Nickel and platinum group metal nanoparticle production by Desulfovibrio alaskensis G20

New Biotechnology • Volume 32, Number 6 • December 2015 RESEARCH PAPER

Research Paper

Nickel and platinum group metal nanoparticle production by *Desulfovibrio alaskensis* G20

M.J. Capeness, M.C. Edmundson and L.E. Horsfall

School of Biological Sciences, University of Edinburgh, The King's Buildings, Mayfield Road, Edinburgh EH9 3JR, UK

***Desulfovibrio alaskensis* G20 is an anaerobic sulfate reducing bacteria. While *Desulfovibrio* species have previously been shown to reduce palladium and platinum to the zero-state, forming nanoparticles in the process; there have been no reports that *D. alaskensis* is able to form these nanoparticles. Metal nanoparticles have properties that make them ideal for use in many industrial and medical applications, such as their size and shape giving them higher catalytic activity than the bulk form of the same metal. Nanoparticles of the platinum group metals in particular are highly sought after for their catalytic ability and herein we report the formation of both palladium and platinum nanoparticles by *D. alaskensis* and the biotransformation of solvated nickel ions to nanoparticle form.**

Introduction

The six platinum group metals (PGMs) are ruthenium (Ru), rhodium (Rh), palladium (Pd), osmium (Os), iridium (Ir), and platinum (Pt). PGMs are in high demand and have low abundance, making them highly valuable; platinum, for example, is only found at 0.003–5 ppm in the earth's crust and is considered to be the second most expensive metal (after Rh) at €37.66/g on the London Platinum and Palladium Market.

Despite the high cost of platinum, very little is done to reduce waste or improve recycling in many of its applications. A major use of platinum is in the catalytic converters of vehicles to convert unburnt hydrocarbons and carbon monoxide into CO_2 and water, with each exhaust system containing 1–5 g of platinum. As the vehicle is used over time, platinum lost from the converter is deposited on the road network and then flushed away by rain, leading to contamination of the water table [1]. Palladium and rhodium are also used in some catalytic converters and, in a similar way; they are lost from converters and contaminate the water table.

Nickel is a significant contaminant of soil throughout the world and is often found in sites of Pt and Pd mining operations as a contaminant or co-product. Recently it has been identified as a major contaminant of UK stream sediments, reaching 4.9 g/kg in some locations (compared to the normal background level of 0.036 g/kg) [2]. However, while not as valuable a metal as the PGMs, nickel is still a useful material in its nanoparticle form. Nickel sulphide nanoparticles have unusual superparamagnetic properties and stability compared to elemental state NiNPs, and also exhibit exceptional electrochemical properties and the potential to be used in super capacitors [3,4], while nano-scale elemental nickel is currently employed as a catalyst in the production of hydrogen, as a capture material for PGMs in the refining process and also as potent anti-microbial and anti-cancer agents [5–7].

On the nano-scale, both the cost of PGMs and nickel increase dramatically (1 g of 3 nm Pt nanoparticles: €159) with the bulk of the cost in the process technology used to create the nanoparticles. Their properties include vastly different reactive characteristics compared to the bulk metal due to their increased surface area, different optical properties and new uses as catalysts [8]. Pt nanoparticles (PtNPs), for example, can be used in oxygen reduction reactions in fuel cells [9] or even as highly specific DNA probes [10] and anti-cancer agents [11]. These highly desirable properties are also present when nanoparticles are produced using a bacterial host. Therefore without a loss in quality, the bacterial production of nanoparticles (NPs) could potentially reduce manufacturing

Corresponding author: Horsfall, L.E. (Louise.Horsfall@ed.ac.uk)

http://dx.doi.org/10.1016/j.nbt.2015.02.002

costs whilst also lowering the costs of starting material, as the selective nature of biology allows nanoparticles to be synthesized from impure starting feeds [12].

Recent resource recovery efforts have included the collection of road dust and chemical remediation to harvest the PGMs from it. Like many other chemical forms of remediation, the cost of this process is high compared to the value of the resultant product and the methods used involve chemicals that are toxic, such as the use of hydrazine in the production of nickel NPs [13]. The use of biogenic methods of nanoparticle production addresses these problems; the cost of using metal-reducing bacteria to produce metal nanoparticles is relatively low, and all steps are carried out in keeping with a Green Chemistry approach, utilizing standard non-toxic aqueous solutions and growth media.

