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Platinum(II) reduction to platinum nanoparticles in anaerobic sludge

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Abstract

BACKGROUND: To help mitigate future problems in the supply of platinum group metals (PGM) due to their scarcity and high demand, new recovery processes must be developed. Microbial processes are a great alternative for the recovery of PGM from waste since they are clean and environmentally friendly techniques. This research studied the microbial reduction of Pt(II) using an anaerobic granular sludge under different physiological conditions.

RESULTS: The anaerobic granular sludge was able to reduce Pt(II) to Pt(0) nanoparticles that were deposited intracellularly as well as extracellularly as confirmed by X-ray diffraction (XRD) and transmission electron microscopy (TEM) analyses. Hydrogen (H₂) and formate supported the chemical reduction of Pt(II) while ethanol supported the biologically catalyzed reduction of Pt(II). Increasing initial concentrations of Pt(II), ethanol or biomass were each shown to increase the Pt(II) reduction rates.

CONCLUSIONS: This study reported for the first time the reduction of Pt(II) using anaerobic granular sludge and provided insights that could help develop biorecovery techniques to alleviate future problems in the supply of PGMs.

Keywords

Methanogenesis; Environmental Remediation; Metals; Recovery; Sludge

INTRODUCTION

Platinum group metals (PGM) are considered as critical and strategic metals because of their low concentrations in the Earth's crust and their high demand which could cause future problems in their supply. Recovery of PGM from waste streams can be considered as a strategy to reduce future supply shortages. PGM are strategic elements because they are in high demand in the energy and defense sectors and because they are limiting resources¹. Some of the applications of PGM are in catalysis, electronics, jewelry, glass making equipment, and fuel cells². The United States and the European Union are aware of the potential impact that PGM shortages could have in the world's economy^{1, 3, 4}, especially in the previously mentioned industrial sectors. Recovery of PGM from waste streams is a

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potential alternative to reduce future supply risks. In regard to the recovery of PGM, traditional physicochemical processes present some disadvantages such as generation of large volume of sludge and high operational costs, among others ⁵. These problems can be overcome by using microbial processes.

Microorganisms can be utilized to recover PGM from aqueous streams. Microorganisms have different mechanisms that can be utilized for the recovery of metals from wastewater such as: biosorption ^{6, 7}, and precipitation with biogenic ligands like sulfide ^{8–10}. Nonetheless, the best mechanism for the recovery of PGM is reductive precipitation which consists in the reduction of a soluble metal specie to an insoluble lower valent state by the action of the microorganisms. The use of metals as the electron acceptors by microorganisms, causing their reduction, has been well studied ¹¹. Some of the metals that can be reduced by microorganisms include PGM such as palladium (Pd), platinum (Pt) and rhodium (Rh) ^{12–16}, as well as other noble metals such as gold (Au) ¹⁷ and silver (Ag) ¹⁸. In order for these processes to be feasible, further investigations are needed to better understand the mechanisms enabling the development of efficient methods for the recovery of PGM.

Previous studies evaluating the microbial recovery of Pt have mainly focused on the recovery of Pt(IV) and little is known about the biorecovery of Pt(II). The study of the biorecovery of Pt(II) is of importance since there are a lot of applications in which Pt(II) is used. For instance Pt(II) is used for selective cleavage of peptides and proteins ¹⁹ and as a catalyst in many industrial processes ^{20, 21}. In addition, Pt(II) was identified as an intermediate species in the microbial reduction of Pt(IV) to Pt(0) nanoparticles ¹⁴. The main objective of this study was to demonstrate the ability of an anaerobic granular sludge to reduce Pt(II) to elemental Pt(0) nanoparticles and compare this process to the bioreduction of Pt(IV). Furthermore, this study evaluated the effect of different electron donors (*e*-donors) in the microbial reduction of Pt(II). Finally, we studied the inhibitory effects of Pt(II) on the activity of the methanogens in the anaerobic granular sludge.

MATERIALS AND METHODS

Inoculum

Anaerobic granular sludge obtained from a full scale up-flow sludge blanket (UASB) reactor at Mahou (beer brewery in Guadalajara, Spain) wastewater treatment plant was used as the source of inoculum. This biomass contained 0.079 g volatile suspended solids (VSS) g⁻¹ wet weight. The maximum methanogenic activity of the sludge was 569 ± 64 and 571 ± 27 mg COD-CH₄ g⁻¹ VSS d⁻¹ in with acetate and hydrogen (H₂) as substrates, respectively. The sludge was stored at 4°C.

