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Reduction of platinum (IV) ions to elemental platinum nanoparticles by anaerobic sludge

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Abstract

Background—The future supply of platinum group metals (PGM) is at risk because of their scarcity combined with a high demand. Thus recovery of platinum (Pt) from waste is an option worthy of study to help alleviate future shortages. This research explored the microbial reduction of platinum (Pt). The ability of anaerobic granular sludge to reduce Pt(IV) ions under different physiological conditions was studied.

Results—X-Ray diffraction (XRD) and transmission electron microscope (TEM) analyses demonstrated the capacity of the microbial mixed culture to reduce Pt(IV) to Pt(0) nanoparticles, which were deposited on the cell-surface and in the periplasmic space. Ethanol supported the biologically catalyzed Pt(IV) reduction, meanwhile other electron donors; hydrogen (H₂) and formate, promoted the chemical reduction of Pt(IV) with some additional biological stimulation in the case of H₂. A hypothesis is proposed in which H₂ formed from the acetogenesis of ethanol is implicated in subsequent abiotic reduction of Pt(IV) indicating an integrated bio-chemical process. Endogenous controls also resulted in slow Pt(IV) removal from aqueous solution. Selected redox mediators, exemplified by riboflavin, enhanced the Pt(IV) reduction rate.

Conclusion—This study reported for the first time the ability of an anaerobic granular sludge to reduce Pt(IV) to elemental Pt(0) nanoparticles.

Keywords

anaerobic; bioprocess; environmental remediation; metals; recovery; sludge

INTRODUCTION

Platinum group metals (PGM) are critical elements for advanced technology; however, their future supply is at risk because of their scarcity and their high demand. Thus recovery of PGM from waste streams is an option worthy of study to help alleviate future supply risks. PGM are considered strategic elements because they are utilized in a wide range of applications in the energy and defense sectors and because they are limiting mineral resources¹. The United States and the European Union are concerned that potential PGM shortages could impact the world's economy^{1–4}, especially in sectors that greatly rely on the

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supply of these materials. PGM are used as catalysts in a wide range of chemical processes as well as in catalytic converters in vehicles⁵. PGM are also used in electronics, jewelry, glass making equipment and fuel cells⁵. Recovery of PGM from waste streams is a potential alternative to help remedy the supply risk problems. Traditional physicochemical methods for the recovery of PGM and other metals present some disadvantages such as generation of large volumes of sludge, high operational costs and difficulties in the treatment of large volumes of waste water containing low concentrations of metals⁶. Biotechnology can offer some important advantages in the recovery of PGM from waste streams.

Microorganisms can be utilized to biotransform PGM into non-aqueous phases that can be separated from aqueous streams. Microorganisms have multiple mechanisms that can be applied to the recovery of metals from wastewater streams: biosorption^{7, 8}; and precipitation with biogenic ligands such as sulfide^{9–11}. However, reductive precipitation is the most appropriate technique for the removal of PGM. Reductive precipitation is the reduction of soluble metals to insoluble lower valent metals. The use of metals as electron acceptors in microbially-mediated reductions has been well studied¹². The metals that are reduced by bacteria including PGM such as palladium (Pd) and to a lesser extent studies have evaluated (Rh) and platinum (Pt)^{13–17}. In most cases, the PGM were reduced to zero-valent nanoparticles (NPs). Further studies are needed in order to better understand the mechanisms responsible for these processes and develop new and efficient microbial recovery technologies. Many of the previous studies have evaluated the microbial reduction of PGM using pure cultures, but an important question is whether natural anaerobic consortia from waste (water) bioreactors can be harnessed for the reductive precipitation of PGM.

The main objective of this study was to demonstrate the ability of an anaerobic granular sludge to reduce Pt(IV) to elemental Pt(0) NPs. Furthermore, the study evaluated whether Pt(IV) reduction also occurs under different physiological conditions (in the presence of different electron donors (e-donors) and redox mediators). Lastly, we examined the inhibitory effects of Pt(IV) on the activity 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 (IV) (as K₂PtCl₆, 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): NaHCO₃ (2500), NH₄Cl (250), KCl (100) and NaH₂PO₄·H₂O (6).