The use of bacteria to produce nanoparticles of various metals such as Au, Ag, Cu, Fe, Ti and Zr has previously been reported (reviewed by Edmundson *et al.* [12]); with *Desulfovibrio* sp. in particular having received a large amount of attention as they can reduce Cr, Mg, Fe, Te and even Ur to nanoparticle forms [14–18]. However, it is with the PGMs that *Desulfovibrio* sp. have been most studied, particularly *D. vulgaris* and *D. desulfuricans* that have been shown to be able to reduce Pt and Pd to zero-valent nanoparticles and display them on their outer surface. These nanoparticles can be observed as dark spheres when viewed by electron microscopy. This bio-Pd/Pt can be used directly in some applications without isolation from the cells, and has previously been shown to act as a nano-catalyst in proton exchange membranes to power fuel cells [19] or in Cr decontamination [20].

The reduction of PGMs in *Desulfovibrio* sp. is hypothesized to occur due to the bacteria incorporating the metals into their energy production pathways. The PGMs act as terminal electron acceptors in respiration, thus being reduced to the zero-state, often in the presence of hydrogen (Fig. 1) [21,22]. This occurs in the periplasm using hydrogenases/cytochromes, with the NPs being exported out of the cell and subsequently attaching to the cell surface, preventing the metal from re-entering the periplasm, and acting as a catalyst for further reduction of the metal [23]. This process may be of added benefit to the cell as PGMs have been observed to cause DNA lesions and inhibit growth in other bacteria such as *E. coli* and *Pseudomonas* sp. [24,25]; therefore the reduction and display of the metal on the cell surface could form part of a bacterial survival mechanism.

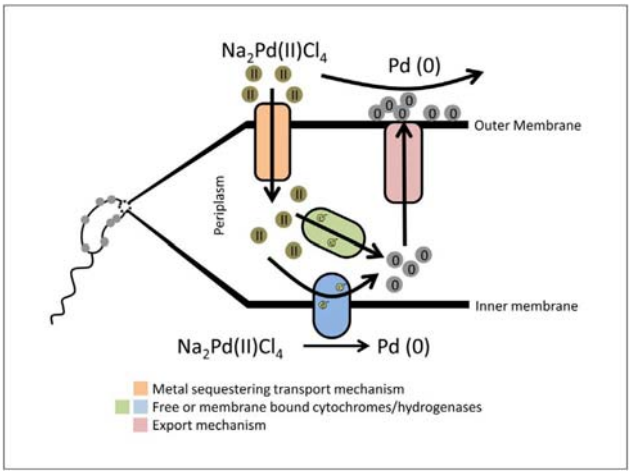

FIGURE 1

Proposed method of palladium reduction to nanoparticle form in the periplasm of *Desulfovibrio*. Pd^{2+} ions are taken up by the *Desulfovibrio* across the outer membrane to the periplasm where it is reduced by cytochromes and/or hydrogenases forming nanoparticles. The NPs are then exported to the outside of the cell where they are deposited on the cell surface and act as a catalytic site for increased palladium reduction. It is thought that both Pt also works in a similar way to the Pd, though the Ni does not bind to the cell surface.

Desulfovibrio alaskensis G20, formerly *D. desulfuricans* G20, is an anaerobic sulfate reducing bacterium with a publically available sequenced genome (GenBank CP000112). Herein we report the formation of palladium and platinum nanoparticles by *D. alaskensis* and the biotransformation of solvated nickel ions to nanoparticle form by both *D. desulfuricans* 8307 and *D. alaskensis* G20, with the efficiency quantified by the novel application of an established assay [26].

Materials and methods

Growth of Desulfovibrio *sp.*

Both the *D. alaskensis* G20 and *D. desulfuricans* 8307 strains were purchased from DSMZ (DSM No. 17464 and 642 respectively). Both were grown on Postgate Media C (PGMC) using lactate as a carbon source [27]. All growth and manipulation of the *Desulfovibrio* strains was carried out at 30°C in an anaerobic hood fed with 10% CO_2, 10% H_2 in nitrogen. For the $NiCl_2$ resistance assay cultures of both *Desulfovibrio* strains were grown to an OD_{600} of 1.0. 10 μl was spotted onto a series of PGMC plates (1% agar) containing 0–20 mM of $NiCl_2$ and grown for 5 days anaerobically.

