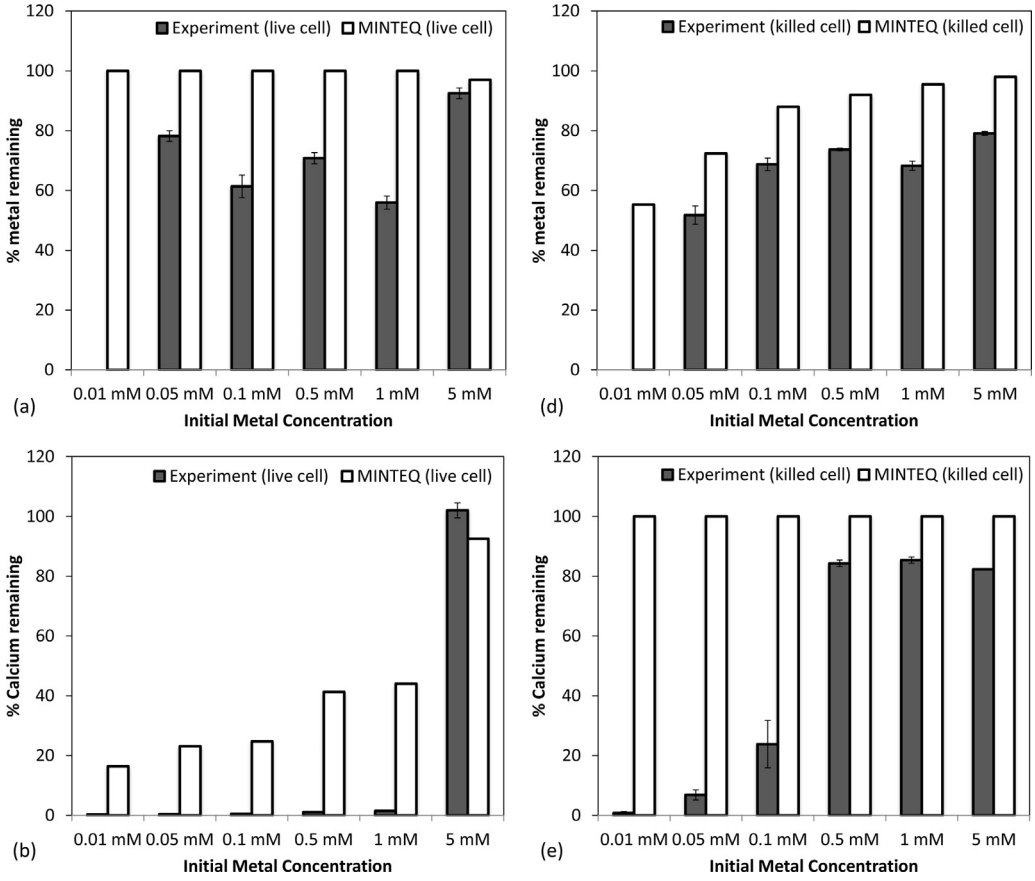

Fig. 10. Comparison of actual and predicted (Visual MINTEQ) dissolved copper and calcium concentrations after 7 days for live cell (A, B) and control (C, D) experiments. [Error bars ± 1 SE].

and recrystallization allowed sequestration of metals in solid solution within calcium carbonate.

5. Conclusions

Heavy metal sequestration in bioprecipitated calcium carbonate deposits has been demonstrated for a range of metals using a urea hydrolysis mechanism. This simple system performed well with zinc and cadmium, with calcium carbonate formation correlating well with metal removal. In the case of lead, however, an abiotic mechanism may have caused the majority of removal and so the evidence for enhancement of lead remediation is less strong. Copper removal was limited, even when calcium carbonate generation was extensive, indicating a lack of interaction between copper and the mineral. The structure of calcium carbonate minerals is known to permit the sequestration of particular metals (*e.g.* cadmium) well, whilst other metals (such as copper) are less strongly linked, explaining these observations. In all cases, microbial activity was observed at higher metal concentrations when the urea hydrolysis mechanism was present, indicating that this particular bioremediation method may operate at higher concentrations than expected.

Acknowledgements

The authors thank the Iraqi Ministry of Higher Education and Scientific Research for funding the studentship of the first author.

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