Microbial reduction assays were performed duplicate using 160-mL serum flasks supplied with 50 mL of basal medium containing, inoculum (0.5 g VSS L⁻¹), e-donor (ethanol, H₂, formate, lactate, acetate or pyruvate at a concentration of 0.368 g COD L⁻¹), and the Pt(IV) salt (25 mg Pt L⁻¹). In assays using H₂ as the e-donor, H₂ was injected at a pressure of 3.27 atm. Non-inoculated-, endogenous (no exogenous e-donor) and heat-killed inoculum controls were run to account for possible removal of Pt due to biosorption or other mechanisms. 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 organisms that survived the first autoclaving. The medium was flushed with a gas mixture of N₂/CO₂ (80%/20%) 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.

The effect of the redox mediators in the reduction of Pt(IV) was carried out in duplicate using 160-mL serum flasks supplied with basal medium (25 mL), inoculum (0.5 g VSS L⁻¹), and Pt(IV) salt (25 mg Pt L⁻¹). H₂ was used as e-donor. AQDS, riboflavin and lawsone were supplied to the medium using concentrated stocks. H₂ supplementation, flushing of the liquid and headspace with N₂/CO₂ (80/20, v/v), and incubation conditions were as described above for the bioreduction tests. Aliquots of the different flasks were taken periodically for metal 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 g COD L⁻¹) and granular sludge (0.5 g VSS L⁻¹). Flushing of the liquid and headspace with N₂/CO₂ (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 25 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) at a wavelength of 214.424 nm. Before the analysis, samples were centrifuged (13000 rpm, 10 min) and diluted into 2% nitric acid. CH₄ analysis was carried using a gas chromatography (Agilent 5890) with a Stabilwax-DA fused silica gel capillary column (30 m × 0.53 mm, Restek Corp., Bellefonte, PA) 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 analysis (XRD)

XRD analyses were carried out to study the oxidation state of the Pt products obtained from the microbial reduction assays. Samples were collected after 120–144 hours of incubation for the experiments with ethanol and H₂ and after 168–192 hours for the experiments with formate, acetate, pyruvate and lactate. 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 Cu K α , $\lambda = 1.5418 \text{ \AA}$. 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

Samples for TEM analysis were collected from experiments with H₂ after 120–144 hours of incubation and prepared as described elsewhere¹⁸ and viewed using a Tecnai Spirit Biotwin from FEI (Hillsboro, OR, USA) operated at 100 kV.

RESULTS AND DISCUSSION

Microbial reduction of Pt(IV)

The effect of the addition of ethanol as an exogenous e-donor on the time course of Pt(IV) concentration is shown in Figure 1. Microorganisms in the sludge were able to reduce Pt(IV) with an estimated maximum reduction rate of $1.02 \pm 0.04 \text{ mg Pt g}^{-1} \text{ VSS h}^{-1}$. After 44 hours most of the Pt was removed from solution and the soluble Pt concentration after 91 h was 0.52 mg L^{-1} . During the reduction process, the color of the sludge changed from light brown to black which is consistent with Pt(0) formation by Pt(IV) reduction.

Pt(IV) reduction was not observed in treatments lacking the sludge but containing ethanol. This suggests that no chemical reduction occurred in the presence of ethanol and therefore microorganisms are required in order to reduce Pt(IV) to Pt(0). In the case of the endogenous control, a gradual decrease in the concentration of Pt(IV) was observed, even in the absence of exogenous e-donor. Nonetheless, the rate at which Pt(IV) was removed in the endogenous control was lower compared to the complete treatment with ethanol and sludge. The maximum reduction rates for endogenous control was $0.34 \pm 0.02 \text{ mg Pt g}^{-1} \text{ VSS h}^{-1}$ was only 33.3% compared with the rate in the full treatment with ethanol. The removal of soluble Pt(IV) in the endogenous control did not result in a darkening of the sludge, suggesting that the endogenous removal of Pt(IV) may be due to another mechanism not involving reduction. Potentially other mechanisms such as adsorption of Pt(IV) to sludge, may be involved. However, this hypothesis was shown not to be valid since the heat killed cell control provided very little Pt(IV) removal compared to the endogenous control ($0.08 \pm 0.01 \text{ mg Pt g}^{-1} \text{ VSS h}^{-1}$).