Chemicals

Platinum (II) (as K₂PtCl₄, purity 99.9%), 9,10-anthraquinone-2,6-disulfonic acid (AQDS, 98%) and rivo flavin (98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and lawsone (2-hydroxy-1,4-naphthoquinone, > 98%) from TCI (Toshima, Kita-ku, Tokyo, Japan).

Bioassays

The basal medium used in all bioassays contained (mg L^{-1}): NaHCO_3 (2500), NH_4Cl (35), KCl (100) and $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (6).

Microbial reduction assays were performed in duplicate using 160-mL serum flasks with 50 mL of liquid phase containing basal medium, inoculum (0.5 g VSS L^{-1}), e -donor (ethanol, H_2 , formate, lactate, acetate or pyruvate at a concentration of $0.368 \text{ g COD L}^{-1}$), and the Pt(II) salt (25 mg Pt L^{-1}). In assays using H_2 as the e -donor, H_2 gas was injected at a pressure of 3.27 atm. Non-inoculated, endogenous (no exogenous e -donor) and no sludge and no e -donor controls were run to account for possible removal of Pt due to other mechanisms different than bioreduction. The heat-killed inoculum controls were autoclaved (121°C , 30 min). Then the flasks were left overnight to cool down and the same procedure was repeated once again to kill any sporulating microorganisms that survived the first autoclaving step. For all the treatments, the medium was flushed with a gas mixture containing N_2/CO_2 (80/20, v/v) for 5 min. The flasks were sealed using a butyl rubber septum and an aluminum seal, and the headspace was flushed for 5 min to ensure anaerobic conditions. Flasks were incubated in a shaker (110 rpm) at 30°C . Samples of the medium were taken periodically for Pt analysis.

Methanogenic toxicity assays were carried out in duplicate in 160-mL serum flasks with basal medium (50 mL) spiked with sodium acetate ($0.368 \text{ g COD L}^{-1}$) and granular sludge (0.5 g VSS L^{-1}). Flushing of the liquid and headspace with N_2/CO_2 (80/20, v/v), and incubation conditions were as described above for the bioreduction tests. After overnight incubation, the headspace was flushed again and then aliquots from a 500 mg Pt L^{-1} stock solution were added into some flasks to reach different Pt concentrations ranging from 0 to 10 mg L^{-1} . Finally, the headspace was analyzed periodically for methane content.

Analytical techniques

Analyses of soluble Pt were carried out using a 5100 ICP-OES from Agilent Technologies (Santa Clara, CA, USA) at a wavelength of 214.424 nm. Before the analysis, samples were centrifuged (13000 rpm, 10 min) and diluted into 2% nitric acid. CH_4 analysis was carried using a gas chromatograph (Agilent 5890) equipped with a Stabilwax-DA fused silica gel capillary column ($30 \text{ m} \times 0.53 \text{ mm}$, Restek Corp., Bellefonte, PA, USA) and a flame ionization detector. The temperature of the column, injector and detector were 140°C , 140°C and 250°C , respectively. Helium was used as the carrier gas.

X-ray diffraction (XRD) analysis

XRD analyses were carried out to study the oxidation state of the Pt products obtained from the microbial reduction assays. The analyses were performed using a PANalytical X'pert Plus Instrument (Westborough, MA, USA) equipped with a programmable incident beam slit with the slit fixed at 2° , and an X'Celerator Detector. The X-ray radiation used was $\text{Cu K}\alpha$, $\lambda = 1.5418 \text{ \AA}$. The generator settings were 45 kV and 40 mA; scan step size of 0.017 from 10° to 80° . The samples were dried with He and scattered onto a zero-background plate (Si wafer) before the analysis. The obtained diffraction patterns were analyzed using the

Panalytical High Score software and compared with patterns for crystalline platinum metal deposited in the ICDD database.

