



Today's wastes, tomorrow's materials for environmental protection

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Today's wastes, tomorrow's materials for environmental protection

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Abstract. Over the past 30 years the literature has burgeoned with bioremediation approaches to heavy metal removal from wastes. The price of base and precious metals has dramatically increased. With the resurgence of nuclear energy uranium has become a strategic resource. Other 'non-carbon energy' technologies are driven by the need to reduce CO₂ emissions. The 'New Biohydrometallurgy' we describe unites these drivers by the concept of conversion of wastes into new materials for environmental applications. The new materials, fashioned, bottom-up, into nanomaterials under biocontrol, can be termed 'Functional Bionanomaterials'. This new discipline, encompassing waste treatment along with nanocatalysis or other applications, can be summarized as 'Environmental Bionanotechnology'. Several case histories illustrate the scope and potential of this concept.

Introduction

Heavy metals become dispersed into the environment in many ways, e.g. in run-offs from disused mines, wastes from metal processing or from industrial processes (e.g. tanning) that use metallic components and from deliberately-applied chemical agents (e.g. wood preservatives). Man's mining of traditional energy sources (e.g. coal) can result in iron-rich wastes [1] while the nuclear industry has still to resolve the problem of long-term containment of radionuclide wastes and the environmental impact of nuclide migration in the geosphere. Man's lifestyle also impacts on the environment via automobiles; here the use of precious metal (PM)-containing catalytic converters for atmospheric protection has resulted in substantial losses of PMs into road dusts [2] and the car industry is a major global consumer of scarce precious metals [3]. Even base metals, previously considered uneconomic to recover, are rising in price such that the economics of metal recovery from wastes should be revisited. More than 20 years ago Brierley & Brierley [4] set an 'economic threshold' for solely metal waste remediation using biosorption/bioaccumulation of metals and this has been met or exceeded by only a few bioremediation systems.

Microorganisms accumulate metals via several mechanisms e.g. biosorption, sequestration or various enzymatically-driven mechanisms such as metal oxidation/reduction and precipitation via enzymatic ligand production. These can lead to the formation of biomineral deposits which have useful properties [5,6]. This paper presents various examples of applications of biomineralization

approaches to convert metal-containing wastes into new nanomaterials for environmental protection.

Case histories illustrating bioconversion of wastes into new materials

Case History 1. Bioremediation of uranium mine-water and nanofilter fabrication for nuclear waste remediation. A *Serratia* sp. originally isolated from a metal-contaminated site has an atypical phosphatase localized within the cellular exopolymeric matrix (Fig. 1A). When provided with a suitable organic phosphate source inorganic phosphate is cleaved enzymatically to deposit heavy metals as their phosphates [7]. Cells immobilized as a biofilm on a suitable porous support comprise a filter that was successfully applied to the removal of uranyl ion, $(\text{UO}_2)^{2+}$, from U-mine wastes [8]. The uranium is retained as cell-bound deposits of hydrogen uranyl phosphate, $\text{H}_2\text{UO}_4 \cdot n\text{H}_2\text{O}$ (HUP) and continuous HUP deposition leads to the accumulation of a biomineral-encrusted meshwork of interwoven cells, with stacked HUP crystals clearly visible (Fig. 1E).