Nanoparticle production

Cells were grown as previously in PGMC to an OD_{600} of 1.0 and centrifuged for 10 min at 4000 rpm. The cell pellets were then washed with an equivalent volume of 10 mM 3-(N-morpholino)-propanesulfonic acid (MOPS) buffer, pH 7.0, three times and re-suspended after the third wash in the buffer. Solutions of $PtCl_4$, Na_2PdCl_4 or $NiCl_2$ were then added to the cell suspension to a final concentration of 2 mM, 2 mM and 1 mM respectively (in water). The cells in the presence of Pd and Pt were left for 2 hours while cells in the presence of Ni were left for 30 min at 30°C anaerobically.

Samples were then taken for analysis by dimethylglyoxime (DMG) assay (Ni) and by electron microscopy and EDX (energy dispersive X-rays) (Ni, Pd and Pt) and EELS (electron energy loss spectroscopy) (Ni).

Electron microscopy and EDX (energy dispersive X-rays)

For the conventional imaging of cells by EM: samples were placed on a 200 mesh copper grid for 5 min and the excess liquid was removed, the samples were then imaged on a Philips CM120 transmission electron microscope. Images were captured with a Gatan Orius CCD camera.

For EDX analysis of nanoparticles: the cells were removed from the sample by centrifugation (4000 rpm for 15 min) and the supernatants were analysed on a JOEL JEM 2011 TEM fitting with an ISIS system and viewed at an accelerating voltage of 200 kV. Readings were compared to a reference database for the specific metal. Further analysis of the particles was done by EELS.

Dimethylglyoxime assay for nickel reduction

Nickel reduction was measured using dimethylglyoxime (DMG) as a chelator of the Ni^{2+} in the solution containing cells or MOPS buffer only [26]. The cell suspension from the nickel-containing nanoparticle production method was taken and the cells were removed by centrifugation. The supernatant was then either taken immediately for the assay or filtered through a 0.20 μm filter and then used. The DMG was added to the solution, forming DMG-Ni^{2+}, which was then phase-extracted using chloroform. The absorbance of the extracted phase was measured at 360 nm, cell supernatants were compared to a solution of MOPS with $NiCl_2$ solution added that had also been centrifuged and filtered. This novel method of measuring the amount of reduced nickel has not previously been applied to nanoparticle formation, and works on the principle DMG does not bind to nickel unless it is present in an aqueous ionic state (i.e. it will not bind with nickel in nanoparticles).

Results

D. alaskensis *G20 produces nickel nanoparticles*

When challenged with $NiCl_2$ (1 mM final concentration) *D. alaskensis* G20 produces a black precipitate after just 30 min of incubation (Fig. 2). This precipitate is made up of extracellular nanoparticles that, unlike the Pt and Pd nanoparticles made by this strain (Fig. 3a,b), are not attached to the cells when viewed using TEM (Fig. 3c,d) and the nanoparticles had a diameter of ~10 nm. EELS analysis confirmed that these nanoparticles are composed of nickel and sulphur in all particles analysed suggesting they have the composition of NiS (Fig. 4) and not elemental Nickel. The formation of these particles did not occur in the MOPS buffer or the PGMC media supplemented with $NiCl_2$, only as a result of the presence of *D. alaskensis* G20 being incubated with $NiCl_2$.

