Biotechnology Letters (2005) 27: 1649–1655
DOI 10.1007/s10529-005-2722-9

A new method for mercury removal

Ashraf M.M. Essa, Lynne E. Macaskie* & Nigel L. Brown
School of Biosciences, The University of Birmingham, Edgbaston, B15 2TT, Birmingham, UK
**Author for correspondence (Fax: +44-121-414-5925; E-mail: L.E.Macaskie@bham.ac.uk)*

Received 10 March 2005; Revisions requested 8 April 2005; Revisions received 15 August 2005; Accepted 22 August 2005

Key words: Klebsiella pneumoniae, mercury organosulphide, mercury removal

Abstract

A method is described for the removal of mercury from solution by using the off-gas produced from aerobic cultures of *Klebsiella pneumoniae* M426. Cells growing in Hg-supplemented medium produced a black precipitate containing mercury and sulphur. The ratio of Hg:S was determined as ~1:1 by analysis using proton-induced X-ray emission, suggesting precipitation of HgS within the culture. The outlet gases produced by a mercury-unsupplemented aerated culture were bubbled into an external chamber supplemented with up to 10 mg $HgCl_2$/ml. A yellowish-white precipitate formed, corresponding to 99% removal of the mercury from solution within 120 min. Energy dispersive X-ray microanalysis showed that this metal precipitate consisted of mercury, carbon and sulphur. Formation of mercury carbonate was discounted since similar precipitation occurred at pH 2 and no oxygen was detected in the solid, which gave an X-ray powder pattern suggesting an amorphous material, with no evidence of HgS. Precipitation of mercury with a volatile organosulphur compound is suggested. Bio-precipitation of heavy metals by using culture off-gas is a useful approach because it can be used with concentrated or physiologically incompatible solutions. Since the metal precipitate is kept separate from the bacterial biomass, it can be managed independently.

Introduction

The discharge of heavy metals into the environment due to agricultural, industrial and military operations and the effect of this pollution on the ecosystem and human health are growing concerns. Some heavy metals serve no biologically relevant function and cause damage due to their avidity for the sulphydryl groups of proteins (Leach 1960). In the case of mercury, the metal and organomercurials can pass through biological membranes (Gutknecht 1981), and organic mercury compounds, e.g. methylmercury, produce irreversible damage to nucleic acids (Sletten & Nerdal 1997).

Different procedures for the removal of toxic metal species from contaminated environments have been developed; most are based on ion-exchange technologies and/or chemical precipitation of cations in an inert form. Ion exchange methods are usually expensive, require additional products for desorption of metals for regeneration of the inorganic matrix and lead to the formation of concentrated secondary wastes. Remediation technologies using micro-organisms are feasible alternatives to the concentration of metals in polluted waters by physical or chemical means (Gadd 1992 & 1993, Lloyd 2003, Lloyd *et al.* 2005). For example sulphate-reducing bacteria have been used to precipitate metals as metal sulphides (Lloyd *et al.* 2005) but this requires control of emission of toxic excess H_2S generated by use of sulphate as the electron acceptor in anaerobic respiration.

As an alternative chemical process, S-based precipitation via organosulphur compounds has

been considered. These are too expensive for routine use but are highly effective (Mühlbacher 1994). For example, the organic precipitant TMT (trimercaptotriacin: trimercapto-*S*-triazine-trisodium) is non-toxic, has no offensive smell and forms compounds of low solubility with divalent metal ions. The Hg-TMT compound is very stable and can form a precipitate in the presence of complexing agents which bind mercury and prevent formation of HgS with Na_2S (Mühlbacher 1994). Hartinger (1991) describes examples of use of organosulphur compounds for metal precipitation. One striking example highlights the use of TMT for removal of 3600 mg mercury/l from a wood preservative waste to a final concentration of 0.02 mg/l (Mühlbacher 1994).