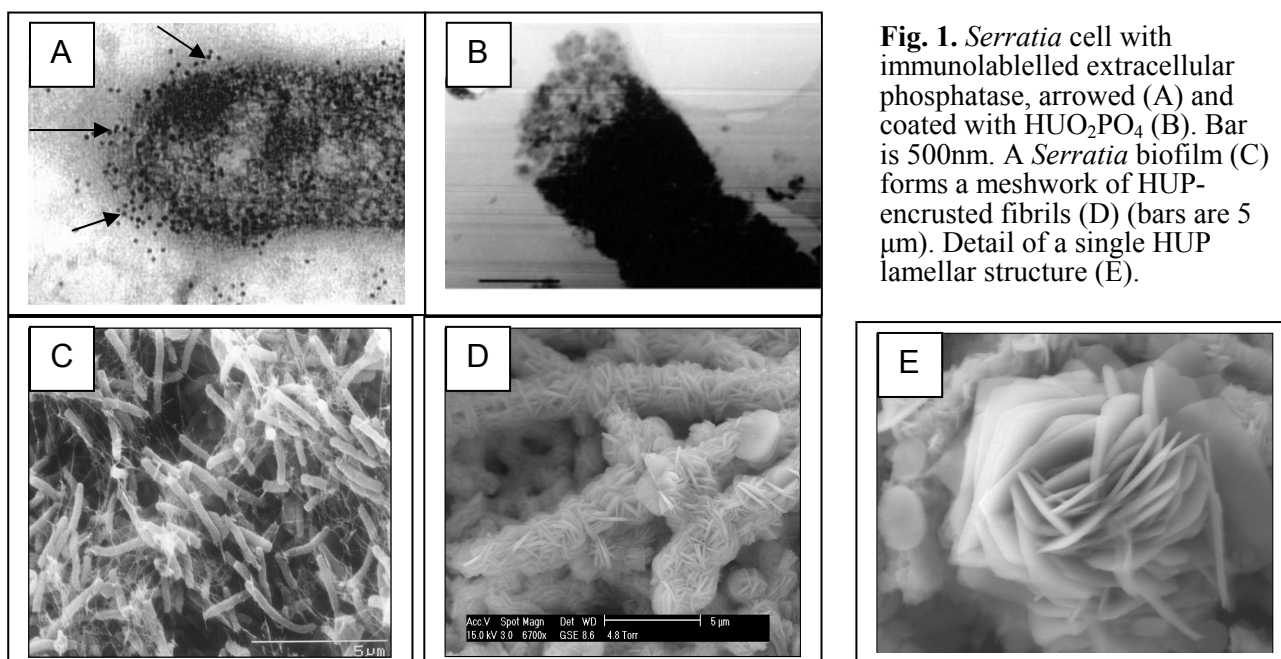


Fig. 1. *Serratia* cell with immunolabelled extracellular phosphatase, arrowed (A) and coated with H_2UO_4 (B). Bar is 500nm. A *Serratia* biofilm (C) forms a meshwork of HUP-encrusted fibrils (D) (bars are 5 μm). Detail of a single HUP lamellar structure (E).

A noteworthy feature of HUP is that it comprises stacks of lamellae with water molecules bridging adjacent HUP chains. These contain mobile protons that can exchange for metal cations like Ni^{2+} , removing the Ni^{2+} from solution into the HUP lattice via intercalative ion exchange [9]. This approach was adopted for the removal of radionuclides from contaminated waters [10].

Initial tests used Co^{2+} , Sr^{2+} and Cs^+ as 'surrogates' for the problematic fission products ^{60}Co , ^{90}Sr and ^{137}Cs . Bioreactors (11 ml) containing HUP (~ 107 (for Co, Sr) or ~ 61 (for Cs) mg of U) were challenged with flows (10 ml/h) of 1 mM Co^{2+} , Sr^{2+} or Cs^+ . Complete removal of the target metals into HUP was maintained for ~ 250 (Co, Sr) and ~ 350 (Cs) ml until column saturation. The capacity (bed volumes) under realistic flow concentrations at ~ 10^8 - 10^9 bed volumes, was more than 1000-fold better than current commercial materials, and the biomaterial was active at pH 3.5-9.3, whereas commercial materials tend to be pH-specific (e.g. for Sr pH > 10 and for Co pH 4-8). Finally the bio-HUP was tested against a real nuclear waste containing ^{137}Cs at 1.78 Bq/ml (0.26 pM) and ^{60}Co at 0.15 Bq/ml (0.71 pM), where the respective radionuclide removal by the bio-derived filter was 99% and 85%, respectively.