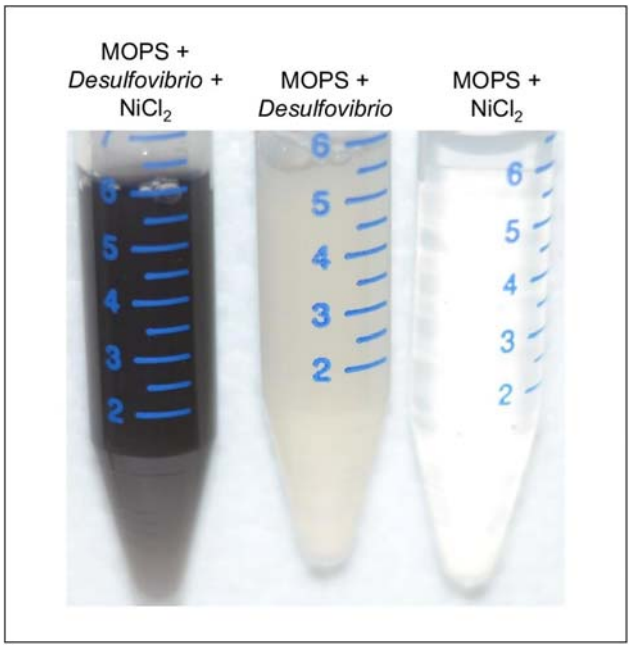

FIGURE 2

Formation of the black precipitate in liquid culture. Comparison between *D. alaskensis* G20 cells in MOPs buffer incubated for 30 min with and without 1 mM final $NiCl_2$ and the buffer control.

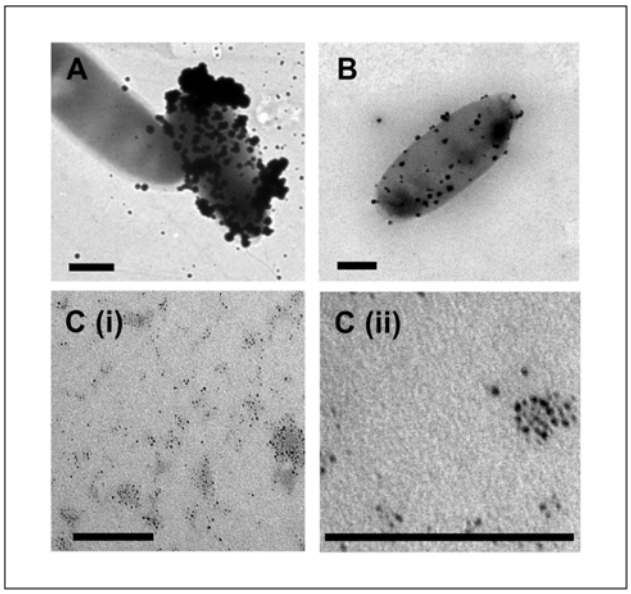

FIGURE 3

Representative electron micrographs of nanoparticles produced by *D. alaskensis* G20. **(a)** Pd NPs exported and found on the surface of the cell as are Pt NPs **(b)**. **c(i), (ii)**. NiS were found in the surrounding media. Scale bars = 200 nm.

Using the DMG assay for Ni^{2+} quantification it was found that up to 90% of the $NiCl_2$ is transformed to the solid state by the *D. alaskensis* G20 in 30 min, while the *D. desulfuricans* 8307 transformed 85% of the Ni^{2+} (Fig. 5). Although there is only a slight difference in the amount of Ni^{2+} removed between the G20 and 8307 strains, the assay used was reliable enough to give little variance, indicating a significant difference between the strains for filtered and non-filtered ($p = <0.005$, 2-tailed *t*-test).

The plate assay to find the resistance of the *Desulfovibrio* strains to $NiCl_2$ showed that growth was sustained up to 20 mM and there was a black discolouration of the colony observed that increased with increasing amounts of $NiCl_2$, which correlates with the production of NPs and the colour observed when $NiCl_2$ was present in buffer containing cells (Fig. 2).

Both strains of *Desulfovibrio* showed high resistance to $NiCl_2$ on PGMC agar plates with growth continuing up to 20 mM $NiCl_2$ (Fig. 6).

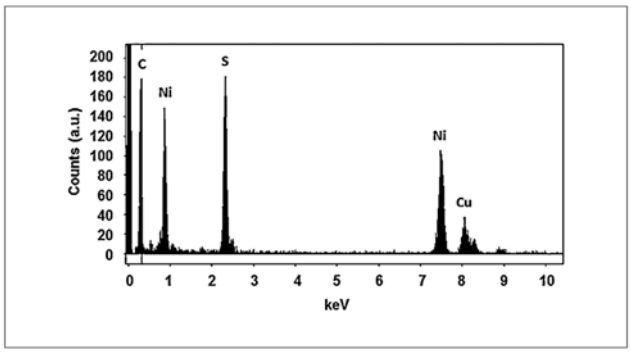

FIGURE 4

EELS data showing the composition of the Ni-containing nanoparticles. Peaks for both nickel and sulphur were present in every nanoparticle analysed suggesting a composition of NiS.