The use of microbially produced organosulphur compounds in the context of bioremediation is little-explored. A previous study (Glendinning *et al*. 2005) described the formation of HgS by a strain of *Bacillus* that did not produce volatile H_2S gas but the thiol precursors were not identified. The formation of methanethiol from methionine by cell extracts of *Lactococcus lactis* subsp. *cremoris* B78 was proposed by Engels *et al*. (2000). The first step is a transamination yielding 4-methylthio-2-ketobutyric acid which probably, after decarboxylation, is converted to methanethiol which, under aerobic conditions, is converted rapidly to dimethyldisulfide and/or dimethyltrisulphide.

Formation of insoluble metal sulphides may be an alternative mercury tolerance mechanism to the well-described mercuric reductase system (Hobman & Brown 1997, Barkay *et al*. 2003, Glendinning *et al*. 2005), and provides the possibility to achieve better metal removal than by using biogenic H_2S alone (see above). With the aim of obtaining bacteria which are metal tolerant by alternative mechanisms, this study focused on mercury-resistant enterobacterial strains isolated in the pre-antibiotic era. The ubiquitous *mer* genes are usually carried on plasmids which may encode for antibiotic resistance (Hobman & Brown 1997) and therefore could be less common before widespread antibiotic usage introduced a strong selection.

In this study we describe a novel approach for the removal of mercury by using the volatile sulphur-containing gases produced during the aerobic culture of *Klebsiella pneumoniae* M426.

Materials and methods

Bacterial strains and bioreactor operation

Klebsiella pneumoniae M426, *Morganella morganii* M567 and *Escherichia coli* M634 were originally obtained from the National Collection of Type Cultures, Colindale, London, UK. Cultures were routinely grown aerobically in Luria Broth medium [LB; agitation at 200 rpm; 37 °C (Essa *et al*. 2003)]. The effect of different $HgCl_2$ concentrations on the growth of the mercury resistant strains was studied in liquid culture. The bacterial strains were grown in LB medium supplemented with $HgCl_2$ (up to 100 μg/ml) and incubated at 37 °C for 48 h with bacterial growth monitored as OD_{600}. For metal precipitation tests a batch bioreactor was constructed (Figure 1) comprising two chambers; one was used for bacterial growth ($\sim$1 litre, maintained under aerobic conditions by pumping in filtered compressed air; 37 °C), and the other chamber (50 ml) was used for metal precipitation by passing the culture exit gases through metal solution (aq.) via a 0.2 μm filter to prevent bacterial contamination. Cultures were inoculated (100 ml) using cells in the mid-exponential growth phase (6 h) and bacterial growth was monitored by measuring the optical density at 600 nm.

Electron microscopy and analysis of the precipitate deposited in cultures grown with Hg^{2+}

Electron microscopy was carried out as described by Badar *et al*. (2000), using osmium-stained sections for transmission electron microscopy and parallel, non-osmium-treated cells for analysis using energy dispersive X-ray microanalysis (EDX). Proton induced X-ray emission analysis (PIXE) was carried out under vacuum, using the Oxford Scanning Proton Microprobe (Department of Nuclear Physics, University of Oxford, UK). Dried samples (a few mg) were washed with acetone three times. The samples were air-dried, ground, suspended in a few drops of distilled water and transferred to a 'pioloform' film spanning a hole in an aluminium target (custom-made in the Department of Nuclear Physics, University of Oxford). The samples were examined in a high energy proton beam (PIXE: Johansson & Campbell 1988) as described

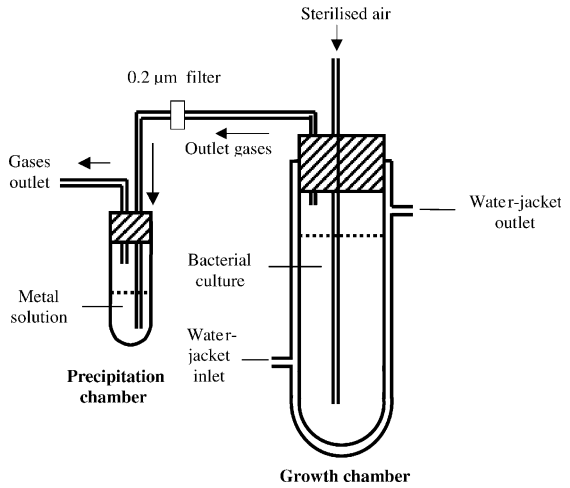

Fig. 1. Bioreactor used for metal precipitation via the culture off-gas consisting of two chambers. The large chamber is the bacterial growth chamber with forced aeration via a filtered compressed air supply. The small second chamber contains a solution of $HgCl_2$ (aq). Contamination by bacteria is prevented by the inclusion of a 0.2 μm filter (see text).