A previous study [11] identified that the limiting economic factor in the fabrication of the bio-HUP from U-wastewater was the cost of the organophosphate feed (glycerol 2-phosphate). Recent studies have shown that phytic acid (inositol phosphate), a natural plant product containing 6 mols phosphate/mol can support HUP deposition and subsequent removal of the surrogate nuclides into the phytic acid-derived HUP; the process then becomes economic [12].

A future possibility yet to be explored is use of the bio-HUP as a precursor for the fabrication of nuclear fuels; at a loading of about tenfold by mass of uranium to the biomass component routinely achieved and with no mineral processing costs the economics of fuel manufacture may be attractive.

Case history 2 Bioconversion of palladium and platinum-containing industrial and automotive catalysts wastes into catalysts for (a) treatment of environmental contaminants and (b) clean electricity generation. An early study [13] showed that bacteria can utilize hydrogenase enzymes for the reduction of soluble Pd(II) ions to generate cell-bound nanoparticles of Pd(0). A process was developed for the continuous removal of Pd as well as other precious metals (PMs) from spent automotive catalyst leachates [14]. This involved a continuous-flow electrobioreactor with hydrogen generated electrochemically and fed to the bacteria via a Pd-thimble electrode. The bacteria formed a biofilm on the electrode and Pd(0) was removed from leachates developed via *aqua regia*-leaching of ground spent automotive catalysts [14]. The recovered PMs sank to the bottom for recovery under gravity. Another approach involved hydrogen gas bubbled from below into a reactor for PM recovery from waste printed circuit board scrap containing 33 ppm of Pd and 79 ppm of Au [15]. Other studies showed that ‘palladised’ bacteria were active in the reduction of Cr(VI) [16], the dehalogenation of chlorinated aromatic compounds [17] including the pesticide lindane [18] and the dehalogenation of tris (chloroisopropyl) phosphate (TCPP) [18], a flame retardant introduced as a substitute for polybrominated diphenyl ethers which is starting to accumulate in groundwaters [19]. Since bio-bound Pd is nanoparticulate the obvious question to address was whether Bio-Pd recovered from leachates has catalytic activity against potential environmental contaminants. Zero-valent iron has long been known to have potential in this respect but reports suggest that a Fe/Pd bimetallic is superior [20].

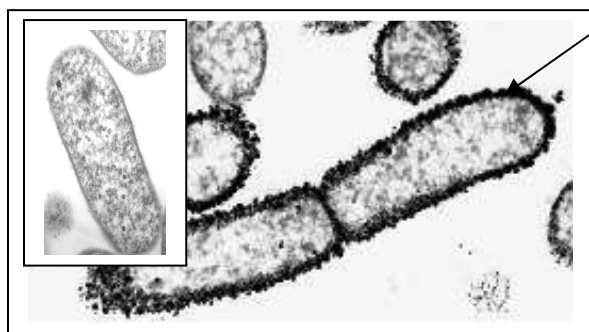


Fig. 2. Palladized cells of *Desulfovibrio desulfuricans*. A non-palladized cell is also shown (inset). Palladization involves pre-incubation with Pd(II) to saturate biosorption sites, followed by addition of H₂ to achieve reduction of Pd(II) to Pd(0) via hydrogen-ase [13]. Particle growth to the correct size is achieved via autocatalysis via the Pd nanoclusters; cellular viability is not required after the initial nucleation. The laboratory ‘workhorse’ *Escherichia coli* and other bacterial strains can be similarly used to make catalysts with similar catalytic activities.

An initial study showed catalytic activity (in the reduction of Cr(VI) to Cr(III)) of Bio-Pd made from an industrial processing waste [21], while subsequent work [22] showed activity using Bio-Pd made from a leachate from a secondary waste source.