Transmission electron microscopy (TEM) analysis

The culture was centrifuged at 12000 rpm for 10 min and the cell pellet was transferred to a plastic vial. Washed cells exposed to nanoparticles were pre-fixed with 2.5% glutaraldehyde overnight and then fixed with 1% osmium in 0.1M PIPES at pH 7.4 for 30 min. After fixation, the cells were washed twice with demineralized water (DI) for 5 min, and centrifuged at 3200 rpm for 10 min. The cells were stained with 2% aqueous uranyl acetate for 20 min, washed with DI water for 5 min, and then dehydrated by immersion in aqueous solutions containing increasing ethanol concentration (50, 70, 90 and 100% each for 5 min, followed by washing with 100% ethanol for 20 min). Finally, the cell pellet was embedded in resin Embed 812 (resin/acetonitrile, 1:1 v/v) overnight, followed by incubation in resin at room temperature for 60 min (three times), and polymerization at 60°C for 24 h). Ultra-thin slices (70–100 nm) of embedded cells were obtained using a microtome (Leica EM UC7m Buffalo Grove, IL, USA) and collected on 200 mesh grids. Sections were examined with a Tecnai G2 TEM (FEI Company, Hillsboro, OR, USA) operated at a power intensity of 100 kV.

RESULTS AND DISCUSSION

Microbial reduction of Pt(II)

Bioreduction of Pt(II) was studied in the presence of ethanol and the results are shown in Figure 1. As shown in the figure, the microorganisms in the sludge were able to completely reduce an initial Pt(II) concentration of 25 mg L⁻¹ in 24 hours at a rate of 6.52 ± 0.33 mg Pt L⁻¹ h⁻¹. The color of the sludge changed from light brown to black during the process which provided visual confirmation of Pt(0) formation by Pt(II) reduction. Monitoring Pt nanoparticles formation by visual inspection was also reported in a study with a consortium of sulfate reducing bacteria¹⁴. A much slower Pt(II) reduction (0.54 ± 0.02 mg Pt L⁻¹ h⁻¹) occurred in the endogenous control lacking *e*-donor. Since no color change in the sludge occurred in the endogenous controls, we are assuming that no Pt(II) bioreduction took place during the process. Instead we considered the possibility of biosorption as the responsible mechanism for the removal of Pt(II) in the endogenous controls. However, this hypothesis was discarded since the Pt(II) removal rate in the heat-killed cells control was 65% of the rate obtained in the endogenous control (data shown in Figure S1 in the Supplementary Information section). Thus the results suggest that while biosorption may have taken place, another mechanism must have also played a role in the observed removal of Pt(II) by the endogenous controls. Limited removals were observed in the abiotic control, lacking inoculum (0.24 ± 0.00 mg Pt L⁻¹ h⁻¹) and in the inoculum-free control lacking *e*-donor and microorganisms (0.25 ± 0.00 mg Pt L⁻¹ h⁻¹). The reason for this observation will be explained in more detail below.

To the best of our knowledge, this is the first study that has reported on the microbial reduction of Pt(II) using anaerobic sludge. The microbial reduction of Pt(II) was previously reported using an uncharacterized consortium of sulfate reducing bacteria (SRB)¹⁴. That

study reported the microbial reduction of Pt(II) to Pt(0) as a second step in the microbial reduction of Pt(IV). The use of an anaerobic granular sludge for metal recovery was previously reported for Pt(IV) by our research group ²². The reduction rates for assays containing biomass and ethanol as *e*-donor were $13.04 \pm 0.67 \text{ mg Pt g}^{-1} \text{ VSS h}^{-1}$ in the case of Pt(II) and $1.02 \pm 0.04 \text{ mg Pt g}^{-1} \text{ VSS h}^{-1}$ in the case of Pt(IV). As the Pt(II) reduction rate is much faster than the Pt(IV) reduction rate, we can assume that Pt(II) will not be expected to accumulate as an intermediate species in the reduction of Pt(IV) to Pt(0). In addition, the difference between the full treatment and endogenous control reduction rate is much higher in the case of Pt(II). The ratio of full treatment to endogenous control removal rate was 12.0 in the case of Pt(II) and 1.8 in the case of Pt(IV) supporting the idea that the reduction of Pt(II) with *e*-donor was an even more important mechanism of Pt removal for Pt(II) compared to Pt(IV).