previously (Bonthrone *et al.* 1996). Matrix major element composition and thickness, which are needed to calculate PIXE corrections, were determined using simultaneous Rutherford back-scattering (RBS) analysis to enable calculation of the elemental concentrations of mercury and sulphur per unit volume of sample (Tamana *et al.* 1994).

Mercury volatilisation assay with growing cells

LB medium (100 ml) was inoculated with a single colony and shaken overnight (37 °C), diluted with fresh medium and grown to an OD_{600} of 0.4–0.5. $HgCl_2$ was added (1 h), the OD_{600} was adjusted to 0.4 with fresh medium and samples (4 ml) were harvested at 1500 *g* (10 min). The supernatant was filtered (0.2 μm filters; Dynagard) and retained and the cells were washed and resuspended in fresh LB medium (4 ml) and divided into 1 ml aliquots into 50 ml acid (2 M HNO_3)-washed flasks (37 °C, with agitation); each was diluted with 4 ml pre-warmed LB supplemented with 1.25 μM $HgCl_2$ and diluted (to 1:2000 dilution of stock solution) with $^{203}HgCl_2$. Timed samples were withdrawn into 5 ml of scintillation cocktail [OptiPhase Hisafe: dioctyl sulfosuccinate, sodium salt; 2-(2-butoxyethoxy)ethanol; poly(ethyleneglycol); mono(4-nonylphenyl-ether)] and counted in a Packard TRI-CARB 2700 Tr liquid Scinitillation Analyser with the window set at 0.2–22 MeV. Experiments were done in duplicate; representative data are shown.

Chemical analysis of residual soluble mercury in metal precipitation tests

Mercury was prepared as an aqueous solution in the external precipitation chamber (Figure 1: $HgCl_2$; 0.02–10 mg/ml). The metal precipitate was removed by centrifugation (1600 *g*; 15 min). Aliquots (10 ml) of the supernatant were filtered (0.2 μm Nalgene Syringe filters) and analysed using a 1999 Duo HR Iris advanced inductive coupled plasma (ICP) spectrometer (Matlock *et al.* 2001). Mercuric ion concentrations in the solutions were also measured with a mercury analyser (Buck Scientific, model 400A) applying cold vapour atomic absorption spectrometry (Chang *et al.* 1993).

Solid state analysis

Samples from the precipitation chamber were centrifuged as above. The supernatant was removed and precipitates were washed in 20 ml deionised water followed by centrifugation as before (3 times). The precipitate was collected and dried at 30 °C. The metal precipitates were examined with a JEOL JSM 5900 scanning electron microscope with the composition determined by EDX using an Oxford Link ISIS System. X-ray powder diffraction (XRD) analysis was done on duplicate samples in parallel. Precipitates were washed and dried as above and the material (a few mg) was examined by X-ray powder diffraction in a SK-2-12 tube resistance furnace under a flow of nitrogen (100 ml/min). X-ray powder diffraction patterns were obtained on a Dmax Rigaku diffractometer using monochromatic high-intensity CuKα radiation ($\lambda = 0.15418$ nm).

Results

Mercury tolerance and volatilisation

The effect of $HgCl_2$ on the growth of the mercury resistant strains was studied by growth in medium supplemented with $HgCl_2$ up to

100 μg/ml (as Hg). Examination of growth after 48 h showed that *K. pneumoniae* M426 grew with up to 100 μg $HgCl_2$/ml; the other strains grew negligibly when $HgCl_2$ was above 70 μg/ml (Figure 2). Despite having been archived in the pre-antibiotics era, all of the strains carried *mer* genes (Essa *et al*. 2003). Accordingly, all of the strains volatilised mercury but *K. pneumoniae* volatilised the least (Figure 3) despite its higher tolerance to Hg^{2+} (Figure 2). An additional Hg-tolerance mechanism was therefore implicated in this organism.