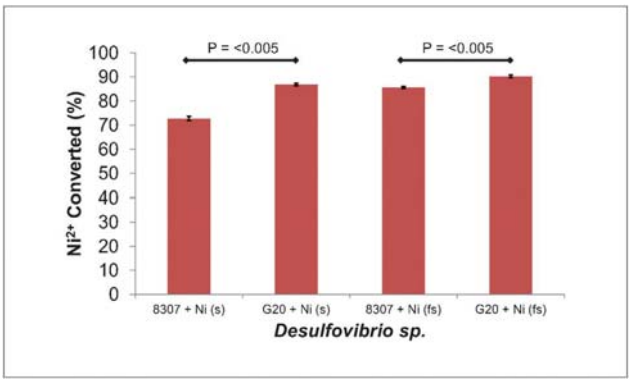

FIGURE 5

Percentage of Ni^{2+} converted to NP form by different species of *Desulfovibrio* in 30 min. Samples were assayed using the chelator dimethylglyoxime to complex with the un-reduced nickel in solution, phase extracted and the absorbance measured at 360 nm. s = supernatant, fs = filtered supernatant. Error bars represent the 95% confidence interval.

Palladium and platinum nanoparticles were also produced by D. alaskensis *G20*

Although platinum nanoparticle formation has been previously reported for *D. desulfuricans* 8307, there has been no documentation that *D. alaskensis* G20 also carried out the reduction, however this was found to be the case. When subjected to $PtCl_4$ or Na_2PdCl_4 the G20 cells reduce the platinum and palladium respectively to form nanoparticles. These were observed on the cell surface when viewed by transmission electron microscopy (Fig. 3a,b), in the same way as reported for *D. desulfuricans* 8307 in the presence of platinum/palladium. As a control 8307 was also subjected to $PdCl_4$ treatment and was seen to present nanoparticles on the cell surface. Both these NPs were subjected to EDX analysis and confirmed they consist of their respective PGM (data not shown). The simple advantage of *D. alaskensis* G20 is that it has a publically available, fully sequenced genome.

Discussion and conclusions

Here we have added to the list of *Desulfovibrio* sp. known to produce metal nanoparticles, demonstrating that *D. alaskensis* G20 can synthesize Ni, Pd and Pt nanoparticles. We have also presented a method for assaying aqueous Ni^{2+} in the presence of NiS NPs and therefore showing that within 30 min *Desulfovibrio* sp. can turnover up to 90% of the $NiCl_2$ solution under anaerobic conditions. These methods are a fast and facile way of producing and detecting nanoparticle formation, and highlight how competitive metal NP production could be against chemical methods.

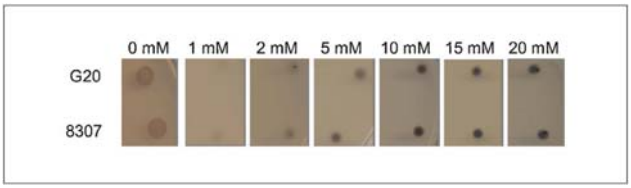

FIGURE 6

Growth of *D. alaskensis* G20 and *D. desulfuricans* 8307 on PGMC-agar plates containing increasing amounts of $NiCl_2$.

Previously it has also been reported that total conversion of Pd^{2+} occurred within 5 min of incubation [28] highlighting how *Desulfovibrio* sp. are suited to bioremediation. *Desulfovibrio* sp. can carry out this conversion at 30°C and in a much reduced time compared to chemically derived methods of production that often require reflux, hazardous chemicals and/or high temperatures, all of which to lead to higher costs.

The NiS NPs produced are not displayed on the cell surface; instead they are suspended in the buffer, allowing for easy removal of the cells by centrifugation. To further purify the sample and remove any remaining cells it is also possible to filter the NP solution through a 0.20 μm filter as the typical size of the NPs is 10 nm. However this purification method is not possible for cells producing Pt or Pd nanoparticles, as they remain attached to the surface and aggregate to form large deposits (Fig. 3a,b).