The effect of different *e*-donors on the biological and chemical reductions of Pt(II) was studied and the results are shown on Figure 2. The addition of different *e*-donors highly impacted the behavior of both chemical and biological Pt(II) reductions. H₂ and formate strongly supported the chemical reduction of Pt(II). The presence of microorganisms increased the reduction rate by 20% in the case of H₂, however; in the case of formate microorganisms did not have a significant role in increasing the rate of Pt(II) reduction beyond the rate determined under abiotic conditions. These results contrasted those obtained in experiments utilizing ethanol as *e*-donor. As explained above, ethanol only supported the biological reduction of Pt(II). A possible explanation is that the acetogenesis of ethanol was carried out by the microorganisms producing acetate and H₂ ²³, and then H₂ was responsible for the chemical reduction of Pt(II) to Pt(0). Interestingly, the addition of lactate had a minimal impact on the reduction of Pt(II) and acetate did not have any effect when compared to the endogenous control. The fact that acetate did not promote the reduction of Pt(II) supports the hypothesis that biogenic H₂ was responsible for the reduction of Pt(II) in the treatment with ethanol. The time course of Pt(II) concentration in treatments with the anaerobic sludge and different *e*-donors is shown in Figure S2 in the Supplementary Information section. The effect of different *e*-donors on the microbial reduction of Pt(IV) was studied previously by our research group ²², enabling a rate comparison of Pt(II) with Pt(IV) under biological and chemical reduction conditions for both Pt(II) and Pt(IV) (Figure 3). Ethanol was the only *e*-donor that worked exclusively under biological reducing conditions. In both cases, H₂ and formate caused mostly a chemical reduction of Pt(II) and Pt(IV) with little or no role of microorganisms. Lastly, lactate and acetate were poor *e*-donors for either Pt(II) or Pt(IV) (data not shown). Most importantly, Figure 3 reveals that the reduction of Pt(II) was consistently faster than the reduction of Pt(IV) with all *e*-donors specially in the case of formate where the biological reduction of Pt(II) was 31 times faster than that of Pt(IV).

As mentioned above, a limited decrease in the Pt(II) concentration was observed in abiotic controls with just mineral medium lacking inoculum and *e*-donors. A possible explanation for this decrease is Pt(II) oxidation to Pt(IV) by the bicarbonate present in the medium coupled to formate formation via reduction of bicarbonate. Subsequently formate is hypothesized to be responsible for the reduction of Pt(IV) to Pt(0) requiring 4 moles of electrons per mol of Pt(IV), however, formate is only able to provide 2 moles of electrons

and that is the reason why the removal of Pt in the control was approximately 50%. A very similar reaction sequence has previously been reported for the reduction of Pd(II)²⁴. If this were true, the Pt(II) concentration would not be expected to decrease in mineral medium controls lacking bicarbonate. This hypothesis was studied and, indeed, the Pt(II) concentration was stable in medium lacking bicarbonate as well as in medium made up without buffer or in phosphate buffer (see Figure S3 in the Supplementary Information section).

Characterization of the end products

To determine whether Pt(II) was in fact reduced to Pt(0), XRD analyses were performed and the results can be seen in Figure 4. As explained above, the best *e*-donors for the Pt(II) reduction were formate, H₂ and ethanol, with formate and H₂ *e*-donors being the most effective for the chemical reduction. On the other hand, ethanol worked exclusively in the biological reduction of Pt(II). These findings are also reflected on the distinctive peaks of Pt(0) observed in samples with ethanol, H₂ and formate as reductants (Figure 4A). In contrast, lactate was a poor *e*-donor, which is in agreement with the low intensity of the Pt(0) peaks in this sample when compared to the samples incubated with formate, H₂ or ethanol (Figure 4B). Finally, the addition of acetate and the absence of *e*-donor (endogenous control) did not have any impact on the reduction of Pt(II), which explains the lack of Pt(0) peaks in the XRD patterns shown in Figure 4B.

The insights provided by XRD analyses are in accordance with the XRD results previously reported for the reduction of Pt(IV)²². However, the peaks characteristic of Pt(0) in the case of Pt(II) reduction are much sharper compared to those obtained in the case of Pt(IV) reduction in accordance to the reduction rate differences between Pt(II) and Pt(IV). Similar to the reduction of Pt(IV), lactate treatment resulted in trace amounts of Pt(0). Acetate and the endogenous treatments did not result in formation of any detectable Pt(0) according to the XRD analyses.