Precipitation of mercury as HgS by growing cultures

During aerobic growth of *K. pneumoniae* M426 with Hg^{2+} a black precipitate formed in the extracellular matrix (Figure 4a). EDX analysis showed that the precipitate contained mercury and sulphur (Figure 4b) but it was not possible to calculate the elemental ratio due to overlapping of the peaks for the Hg and sulphur X-ray emission energies. Complementary analysis using PIXE gave an elemental ratio of Hg:S of 1.28 ± 0.21:1. It was therefore concluded that *K. pneumoniae* M426 produces hydrogen sulphide (H_2S) under aerobic conditions to precipitate Hg^{2+} as insoluble black HgS (Essa *et al*. 2002, 2003). This mechanism was recognised previously by Aiking *et al*. (1985), who described the aerobic formation of CdS by *Klebsiella aerogenes* when grown in continuous aerobic culture in the presence of $CdCl_2$ and by Wang *et al* (2002), who showed the ability of *Pseudomonas aeruginosa* to remove more than 99% of the cadmium from 5 mM cadmium solution by precipitation on the cell wall as cadmium sulphide aerobically. Aerobic precipitation of HgS was also observed using a *Bacillus* sp. (Glendinning *et al*. 2005).

Mercury precipitation using culture off-gases

A major limitation of bioremediation is the need to work within physiologically permissive conditions and often the toxicity of co-contaminants is problematic even if the organism carries a resistance to the primary target species. The potential for using the culture off-gas in a separate precipitation chamber was therefore explored using the bacteria in the primary growth chamber as a gas generator with the off-gases carried in the exit air stream into a mercury solution in the precipitation chamber (Figure 1).

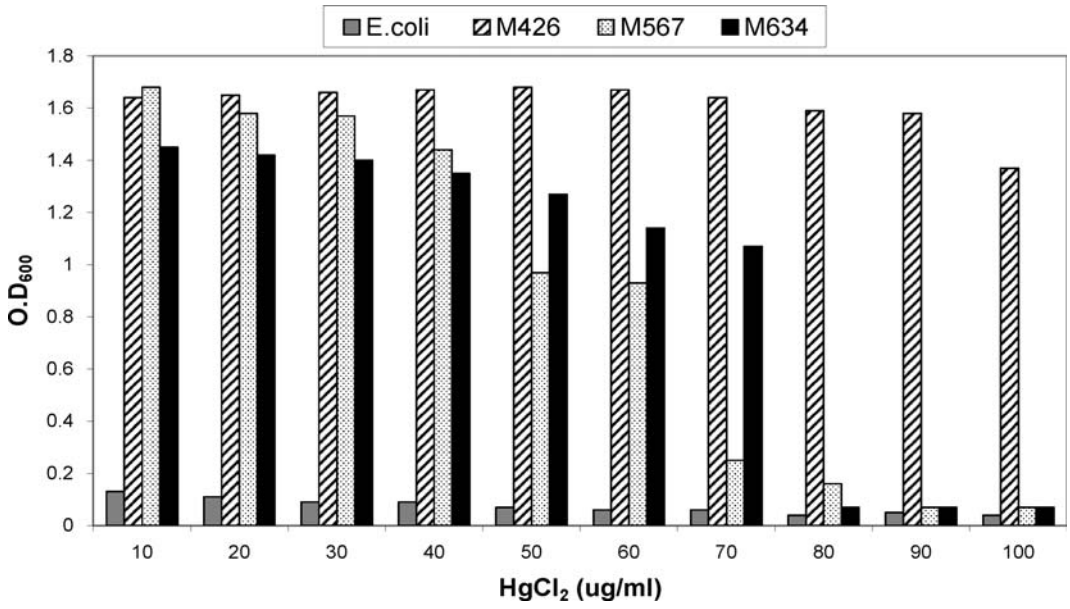

Fig. 2. Effect of $HgCl_2$ on the growth of mercury resistant strains: *E. coli* M634 (solid black), *K. pneumoniae* M426 (crossed hatched) and *M. morganii* M567 (dotted) compared to a mercury sensitive control strain *E. coli* TG2 (solid grey). Cultures were grown in the presence of $HgCl_2$ (μg/ml as Hg) and the OD_{600} was determined after 48 h.