As *D. alaskensis* can transform Ni^{2+} into nanoparticles containing sulphur, this process must be different to that hypothesized for Pd and Pt ions and their subsequent NP formation as no reduction takes place. The formation of NiS NPs has previously been hypothesized to be a method for increasing Ni^{2+} tolerance in another bacterium, *Desulfotomaculum*, which forms NiS using H_2S [29]. The *Desulfovibrio* sp. also produce H_2S, as do most sulphate-reducing bacteria, so this is a possible mechanism for the conversion of $NiCl_2$ to NiS NPs, which may happen extracellularly rather than the aforementioned hypothesized pathways for Pd and Pt ion reduction (Fig. 1). Hydrogen sulphide is also used in the chemical synthesis of NiS NPs in conjunction with high temperatures or sodium hydroxide [30,31], whereas the NiS NP made here require much lower temperatures (30°C) and no application of NaOH. Further work is required to prove that H_2S is involved in the formation of NiS NPs in *D. alaskensis*. The resistance to Ni^{2+} itself is something worthy of note, in comparison *Escherichia coli* MC4100 is only resistant to concentrations up to 10 μM $NiCl_2$ [33]; thus making *D. alaskensis* an ideal organism for nickel bioremediation and NP formation. One possible reason for this heightened tolerance to nickel could be the formation of these NiS NPs. With its sequenced genome, this strain could be a useful source of genes for the construction of bacterial strains that allow the customisation of metal and metal-containing nanoparticles. By selecting, combining and manipulating the genes involved in the production of the nanoparticles, synthetic biology could allow the tailoring of nanoparticle size and production rate in an *E. coli* chassis, in a significant step beyond how *D. alaskensis* arsenic resistance genes have been previously used to increase arsenic tolerance [34]. Although there has been a large amount of work carried out on PGM NP production by *Desulfovibrio* sp. there has been little work carried out into the genetics of NP bio-production due to the lack of genetic tools and genomic data of the species used; the focus of the work so far has mainly been on the production and use of the NPs, rather than the biological aspects of their synthesis. In addition to the advantages biogenic NP production has over traditional physical and chemical NP synthesis methods, advances in Synthetic Biology could potentially allow the tailoring of the *Desulfovibrio* sp. to selectively produce NPs that are for a specific use, that allow the bacteria to target a specific contaminant in a heterogeneous solution, and that broaden the list of metals able to be removed from a contaminated site/solution by their conversion into higher-value NPs [12].

The DMG assay reported here can also be used to assay the reduction rate of Pd as it has previously been reported to be a chelator of Pd ions. As such, perhaps it too can be used in waste recovery, to separate Pd from other PGMs, for example, separating the different PGMs from roads contaminated with PGMs lost from several different types of catalytic converter [35]. Additionally we are exploring the possibility of utilizing heavy metals from contaminated sites as a cost-effective source of the raw materials needed to produce nanoparticles.

All the NPs produced by *D. alaskensis* require further analysis into their properties both catalytically and morphologically to determine if they are comparable to their chemically made alternatives. In time, this proposed method of metal ion conversion to elemental or sulphide-containing states could potentially be used as a generic resource recovery method, encompassing metals reported to be reduced by *Desulfovibrio* and also those yet to be identified. For example until now there has been no evidence to show that any of the *Desulfovibrio* sp. can form NiS NPs and very few examples of bacteria making nickel containing NPs; examples include *Desulfotomaculum*, which produces NiS NPs, and the formation of NiNPs by *Pseudomonas* [29,36]. Perhaps because nickel, while important in cell metabolism (forming the metal centre of hydrogenases which are essential to the aforementioned Pd/Pt NP synthesis [21]), is generally toxic to bacteria in high amounts.

In addition to being greener and more cost-effective than traditional chemical and physical methods of nanoparticle production, the use of bacteria in biogenic NP synthesis has the added advantage that the synthesis could be 'fine-tuned' by the manipulation of the bacteria [12]. This manipulation of the organism at the DNA level has the potential to improve yield and standardize the sample homogeneity, dictating characteristics of the resulting NPs such as size and shape; all features that determine the functionality of the NPs.