To the best of our knowledge, our finding is the first report in which the microbially catalyzed reduction of Pt(IV) was achieved using anaerobic granular sludge. However, the

reduction of a related PGM ion, Pd(II), by granular sludge was studied previously¹⁴. In that study, ethanol was also used as an e-donor to support the microbial reduction of Pd(II). In the same way, Pd(II) reduction was not present in treatments lacking the granular sludge, suggesting that microorganisms are required for the reduction of Pd(II) to Pd(0). Even though the behavior was similar for both metals, the microbial consortium was able to reduce Pd(II) more rapidly than Pt(IV).

The effect of different e-donors on the reduction of Pt(IV) was studied and the results are shown in Figure 2. The different e-donors varied in behavior with respect to chemical and biological reduction of Pt(IV). H₂ and formate caused a very noteworthy chemical reduction of Pt(IV). In the case of H₂, biological conditions stimulated the H₂ chemical rate by 50%. However, biological conditions had a negative impact in the chemical reduction of Pt(IV) by formate causing a 32% decrease in the reduction rate. Ethanol was the only e-donor which served exclusively to catalyze the microbial reduction of Pt(IV), which was much faster than the endogenous controls. A possible mechanistic explanation is that the microorganisms carried out the acetogenesis of ethanol into acetate and H₂¹⁹ and then, H₂ was responsible for the chemical Pt(IV) reduction (Figure 3). Similarly, lactate exclusively supported the biological reduction of Pt(IV) but its impact was limited causing only a small stimulation (36%) beyond the endogenous control rate. On the other hand, the addition of acetate and pyruvate did not affect the Pt(IV) reduction rate when compared to the endogenous controls. This supports the idea that microbially produced H₂ was responsible for the Pt(IV) reduction in the treatments with ethanol.

Interestingly, the contributions of the chemical reduction of Pt(IV) and other PGM and noble metals by H₂ and formate have been overlooked by most researchers who were up to now more focused on the effects of the microbial reduction¹³. To the best of our knowledge, this is the first report in which the H₂ intermediate of acetogenesis is suggested to be responsible for the chemical reduction of Pt(IV) to Pt(0).

The time course of Pt(IV) concentration in the aforementioned incubations with granular sludge using different e-donors is shown in Figure S1 (Supplementary Information). Considering the e-donor treatments that provided a demonstrable rate enhancement due to biological activity (ethanol, H₂, lactate), none of them had a discernible lag phase suggesting that the Pt(IV) reducing ability is an innate capacity of the existing organisms. The exact mechanism responsible for Pt(IV) reduction has not been identified yet. Nonetheless, a previous study suggested the implication of hydrogenases in the reduction of Pt(IV) by sulfate reducing bacteria¹⁵.

There is little information regarding the microbial reduction of Pt(IV) in the literature. Among the existing studies, the majority focus on the effect of pure cultures of bacteria on the reduction of Pt(IV). Pt(IV) reduction was studied using the bacterium *Shewanella algae*²⁰. In that study, *S. algae* was able to reduce an initial concentration of 1mM (410 mg L⁻¹) of H₂PtCl₆ to 0.1 mM (41 mg L⁻¹) in ca. 60 min using sodium lactate as the e-donor (30 mM). Endogenous and non-inoculated treatments did not have any effect suggesting that the bacterial cells catalyzed the reaction. Another report studied the reduction of Pt(IV) using

the lactate grown cells of bacteria *Desulfovibrio alaskensis* G20²¹. That study reported the ability of *D. alaskensis* to reduce 2 mM of PtCl₄ in 2 h.