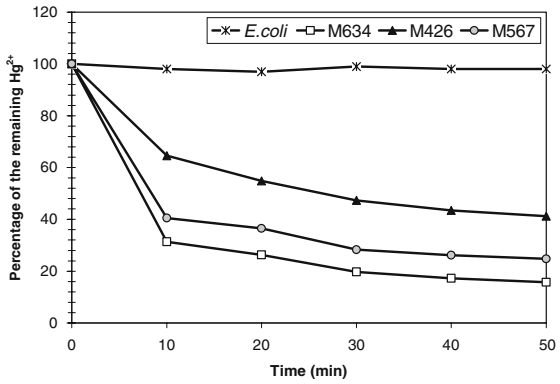

Fig. 3. Mercury volatilisation by the three mercury resistant strains; *E. coli* M634 (□), *K. pneumoniae* M426 (▲), and *M. morganii* M567 (○) compared to a mercury sensitive strain *E. coli* TG2 (✳).

A black precipitate of HgS was anticipated, but more than 99% of the soluble Hg^{2+} by assay was removed (Figure 5) and was recovered in the precipitation chamber as a yellowish white precipitate within 2 h. EDX analysis (Figure 5, inset) showed that the precipitate comprised mercury, sulphur and also carbon but with no detectable phosphorus, confirming the lack of contamination by biomass. The EDX spectrum cannot distinguish between Hg and sulphur (see above) but tests using Cd^{2+} (which was removed comparably to Hg^{2+} in the precipitation chamber) gave clearly separated Cd and S peaks (and also carbon; not shown) and a white precipitate instead of the bright yellow coloration diagnostic of CdS. It could be suggested that metal carbonate was precipitated via CO_2 in the culture off-gas, but similar results were obtained at pH 2, at which pH little bicarbonate would be present in the solution and no carbonate (Mulligan *et al.* 2001), and the EDX spectrum showed no emission peak at 0.5249 keV (Figure 5, inset) corresponding to the oxygen Kα X-ray emission energy (cf. Figure 4b). It was concluded that the bacterial off-gas contained a volatile organic sulphur compound, which precipitated with Hg^{2+}. The absence of a black/grey coloration in the precipitate mitigates against HgS precipitation, while the X-ray powder pattern shows few distinguishing features and none characteristic of HgS (a reference of HgS and a corresponding pattern of biogenic HgS produced by *Bacillus* sp. was shown by Glendinning *et al.* (2005)). Formation of an amorphous organosulphur material is therefore suggested. Full analysis of the culture off-gas was not attempted but GC mass spectral analysis showed the presence of dimethyl disulfide as a distinct component (Essa 2004).

Discussion

A previous study (Glendinning *et al.* 2005) showed that although biogenic HgS was depos-