TEM analyses were performed in order to determine the morphology of the Pt(0) nanoparticles formed by anaerobic reduction of Pt(II) (Figure 5). The Pt(0) nanoparticles formed had a spherical shape and their size ranged from 2 nm to 5 nm. The nanoparticles were formed intracellularly and extracellularly. The extracellular nanoparticles were found attached to the cell surface as well as freely suspended in solution. Nonetheless, the amount of nanoparticles detected freely in suspension was less when compared to the amount found inside the cells and attached to the cell surface.

The morphology of the Pt nanoparticles was very similar to that of some of the nanoparticles obtained in the earlier study regarding Pt(IV) reduction²². In both cases, the nanoparticles had spherical shape and their average size was less than 5 nm. However, the Pt nanoparticles obtained by reduction of Pt(IV) aggregated and formed bigger nano-structures with sizes up to 200 nm. Formation of Pt nanoparticles has been previously reported for different microorganisms and physiological conditions leading to the formation of both intracellular and extracellular Pt(0) nanoparticles. Microbial formation of intracellular Pt(0) nanoparticles has been described for an uncharacterized consortium of SRB supplemented with Pt(IV), and H₂¹⁴ and for *S. alga*e supplemented with Pt(IV) and lactate²⁵. On the other hand,

formation of extracellular Pt(0) nanoparticles was reported in studies with *Desulfovibrio. alaskensis* G20 cells grown under anaerobic conditions supplemented with lactate and Pt(IV)²⁶ and with the fungus *Fusarium oxysporum*²⁷.

In order to recover the nanoparticles formed intracellularly, one would have to lyse the cells. Since the role of microorganisms is to produce H₂ from substrates (e.g. ethanol), one could opt to just promote the chemical reduction of Pt(II) to Pt(0), which would avoid this problem since the nanoparticles will be formed in the liquid medium. As it was mentioned before in this report, H₂ and formate are good reductants promoting the chemical reduction of Pt(II) and, therefore, they would be the best alternative to avoid potential problems with the recovery of intracellular nanoparticles.

Influence of ethanol concentration on the Pt(II) reduction rate

The effect of the initial concentration of ethanol on the reduction of Pt(II) was studied and the results can be seen in Figure 6. It is important to note that the concentrations of ethanol are expressed as excess over the stoichiometric concentration required to generate the biogenic H₂ to reduce Pt(II) which is theoretically 2.95 mg L⁻¹ of ethanol for the added Pt(II) concentration of 25 mg L⁻¹. Figure 6A shows the time course of the Pt(II) concentration in the treatments with different ethanol concentrations. Figure 6 B illustrates the relationship between the maximum Pt(II) reduction rate and the ethanol concentration. It can be seen that Pt(II) reduction follows the typical Michaelis and Menten kinetics in which saturation is reached at high substrate concentrations.

Influence of biomass concentration on the Pt(II) reduction rate

Figure 7A shows the time course of the Pt(II) reduction in treatments with different biomass concentrations. Figure 7B depicts the relationship between the maximum reduction rate and the biomass concentration. The graph shows that increasing biomass concentrations led to increasing reduction rates. However, this relationship is only valid at low biomass concentrations. For biomass concentrations higher than 0.5 g VSS L⁻¹, saturation was observed and increasing biomass concentrations did not provide for further increases in the maximum reduction rate. Rate limitations of the flux of Pt(II) into granules may have been responsible for this pattern.

Influence of Pt(II) concentration on its reduction rate

Increasing Pt(II) initial concentrations led to increasing Pt(II) reduction rates in the full treatments and endogenous controls (Figure 8). However, the reduction rates increased much more in the case of full treatments with e-donor. The influence of the initial concentration of Pt on its reduction rate was previously reported for Pt(IV)^{22, 28}. Similar to the observation in this study with Pt(II), increasing initial concentrations of Pt(IV) led to increasing reduction rates. The influence of the initial PGM concentration on its reduction rate was also reported in a study with Rh(III)²⁹. Again, increasing initial concentrations of Rh(III) led to increasing reduction rates. Taken as a whole the data suggest that the reductive precipitation of PGM ions follows first order reaction kinetics. The interspecies intermediates such as biogenic H₂ and formate chemically react with PGM ions.