Table 1. metal composition and catalytic activity of Bio-Pd made from precious metals (PM) bio recovered from industrial processing waste (A) and acid leachate from secondary waste (B)

A. From Degussa processing waste Waste pH 7 on <i>D. desulfuricans</i>		B. From spent automotive catalyst waste pH 2 on <i>E. coli</i>		Catalytic activity ^c		
Composition of metals on cells (%) ^a		Composition of metals on cells (%) ^b		A	B	
Pd	33%	Pd	28%	Pd metal	1.7	12.5
Pt	17%	Pt	4%	Bio-Pd(0)	2.7	31.0
Ag	6%	Rh	2%	Bio-PM	25	530
Al	42%	Al	37%	x-fold		
Mg	3%	Mg	12%	increase		
		Fe	14%	using		
				waste		
				source	x10	x17

^aMetals were analysed in the solid material by proton induced X-ray emission [21]. ^bMetals were estimated in the solid material by loss from the liquid leachate. ^cActivity is $\mu\text{mol Cr(III)}$ produced/h/mg Pd metal on biomass. 'Pd metal' is commercial Pd(0). 'Bio-Pd' is catalyst made from pure Pd(II) solution under the same conditions. Bio-PM is catalyst made from the liquid wastes.

For clean energy production a major focus is on the use of fuel cells (FCs) to produce clean electricity from hydrogen. The most efficient FCs use a precious metal anode and cathode catalyst, usually Pt, which splits H_2 into 2H^+ and $2e^-$, with the electrons forming the current and the protons being reunited with them at the cathode in air forming water as the only product. Recent studies have shown that precious metal scavenged onto biomass: Pt(0) on yeast [23] and Pd(0) on bacteria [24] have been fashioned into active FC catalysts. The PM mixture recovered from the industrial processing waste (see Table 1) also supported electricity production as the anode catalyst in a FC after further treatment [25]. By using waste biomass from another process [23] (and see below) the catalyst can be made inexpensively while overcoming problems of disposal of organic waste.

Table 2. Electricity production in a fuel cell with anodes made using Bio-Pd(0)s

Pd(0) source		power output _{max} (mWatt)
Commercial Pd (0)		99
<i>D.desulfuricans</i>	(pure Pd(II) solution)	140
<i>E.coli</i>	(pure Pd(II) solution)	114
<i>E. coli</i>	(waste PM)	66

Case History 3. Bioconversion of Au-containing jewellery waste into nano-Au catalyst for glycerol oxidation. Initial studies aimed at biorecovery of gold from printed circuit board scrap [15] revealed that, in addition to hydrogenase-mediated Au(III) reduction, another, undefined mechanism is involved. This is reflected in the different morphology of Bio-Au(0) made in the same way as for Bio-Pd(0) (Fig. 3). To date the secondary 'Au-reductase' is unidentified.

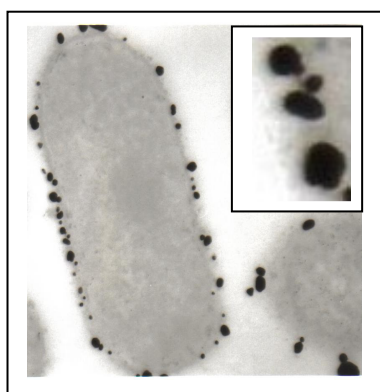


Fig. 3. Formation of Bio-Au(0) on *E. coli*. Similar Au-particles were seen on *D.desulfuricans*. Close inspection (insert) shows the Au-particles to be embedded in the cell surface layers and not extruded as well defined nanocrystals as with Pd(0) shown in Fig. 2.

Gold was recovered from jewellery waste [26]. The concentration of Au(III) in the leachate was ~ 115 ppm and after 100 min no residual soluble Au(III) was detected [26]. Au(0) is a potent oxidation catalyst, with the benefits of nanoscale Au recently becoming realized. Bio-Au functioned in the selective oxidation of glycerol to glycerate [27]; this is noteworthy since commercial glycerol oxidation catalysts (supported Pt and Pd) generally give poor selectivity for glycerate. The conversion by both pure Bio-Au(III) and that made from the jewellery waste was $\sim 30\%$ after 3 h [27].