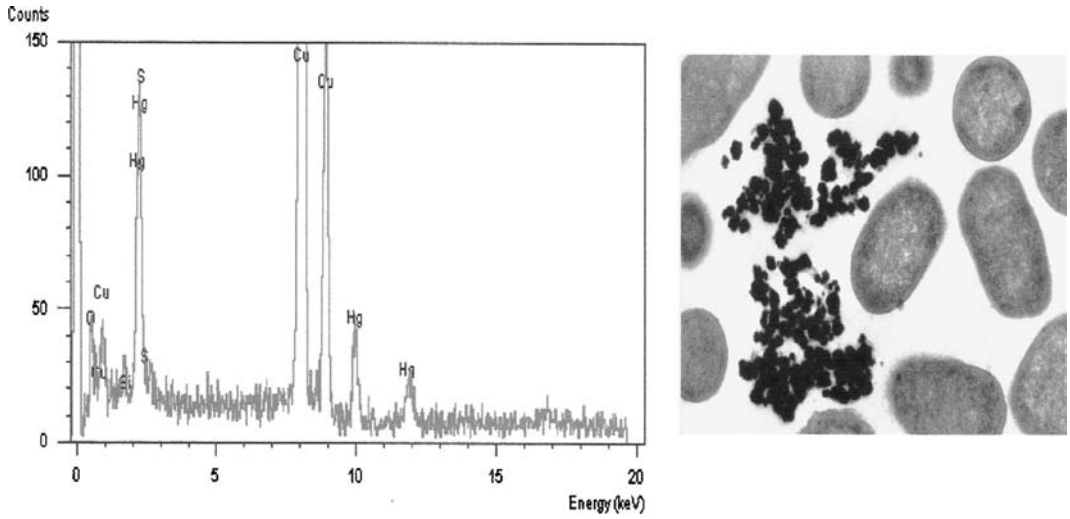

Fig. 4. Metal precipitate produced by *K. pneumoniae* M426 growing in the presence of $HgCl_2$. The extracellular precipitate (right panel) was analysed by EDX (left panel). Copper peaks are from the copper grid. Bar is 0.5 μm.

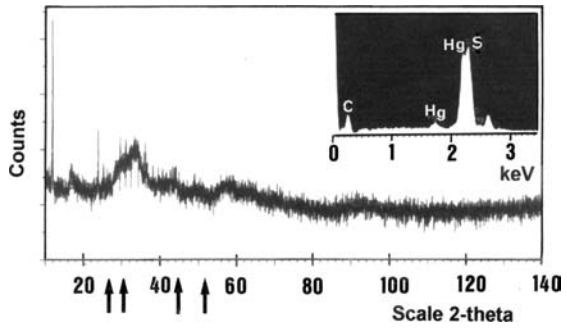

Fig. 5. Characterisation of precipitate collected in the precipitation chamber after passage of culture off-gas through a solution of $HgCl_2$ (see text and Figure 1). Inset: EDX analysis of the precipitate. Main panel: X-ray powder pattern. Positions indicated by arrows are the reference peak positions for the powder pattern of HgS (Glendinning *et al.* 2005).

ited by a *Bacillus* sp. There was no evidence for the production of gaseous H_2S by this strain, suggesting an origin in a biogenic organothiol. In the present case, although some volatile H_2S was detected using a lead acetate test strip (not shown) and HgS was identified in cultures growing in the presence of mercury, its precipitation via off-gas from mercury-unsupplemented culture was predominantly at the expense of a volatile organosulphur compound, possibly dimethyldisulphide (DMDS) (Essa 2004).

There have been previous attempts to engineer aerobic sulphate or thio-sulphate reducing systems for metal removal (Bang *et al.* 2000, Wang *et al.* 2002) but there is little, if any, focus on the production of organosulphur metal precipitants, despite their high potential (see Introduction). This study describes a novel approach, not only for mercury bioremediation, but also for some other heavy metals (Essa 2004) and has certain advantages. First, since culture off-gas is used, it is applicable for concentrated or non-physiologically compatible solutions under a wide range of factors such as pH and salinity (Essa 2004). Second, with this bio-process one organism, *K. pneumoniae*, was used to precipitate different metals from their solutions and could be also used with mixed metal solution, and with some selectivity (Essa 2004). Importantly, during metal precipitation there is no direct contact between the bacterial biomass and the metal precipitate, thus keeping the biomass separate from the contaminated waste water and ensuring that there is no cross-contamination between the biomass and the final metal precipitate. Several sequential precipitation tests were carried out using one batch culture and gas production could be prolonged by a fed-batch approach (A.M.M. Essa, unpublished).