Acknowledgements

We would like to acknowledge the financial support of the EPSRC (EP/K026216/1) for this work, which makes up part of the CL4W project, and the technical help of Steve Mitchell in the School of Biosciences at Edinburgh University EM suite and Ross Blackley at Electron Microscopy Facility at the University of St Andrews and Dr Sam McFadzean at the School of Physics and Astronomy at the University of Glasgow.

References

[1] Gordon RB, Bertram M, Graedel TE. Metal stocks and sustainability. Proc Natl Acad Sci U S A 2006;103(5):1209–14.
[2] Ander EL, Johnson CC, Cave MR, Palumbo-Roe B, Nathanail CP, Lark RM. Methodology for the determination of normal background concentrations of contaminants in English soil. Sci Total Environ 2013;454–455:604–18.
[3] Barry L, Holmes JD, Otway DJ, Copley MP, Kazakova O, Morris MA. Unusual magnetism in templated NiS nanoparticles. J Phys Condens Matter 2010;22(7):076001.
[4] Sun C, Ma M, Yang J, Zhang Y, Chen P, Huang W, et al. Phase-controlled synthesis of alpha-NiS nanoparticles confined in carbon nanorods for high performance supercapacitors. Sci Rep 2014;4:7054.
[5] Crundwell F, Moats M, Ramachandran V, Robinson T, Davenport WG. Extractive metallurgy of nickel, cobalt and platinum-group metals overview; 2011;1–18.
[6] Alonso F, Riente P, Yus M. Nickel nanoparticles in hydrogen transfer reactions. Acc Chem Res 2011;44(5):379–91.
[7] Ahamed M, Alhadlaq HA. Nickel nanoparticle-induced dose-dependent cytogenotoxicity in human breast carcinoma MCF-7 cells. Oncotargets Ther 2014;7:269–80.
[8] Wang P. Nanoscale biocatalyst systems. Curr Opin Biotechnol 2006;17(6):574–9.
[9] Muthuswamy N, de la Fuente JL, Ochal P, Giri R, Raaen S, Sunde S, et al. Towards a highly-efficient fuel-cell catalyst: optimization of Pt particle size, supports and surface-oxygen group concentration. Phys Chem Chem Phys 2013;15(11):3803–13.
[10] Farlow J, Seo D, Broaders KE, Taylor MJ, Gartner ZJ, Jun Y. Formation of targeted monovalent quantum dots by steric exclusion. Nat Methods 2013;10(12):1203–5.
[11] Porcel E, Liehn S, Remita H, Usami N, Kobayashi K, Furusawa Y, et al. Platinum nanoparticles: a promising material for future cancer therapy? Nanotechnology 2010;21(8):85103.
[12] Edmundson MC, Capeness M, Horsfall L. Exploring the potential of metallic nanoparticles within synthetic biology. New Biotechnol 2014;31(6):572–8.
[13] Wu SH, Chen DH. Synthesis and characterization of nickel nanoparticles by hydrazine reduction in ethylene glycol. J Colloid Interface Sci 2003;259(2):282–6.
[14] Lovley DR, Phillips EJ. Reduction of uranium by *Desulfovibrio desulfuricans*. Appl Environ Microbiol 1992;58(3):850–6.
[15] Lloyd JR, Yong P, Macaskie LE. Enzymatic recovery of elemental palladium by using sulfate-reducing bacteria. Appl Environ Microbiol 1998;64(11):4607–9.
[16] Lloyd JR, Ridley J, Khizniak T, Lyalikova NN, Macaskie LE. Reduction of technetium by *Desulfovibrio desulfuricans*: biocatalyst characterization and use in a flowthrough bioreactor. Appl Environ Microbiol 1999;65(6):2691–6.
[17] Chardin B, Dolla A, Chaspoul F, Fardeau ML, Gallice P, Bruschi M. Bioremediation of chromate: thermodynamic analysis of the effects of Cr(VI) on sulfate-reducing bacteria. Appl Microbiol Biotechnol 2002;60(3):352–60.