To the best of our knowledge this is the first report that studied the influence of different initial ethanol, biomass and Pt(II) concentrations on the Pt(II) reduction kinetics.

Methanogenic inhibition by Pt(II)

Figure 9 shows the activity of the acetoclastic methanogenic microorganisms expressed as a percentage of the uninhibited control over a range of initial Pt(II) concentrations. Increasing Pt(II) concentrations resulted in decreasing methanogenic activity. Therefore, Pt(II) caused a concentration dependent inhibition to acetoclastic methanogens in the sludge. The methanogenic activity was fully inhibited at Pt(II) concentrations equal to or higher than 10 mg L⁻¹, and the concentration of Pt(II) causing 50% of methanogenic inhibition (IC₅₀) was very low, approximately 2.4 mg L⁻¹. The time course of the methane production for the treatments with different initial Pt(II) concentration is shown in Figure S4 in the Supplementary Information section.

To the best of our knowledge, this is the first time that the inhibitory effect of Pt(II) to methanogens is reported. However, we recently reported on the inhibitory impact of Pt(IV) on methanogenic microorganisms. The IC₅₀ values determined for Pt(II) (2.4 mg L⁻¹) and Pt(IV) (3.7 mg L⁻¹) are very similar, indicating that both species cause similar inhibition effects. Reduced cofactors required for key steps in the catabolic pathways of methanogens (methyl-coenzyme M, coenzyme B, F420, and methanophenazines) could potentially be oxidized by PGM ions which in turn would interfere with the biochemistry of methanogens