The use of *K. pneumoniae* commercially would be unattractive due to its potential pathogenity. DMDS has been reported to be produced by other organisms, e.g. *Streptomyces* sp. (Segal & Starkey 1969), *Aspergillus* sp. (Ruiz-Herrera & Starkey 1969), *Clostridium sporogenes* (Kreis & Hession 1973), *Pseudomonas putida* (Ito *et al.* 1976), *Proteus* sp. (Hayward *et al.* 1977) and *Pseudomonas fluorescens, Proteus vulgaris*, and *Serratia marcescens* (Pohl *et al.* 1984), isolated from soil, wastewater, and fish, while other bacteria isolated from activated sludge (Tomita *et al.* 1981) e.g. *Lactobacillus* sp., *Corynebacterium* sp. and *Alcaligenes* sp. showed an ability to produce DMDS from the sludge. The use of more innocuous strains will be explored in future studies.

Acknowledgements

This work was supported by the Government of Egypt (support to AMME) and a Royal Society Industrial Fellowship to L.E.M. Thanks are due to Dr J. Dowling (School of Physics) for the XRD analysis, to Mr H. Pflicke for translation from original German into English, and to Drs J.L. Hobman and C. Leang for discussion and advice in setting up assay systems.

References

Aiking H, Govers H, van 't Riet J. (1985) Detoxification of mercury, cadmium and lead in *Klebsiella aerogenes* NCTC 418 growing in continuous culture. *Appl. Environ. Microbiol.* **50**: 1262–1267.

Badar U, Ahmed N, Beswick AJ, Pattanapipitpaisal P, Macaskie L.E. (2000) Reduction of chromate by microorganisms isolated from metal contaminated sites of Karachi, Pakistan. *Biotechnol. Lett.* **22**: 829–836.

Bang SW, Clark DS, Keasling JD (2000) Engineering hydrogen sulphide production and cadmium removal by expression of the thiosulfate reductase gene (*phsABC*) from *Salmonella enterica* serovar *typhimurium* in *Escherichia coli*. *Appl. Environ. Microbiol.* **66**: 3939–3944.

Barkay T, Miller SM, Summers AO (2003) Bacterial mercury resistance from atoms to ecosystems. *FEMS Microbiol. Rev.* **27**: 355–384.

Bonthrone KM, Basnakova G, Lin F, Macaskie LE (1996) Bioaccumulation of nickel by intercalation into polycrystalline hydrogen uranyl phosphate deposited via an enzymatic mechanism. *Nature Biotechnol.* **14**: 635–638.

Chang JS, Hong J, Ogunseitan QA, Olson BH (1993) Interaction of mercuric ions with the bacterial growth medium and its effects on enzymatic reduction of mercury. *Biotechnol. Prog.* **9**: 526–532.

Engels WJM, Alting AC, Arntz MMTG, Gruppen H, Voragen AGJ, Smit G, Visser S ((2000)) Partial purification and characterization of two aminotransferases from *Lactococcus lactis* subsp. cremoris B78 involved in the catabolism of methionine and branched chain amino acids. *Int. Dairy J* **10**: 443–452.

Essa AMM (2004) Mercury resistance in enterobacteria; characterisation of determinants and a new bioprocess for mercury bioremediation. PhD Thesis, The University of Birmingham, UK.

Essa AMM, Macaskie LE, Brown NL (2002) Mechanisms of mercury bioremediation. *Biochem. Soc. Trans.* **30**: 672–674.

Essa AMM, Julian DJ, Kidd SP, Brown NL, Hobman JL (2003) Mercury resistance determinants related to Tn*21*, Tn *1696* and Tn *5053* in enterobacteria from the pre-antibiotic era. *Antimicrob. Agents Chemother.* **47**: 1115–1119.

Gadd GM (1992) Microbial control of heavy metal pollution. Soc. Gen. Microbiol.Symp. No 48 Eds Fry JC, Gadd GM, Herbert RA, Jones CW, Watson-Craik IA Cambridge University Press, Cambridge, UK pp. 59–88.

Gadd GM (1993) Microbial formation and transformation of organometallic and organometalloid compounds. *FEMS Microbiol. Rev.* **11**: 297–316.