Besides Pt(IV), the impact of microorganisms has been studied for the reduction of other PGM such as Pd^{13, 17} and rhodium (Rh)^{22, 23} and noble metals like silver (Ag)²⁴ and gold (Au)²⁵. Studies with Pd(II) demonstrated that the metal can be recovered by chemical reduction and biosorption; however, the rates were highly increased when H₂ and formate are used as e-donors. As it was reported for Pt(IV), the reduction of Pd(II) by a hydrogenase-mediated mechanism is implicated since the reduction was inhibited by Cu²⁺¹⁷. In a similar way, studies with Rh(III) showed the possibility of Rh(III) removal by endogenous and chemical reduction as well as biosorption. Nonetheless, the reduction rate was enhanced in the presence of e-donors especially H₂²². Interestingly, the reduction of Rh(III) was not complete and the reduction efficiency varied with different microorganisms and different physiological conditions. In the case of Ag, it was reported that *Bacillus* sp. was able to reduce Ag(I) to Ag(0) NPs which were deposited in the periplasmic space²⁴. Finally, studies with Au(III) showed that the reduction is only possible under the presence of H₂; no chemical or endogenous reduction occurred and similar with other metals, hydrogenases were implicated in the removal of Au(III)²⁵.

Characterization of the end products

To determine whether Pt(IV) was reduced to elemental platinum, Pt(0), XRD analyses were carried out. The results of these analyses are shown in Figure 4. The best e-donors were formate and H₂ which worked mostly as a chemical reaction albeit that the reaction took place in the complete treatment with sludge, and ethanol which worked exclusively as an e-donor for microbially catalyzed reduction reactions. H₂, formate and ethanol provided the largest XRD peaks corresponding to Pt(0). On the other hand pyruvate and lactate were poor microbial e-donors, and this was reflected by poor development of the key XRD peaks expected for Pt(0). Acetate was not an e-donor because it behaved the same as the endogenous control. As mentioned previously the endogenous control removed Pt(IV) slowly from solution but did not discolor the sludge black. This discoloration corresponded to the lack of detectable formation of characteristic Pt(0) peaks.

The platinum NPs formed were examined by TEM. Figure 5 indicates the formation of both, intracellular and extracellular Pt(0) NPs by reduction of Pt(IV) under anaerobic conditions using H₂ as e-donor. Figure 5A shows that intracellular NPs are bulging the cell membrane which means that the NPs are synthesized inside the cell or in the periplasm. Figure 5B shows that the NPs had a spherical shape and an average particle diameter of 3.52 nm. Extracellular platinum NPs agglomerated to form bigger nanostructures with sizes going up to 199 nm (Figures 5C and 5D).

The formation of Pt(0) NPs has been reported for different microorganism strains and different reaction conditions which led to the formation of both intracellular and extracellular Pt(0) NPs. This suggests that the physiological conditions in which the reaction takes place plays an important role in the localization of the NPs.

The formation of intracellular Pt(0) NPs has been demonstrated in different microorganisms. Intracellular Pt(0) NPs were found in an uncharacterized consortium of sulfate reducing bacteria supplemented with Pt(IV) in the presence and absence of H₂¹⁵. In addition, intracellular Pt(0) NPs from Pt(IV) was also observed in studies with the bacterium *S. algae* and using lactate as e-donor²⁰.

The formation of extracellular Pt(0) NPs has also been demonstrated. Formation of extracellular Pt(0) NPs from Pt(IV) was observed in experiments with the fungus *Fusarium oxysporum*³ and the bacterium *Desulfovibrio. alaskensis* G20 cells under anaerobic conditions using lactate as a carbon and energy source²¹.

Besides Pt, formation of nanoparticles has also been demonstrated for other PGM and noble metals in microbial studies. Multiple studies have shown that Pd(0), Rh(0) and Au(0) NPs formed from reduction of their corresponding ions can occur extracellularly¹⁷, or intracellularly^{23, 25} or both²². One study reported the change in Pd(0) NPs size and location depending on the e-donor used, suggesting that the physiological conditions could impact how and where the NPs are formed¹³. This fact could be of importance for future industrial applications in which the separation of the final product is of key concern.