CONCLUSIONS

The anaerobic microbial consortium used in this study was able to reduce Pt(II) to Pt(0) nanoparticles as confirmed by XRD and TEM analyses. Pt(II) reduction to Pt(0) nanoparticles was much faster than the reduction of Pt(IV) (up to 31-fold higher when formate was utilized as *e*-donor), indicating that Pt(II) is not likely to accumulate as an intermediate species in the reduction of Pt(IV) to Pt(0) nanoparticles. Both chemical and biological reduction was observed under different experimental conditions. H₂ and formate supported the chemical reduction of Pt(II). On the other hand, ethanol supported the biological reduction of Pt(II). Interspecies intermediates such as H₂ and formate formed from ethanol acetogenesis were found to chemically react with Pt(II). The influence of the initial concentrations of ethanol, biomass and Pt(II) in the reduction rates were studied and the results showed that increasing concentrations of each of these items led to increasing Pt(II) reduction rates albeit in some cases with saturation kinetics or mass transfer limitations at the highest concentrations. Finally, the methanogenic toxicity of Pt(II) was studied and complete inhibition of the methanogenic activity occurred at low Pt(II) concentrations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Hennebel T, Boon N, Maes S and Lenz M, Biotechnologies for critical raw material recovery from primary and secondary sources: R& D priorities and future perspectives. *New Biotechnology* 32: 121–127 (2015). [PubMed: 23994422]
2. Renner H, Schlamp G, Drost I, Lüschoff HM, Tews P, Panster P, Diehl M, Lang J, Kreuzer TKA, Starz KA, Dermann K, Rothaut J, Drieselmann R, Peter C, Schiele R, Platinum Group Metals and Compound, in Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp. 317–388 (2001).
3. DOE, Critical Materials Strategy, U.S. Department of Energy (2012).
4. NRC, Minerals, Critical Minerals, and the U.S. Economy. National research Council, National Academies Press, Washington DC pp 264 (2008).
5. Ahluwalia SS and Goyal D, Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresource Technology* 98: 2243–2257 (2007). [PubMed: 16427277]
6. Chen JZ, Tao XC, Xu J, Zhang T and Liu ZL, Biosorption of lead, cadmium and mercury by immobilized *Microcystis aeruginosa* in a column. *Process Biochemistry* 40: 3675–3679 (2005).
7. Creamer NJ, Baxter-Plant VS, Henderson J, Potter M and Macaskie LE, Palladium and gold removal and recovery from precious metal solutions and electronic scrap leachates by *Desulfovibrio desulfuricans*. *Biotechnology Letters* 28: 1475–1484 (2006). [PubMed: 16909331]
8. Essa AMM, Creamer NJ, Brown NL and Macaskie LE, A new approach to the remediation of heavy metal liquid wastes via off-gases produced by *Klebsiella pneumoniae* M426. *Biotechnology and Bioengineering* 95: 574–583 (2006). [PubMed: 16958139]
9. Gadd GM, Bioremediation potential of microbial mechanisms of metal mobilization and immobilization. *Current Opinion in Biotechnology* 11: 271–279 (2000). [PubMed: 10851150]
10. White C and Gadd GM, Accumulation and effects of cadmium on sulphate-reducing bacterial biofilms. *Microbiology-Uk* 144: 1407–1415 (1998).
11. Lovley DR, Dissimilatory metal reduction. *Annual Review of Microbiology* 47: 263–290 (1993).
12. De Windt W, Aelterman P and Verstraete W, Bioreductive deposition of palladium (0) nanoparticles on *Shewanella oneidensis* with catalytic activity towards reductive dechlorination of polychlorinated biphenyls. *Environmental Microbiology* 7: 314–325 (2005). [PubMed: 15683392]
13. Pat-Espadas AM, Field JA, Otero-Gonzalez L, Razo-Flores E, Cervantes FJ and Sierra-Alvarez R, Recovery of palladium(II) by methanogenic granular sludge. *Chemosphere* 144: 745–753 (2016). [PubMed: 26408982]
14. Riddin TL, Govender Y, Gericke M and Whiteley CG, Two different hydrogenase enzymes from sulphate-reducing bacteria are responsible for the bioreductive mechanism of platinum into nanoparticles. *Enzyme and Microbial Technology* 45: 267–273 (2009).
15. Tamaoki K, Saito N, Nomura T and Konishi Y, Microbial recovery of rhodium from dilute solutions by the metal ion-reducing bacterium *Shewanella algae*. *Hydrometallurgy* 139: 26–29 (2013).
16. Yong P, Rowson NA, Farr JPG, Harris IR and Macaskie LE, Bioreduction and biocrystallization of palladium by *Desulfovibrio desulfuricans* NCIMB 8307. *Biotechnology and Bioengineering* 80: 369–379 (2002). [PubMed: 12325145]
17. Konishi Y, Tsukiyama T, Ohno K, Saitoh N, Nomura T and Nagamine S, Intracellular recovery of gold by microbial reduction of AuCl₄⁻ ions using the anaerobic bacterium *Shewanella algae*. *Hydrometallurgy* 81: 24–29 (2006).
18. Pugazhenthiran N, Anandan S, Kathiravan G, Prakash NKU, Crawford S and Ashokkumar M, Microbial synthesis of silver nanoparticles by *Bacillus* sp. *Journal of Nanoparticle Research* 11: 1811–1815 (2009).

19. Milovic NM, Dutca LM and Kostic NM, Combined use of platinum(II) complexes and palladium(II) complexes for selective cleavage of peptides and proteins. *Inorganic chemistry* 42: 4036–4045 (2003). [PubMed: 12817959]
20. Carroll HK, Parlane FGL, Reich N, Jelier BJ and Montgomery CD, Phosphoramidite complexes of Pd(II), Pt(II) and Rh(I): An effective hydrosilylation catalyst of 1-hexyne and 1-octene. *Inorganica Chimica Acta* 465: 78–83 (2017).
21. Rezaei SJT, Khorramabadi H, Hesami A, Ramazani A, Amani R and Ahmadi R, Chemoselective reduction of nitro and nitrile compounds with magnetic carbon nanotubes-supported Pt(II) catalyst under mild conditions. *Industrial & Engineering Chemistry Research* 56: 12256–12266 (2017).
22. Simon-Pascual A, Sierra-Alvarez R, Ramos-Ruiz A and Field JA, Reduction of platinum (IV) ions to elemental platinum nanoparticles by anaerobic sludge. *Journal of Chemical Technology & Biotechnology* 0.
23. Madigan TM, Martinko JM, Bender KS, Buckley DH and Stahl DA, *Brook Biology of Microorganisms* (2015).
24. Sullivan BP, Reduction of Carbon Dioxide with Platinum Metals Electrocatalysts. *Platinum Metals Rev* 33:2–8 (1989).
25. Konishi Y, Ohno K, Saitoh N, Nomura T, Nagamine S, Hishida H, Takahashi Y and Uruga T, Bioreductive deposition of platinum nanoparticles on the bacterium *Shewanella algae*. *Journal of Biotechnology* 128: 648–653 (2007). [PubMed: 17182148]
26. Capeness MJ, Edmundson MC and Horsfall LE, Nickel and platinum group metal nanoparticle production by *Desulfovibrio alaskensis* G20. *New Biotechnology* 32: 727–731 (2015). [PubMed: 25686718]
27. Syed A and Ahmad A, Extracellular biosynthesis of platinum nanoparticles using the fungus *Fusarium oxysporum*. *Colloids and Surfaces B-Biointerfaces* 97: 27–31 (2012).
28. Rashamuse K, The bioaccumulation of platinum (IV) from aqueous solution using sulphate reducing bacteria : role of a hydrogenase enzyme (2003).
29. Ngwenya N and Whiteley CG, Recovery of rhodium(III) from solutions and industrial wastewaters by a sulfate-reducing bacteria consortium. *Biotechnology Progress* 22: 1604–1611 (2006). [PubMed: 17137308]
30. Ferry JG, Biochemistry of methanogenesis. *Abstr Pap Am Chem Soc* 203: 3-BIOT (1992).