Glendinning KJ Macaskie LE, Brown NL (2005) Mercury tolerance of thermophilic Bacillus sp. and Ureibacillus sp. Biotechnol. Lett. (following paper).

Gutknecht J (1981) Inorganic mercury (Hg^{2+}) transport through lipid bilayer membranes. *J. Membr. Biol.* **61**: 61–66.

Hartinger H (1991) *Handbach der Abwässer und Recyclingtechnik für die Metallverarbeiterde Industrie*, München: Carl Hanser Verlag.

Hayward NJ, Jeavons TH, Nicholson AJC, Thornton AG (1977) Methyl mercaptan and dimethyldisulfide production from methionine by *Proteus* species detected by headspace gas liquid chromatography. *J. Clin. Microbiol.* **6**: 187–194.

Hobman JL, Brown NL (1997) Bacterial mercury resistance genes. *Met. Ions Biol. Syst.* **34**: 527–568.

Ito S, Nakamura T, EguchiY (1976) Purification and characterization of methioninase from *Pseudomonas putida*. *J. Biochem.* **79**: 1263–1272.

Johansson SAE, Campbell JL (1988) *PIXE: A Novel Technique for Elemental Analysis*, Chichester, UK: Wiley.

Kreis W, Hession C (1973) Isolation and purifcation of L-methionine-α-deamino-γ-mercaptomethane-lyase (L-methioninase) from *Clostridium sporogenes*. *Cancer Res.* **33**: 1862–1865.

Leach SJ (1960) The reaction of thiol and disulphide groups with mercuric chloride and mercuric iodide. *J. Aust. Chem. Soc.* **13**: 520–528.

Lloyd JR (2003) Microbial reduction of metals and radionuclides. *FEMS Microbiol. Rev.* **27**: 411–425.

Lloyd JR, Anderson RT, Macaskie LE (2005) In Bioremediation of Metals and Radionclides In Bioremediation: Applied Microbial Solutions to Real-World Environmental Cleanup eds R. Atlas, J. Philp ASM Press, Washington (in press).

Matlock MM, Howerton BS, Atwood. DA (2001) Irreversible precipitation of mercury and lead. *J. Haz. Mats.* **84**: 73–82.

Mühlbacher R (1994) Abtrennung von Schwermetallen aus Abwässern. Diploma Thesis, Institut Für Thermische Verfahrenstechnik und Umwelttechnik, Technical University of Graz, Austria.

Mulligan CN, Yong RN, Gibbs BF (2001) Heavy metal removal from sediments by bacteria. *J. Haz. Mats.* **85**: 111–125.

Pohl M, Bock E, Rinken M, Avvdin M, Konig. WA (1984) Volatile sulfur compounds produced by methionine degrading bacteria and their relationship to concrete corrosion *Z. Naturforsch. Teil C.* **39**: 240–243.

Ruiz-Herrera J, Starkey RL (1969) Dissimilation of methionine by fungi. *J. Bacteriol.* **99**: 544–551.

Segal W, Starkey RL (1969) Microbial decomposition of methionine and identity of the resulting sulphur compounds. *J. Bacteriol.* **98**: 908–913.

Sletten E, Nerdal W (1997) Interaction of mercury with nucleic acids and their components. *Met. Ions Biol. Syst.* **34**: 479–501.

Tamana H, Criddle A, Grime GW, Vaughan D, Spratt J (1994) Trace elements in platinum group minerals studied using nuclear microsopy. *Nucl. Instr. Meth.* **89**: 213–218.

Tomita B, Hamamura N, Ose Y (1981) Studies on the control index of activated sludge. The mechanism on the formation of dimethyl disulfide in mixed liquor of activated sludge. *Eisei Kagaku.* **27**: 98–104.

Wang CL, Maratukulam PD, Lum AM, Clark DS, Keasling JD ((2002)) Metabolic engineering of an aerobic sulphate reduction pathway and its application to precipitation of cadmium on the cell surface. *Appl. Environ. Microbiol* **66**: 4497–4502.