The catalytic oxidation of glycerol is foreseen as having an impact in the energy sector. Low-grade glycerol is produced in large quantities from the manufacture of biodiesel (~ 1 tonne low grade glycerol/tonne of biodiesel) and it has little market value *per se*. Other studies have shown that organic acids can be separated from the mother liquor rapidly and selectively using anion-selective

electrodialysis. Organic acids provide an excellent feedstock for the photosynthetically-mediated generation of hydrogen by *Rhodobacter sphaeroides* (via side reaction of nitrogenase) [28]. The bio-H₂ is fuel-cell quality (i.e. it does not require clean-up) and, furthermore, the residual *R. sphaeroides* is active in the manufacture of catalytically-active Bio-Pd [29] or Bio-Au (unpublished).

The foregoing examples show real applications of biorecovered materials in the remediation of environmental contaminants or in the production of ‘non-carbon’ energy. Several other possibilities exist which have not yet been demonstrated in ‘real life’ but nevertheless provide good potential for future focus.

Case history 4. Bioconversion of selenium oxyanions into optically active chalcogenide materials. Selenium is found in waste materials from a broad spectrum of anthropogenic operations including mining, agricultural, petrochemical, electronics, and industrial manufacturing operations [30]. The developing field of nanotechnology includes many Se-based materials, so wastes of the future are also likely to be seleniferous. Bacterially-mediated processes in the environment result in Se transformations, including changes in valence or chemical form [31]. These processes can be used to develop stabilization strategies, in which toxic, bioavailable Se oxyanions are reduced to less available elemental selenium, Se(0), removing them from aqueous waste streams, e.g. drinking water, groundwater or wastewater. The products of these reductive transformations include a range of functional bionanominerals with interesting physical properties of potential technological significance [32-35]. For instance, these biogenic elemental Se nanospheres have photooptical and semiconducting physical properties, different from their chemically formed counterparts, for application in photocopiers, microelectronic circuits and solar cells [35]. Element Se is further reduced by some bacterial strains e.g. *Veillonella atypica*, to form reactive soluble selenide, which can be precipitated to produce nanoparticles such as the II-VI semiconductors ZnSe and CdSe, potentially stabilized by proteins present in the biogenic starting material. These materials can be fabricated into optoelectronic devices, light sensors and photovoltaics [33].

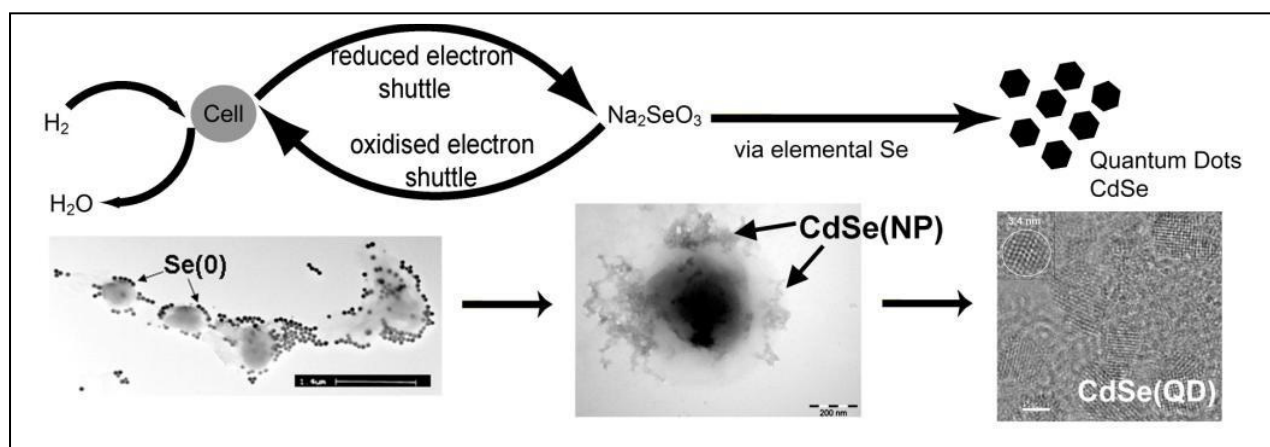


Fig. 4. Microbial synthesis of chalcogenide-based nanoparticles via the reduction of sodium selenite using the metal-reducing bacterium *Veillonella atypica*