Figure 1.

Panel A: time course of Pt(II) concentration in a methanogenic granular sludge using ethanol as *e*-donor. Legend: (◆) abiotic control, (▲) endogenous control, (■) complete inoculated treatment supplemented with ethanol (0.368 g COD L⁻¹), and (X) control lacking sludge and *e*-donor. Panel B: Maximum Pt(II) reduction rates determined in the treatments mentioned above.

Figure 2. Maximum rates determined in the biological reduction (□) and chemical reduction (■) of Pt(II) in treatments with different e-donors.

Figure 3. Maximum reduction rates for Pt(II) and Pt(IV) reduction with different e^- donors in the presence and absence of microorganisms.

Figure 4.

Results of XRD analyses. Panel A: analyses of samples from (1) complete treatment with H_2 , (2) complete treatment with formate, and (3) complete treatment with ethanol. Panel B: analyses of samples from (4) complete treatment with lactate, (5) complete treatment with acetate, and (6) endogenous treatment. (†) Representative peaks for Pt(0).

Figure 5.
TEM images showing the formation of Pt(0) nanoparticles in biological assays supplemented with ethanol.

Figure 6.

Influence of the ethanol concentration in the Pt(II) reduction. Panel A: Pt(II) reduction kinetics in full treatments with different ethanol concentrations: (◆) 1.48 mg L⁻¹, (■) 2.95 mg L⁻¹, (▲) 14.75 mg L⁻¹, (●) 88.50 mg L⁻¹, (○) endogenous control, (□) no sludge & no e-donor, and (X) abiotic with 88.50 mg L⁻¹ of ethanol (the dotted line represents the concentration that provides sufficient amount of biogenic H₂ to reduce 25 mg L⁻¹ of Pt(II)). Panel B: Relationship between ethanol concentration and Pt(II) reduction rate. Note: the Pt(II) reduction rate at the highest ethanol concentration is expected to be higher but not enough data points were captured to calculate the reduction rate.

Figure 7. Influence of biomass concentration in Pt(II) reduction. Panel A: Time course of Pt(II) concentration in methanogenic sludge using ethanol as *e*-donor and different biomass concentrations: (X) 0.5 g VSS L⁻¹, (▲) 0.25 g VSS L⁻¹, (■) 0.1 g VSS L⁻¹ and (◆) 0 g VSS L⁻¹. Panel B: Maximum reduction rates for the treatments mentioned above.

Figure 8.
Maximum reduction rates for Pt(II) reduction in (▲) full treatment with ethanol and (■) endogenous control with different initial Pt(II) concentrations.

Figure 9. Normalized rate of acetoclastic methanogenic activity at different concentrations of Pt(II) (in mg L⁻¹): (□) no Pt(II), (X) 0.025 mg L⁻¹ (▲) 0.5, (■) 1.0, (●) 2.5, (○) 5, and () 10.