[18] Payne RB, Gentry DM, Rapp-Giles BJ, Casalot L, Wall JD. Uranium reduction by *Desulfovibrio desulfuricans* strain G20 and a cytochrome c3 mutant. Appl Environ Microbiol 2002;68(6):3129–32.
[19] Yong P, Farr JPG, Harris IR, Macaskie LE. Palladium recovery by immobilized cells of *Desulfovibrio desulfuricans* using hydrogen as the electron donor in a novel electrobioreactor. Biotechnol Lett 2002;24(3):205–12.
[20] Mabbett AN, Yong P, Farr JP, Macaskie LE. Reduction of Cr(VI) by palladized biomass of *Desulfovibrio desulfuricans* ATCC 29577. Biotechnol Bioeng 2004;87(1):104–9.
[21] Rapp-Giles BJ, Casalot L, English RS, Ringbauer JA, Dolla A, Wall JD. Cytochrome c(3) mutants of *Desulfovibrio desulfuricans*. Appl Environ Microbiol 2000;66(2):671–7.
[22] Elias DA, Suflita JM, McInerney MJ, Krumholz LR. Periplasmic cytochrome c3 of *Desulfovibrio vulgaris* is directly involved in H_2-mediated metal but not sulfate reduction. Appl Environ Microbiol 2004;70(1):413–20.
[23] Bunge M, Søbjerg LS, Rotaru A, Gauthier D, Lindhardt AT, Hause G, et al. Formation of palladium(0) nanoparticles at microbial surfaces. Biotechnol Bioeng 2010;107(2):206–15.
[24] Rosenberg B, Renshaw E, Vancamp L, Hartwick J, Drobnik J. Platinum-induced filamentous growth in *Escherichia coli*. J Bacteriol 1967;93(2):716–21.
[25] Gopal J, Hasan N, Manikandan M, Wu HF. Bacterial toxicity/compatibility of platinum nanospheres, nanocuboids and nanoflowers. Sci Rep 2013;3:1260.
[26] Mohanapriya S, Lakshminarayanan V. Simultaneous purification and spectrophotometric determination of nickel present in as-prepared single-walled carbon nanotubes (SWCNT). Talanta 2007;71(1):493–7.
[27] Postgate JR, Kent HM, Robson RL, Chesshyre JA. The genomes of *Desulfovibrio gigas* and *D. vulgaris*. J Gen Microbiol 1984;130(7):1597–601.
[28] Yong P, Rowson NA, Farr JP, Harris IR, Macaskie LE. Bioreduction and biocrystallization of palladium by *Desulfovibrio desulfuricans* NCIMB 8307. Biotechnol Bioeng 2002;80(4):369–79.
[29] Fortin D, Southam G, Beveridge TJ. Nickel sulfide, iron-nickel sulfide and iron sulfide precipitation by a newly isolated *Desulfotomaculum* species and its relation to nickel resistance. FEMS Microbiol Ecol 1994;14(2):121–32.
[30] Guo H, Ke Y, Wang D, Lin K, Shen R, Chen J, et al. Efficient adsorption and photocatalytic degradation of Congo red onto hydrothermally synthesized NiS nanoparticles. J Nanopart Res 2013;15(3).
[31] Tilley RD, Jefferson DA. The synthesis of nickel sulfide nanoparticles on graphitized carbon supports. J Phys Chem B 2002;106(42):10895–901.
[33] Rodrigue A, Effantin G, Mandrand-Berthelot MA. Identification of rcnA (yohM): a nickel and cobalt resistance gene in *Escherichia coli*. J Bacteriol 2005;187(8):2912–6.
[34] Li XK, Krumholz LR. Regulation of arsenate resistance in *Desulfovibrio desulfuricans* G20 by an arsRBCC operon and an arsC gene. J Bacteriol 2007;189(10):3705–11.
[35] Ayres GH, Berg EW. Separation of palladium from platinum, iridium, and rhodium with dimethylglyoxime. Anal Chem 1953;25(6):980–1.
[36] Srivastava SK, Constanti M. Room temperature biogenic synthesis of multiple nanoparticles (Ag, Pd, Fe, Rh, Ni, Ru, Pt, Co, and Li) by *Pseudomonas aeruginosa* SM1. J Nanopart Res 2012;14(4).