Case History 5. Bioconversion of Fe(III)-oxide waste into a nanoscale magnetic material. Wastes containing iron are produced from various industrial activities including the polishing steps used by the water industries and also from mining activities. Under oxic conditions Fe(II) oxidises to Fe(III) to give a colloidal deposit of Fe(OH)₃ which settles poorly under gravity to give a high volume wet sludge which is difficult to handle and transport. The low recovery value of iron means that a high value chemical product is not usually a primary consideration. However bioconversion of the substrate from a poorly crystalline amorphous Fe(III) oxide, to a structured magnetic spinel

offers a potentially useful remediation strategy for areas impacted with Fe(III)-based industrial reagents and ‘one-pot conversion’ of the waste into a potentially useful product.

Fe(III)-reducing bacteria are a group of micro-organisms found in anoxic subsurface sediments that gain energy for growth by reducing metals such as Fe(III) and Mn(VI) coupled to the oxidation of organic matter, causing changes in the mineralogy of the sediments [36]. *Geobacter sulfurreducens* is a bacterium capable of respiring amorphous Fe(III) oxides, converting the iron minerals to nano-sized magnetic particles of magnetite (Fe₃O₄) (Fig. 5A), with a size of ~ 20 nm (Fig. 5B) [38]. Such particles have electrical, magnetic, and structural properties that differ from coarse grained materials.

Waste iron oxides can contain other important metals such as cobalt, nickel and manganese, which can be incorporated into the spinel structure changing the chemical formula to MxFe_{3-x}O₄, where M is a cation different from iron. This changes the magnetic properties of the particles, therefore it has been important to first study simple synthetic systems in order to understand the more complicated waste nano-magnets. Using *Geobacter sulfurreducens*, we have produced a variety of nanoparticles from iron oxides synthesised in the laboratory, containing just one or two of the above elements [39]. These experiments have confirmed that Fe(III)-reducing bacteria can produce doped nano-sized “designer” magnetic particles via biosynthetic routes, for a range of technological and medical applications. This type of reductive transformation also has potential applications for the recovery and re-use of waste Fe(III) oxides where the magnetic properties of the material do not have to be so carefully controlled. Obvious applications here include the formation of magnetically recoverable functional biominerals for Fe(II)-mediated reactions leading to the bioremediation of redox active organics, metals and radionuclides. To conclude, the method of production of these novel extracellular bionanomagnets is efficient, requires little energy, has no hazardous wastes and can be used to make tailored nano-magnets for a variety of applications.

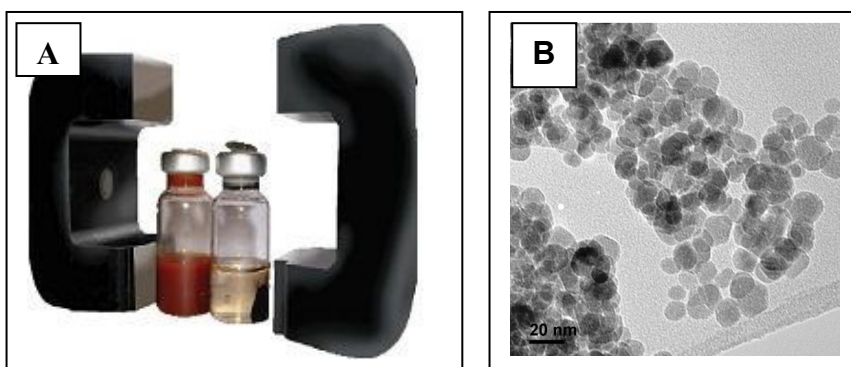


Fig. 5. (A) Conversion of Fe(III)-oxyhydroxide (orange vial) to nanoscale magnetite (black vial) using *G. sulfurreducens* (B) TEM image of nano-scale biogenic magnetite particles.

Acknowledgments

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