Impact of Pt(IV) concentration on its reduction rate

The influence of the initial concentration of Pt(IV) in the microbial reduction with H₂ as e-donor was studied as is shown in Figure 6. A marked increase in the maximum reduction rate was observed with increasing initial Pt(IV) concentration. The relationship between the initial reduction rate and the initial concentration of Pt(IV) followed first-order kinetics. The kinetic evolution of Pt(IV) reduction as a function of concentration can be seen in Figure S2. The curves follow a typical 1st order kinetic pattern. It is important to notice that this relationship is only valid at studied concentration range since higher concentrations may present inhibition, and thus a decrease in the reduction rate could potentially be expected.. Previous studies reported the effect of initial concentrations of Pt(IV)²⁶, Au(III)²⁵ and Rh(III)²² on their respective reduction rates. Interestingly, the previous study with Pt(IV) reported that increasing initial concentrations of Pt(IV) did not cause an increase in the reduction rate implying that the reduction rate was limited by a maximum activity of the microorganisms. Another possibility is that higher concentrations of Pt(IV) are inhibitory to reduction of Pt(IV) albeit we did not observe that. In addition, the study with Au(III) did not find any relationship between the initial concentration of Au(III) and the reduction rate. However, in the case of Rh(III) it was demonstrated that increasing concentrations of Rh(III) led to increasing reduction rates. The relationship between the initial concentration of the metal ion and the reduction rate seems to be specific for the metal and microbial culture being studied. Nonetheless the physiological conditions under which the reaction takes place could play a crucial role.

Impact of redox mediators in the reduction of Pt(IV)

Redox mediators (RM), are molecules that can be reversibly reduced and oxidized, so they can be used as electron carriers in a wide range of reactions. The big advantage of these molecules is that they can serve as electron shuttles increasing the rate of microbial

reactions²⁷. Experiments were performed in order to study the effect of three different redox mediators (riboflavin, lawsone, and AQDS) on the rate of Pt(IV) reduction to Pt(0).

Figure 7 shows the effect of the redox mediators on the microbial and abiotic reduction of Pt(IV) using a molar ratio Pt:RM= 1:1. The addition of redox mediators led to considerable increases in the reduction rates when utilizing H₂ as the e-donor in assays with anaerobic sludge. The results in the graph clearly indicate that the redox mediators were able to increase the reduction rates. Riboflavin was the best redox mediator for both the condition with and without sludge present; increasing the reduction rate by 7- and 5-fold, respectively. The time course evolution of the impact of redox mediators on the microbial and chemical reduction of Pt(IV) is shown in Figure S3. It can be clearly seen that the enhancement occurs over the first hours; thereafter the rates become similar to the endogenous rate.

Once it was demonstrated that redox mediators can increase the maximum rate of the reduction of Pt(IV) and that riboflavin was the best redox mediator, an optimization of the process was studied in order to reduce the amount of riboflavin required. Figure 8 shows the impact of riboflavin concentration on the microbial reduction of Pt(IV) with H₂ using different Pt:RF molar ratios. As can be seen in the graph, decreasing concentrations of riboflavin (higher Pt:RF molar ratio) were associated with a decrease in the reduction rate. The effect of the molar ratio on the rate indicates that there is an important compromise. The treatments with the 30:1 and 100:1 molar ratios are good because the quantity of riboflavin is lowered; however, the consequence is that reduction rates are low compared to the treatments with 1:1 molar ratios. As a result, the best option may be the compromise at the 10:1 molar ratio which required a lowered quantity of riboflavin while still delivering an acceptable rate.

To the best of our knowledge there is no previous information about the influence of redox mediators in the microbial reduction of Pt(IV). Nonetheless the effect of different redox mediators has been studied in the microbial reduction of Te(VI) and Te(IV) to Te(0) NPs in a very recent report¹⁸. In that research, lawsone and riboflavin were found capable of increasing the rate of the microbial reduction of Te(VI) and Te(IV), being lawsone the most effective for the reduction of Te(VI) and riboflavin the most effective for the reduction of Te(IV). In addition, there is one report which studied the effect of the addition of riboflavin in the microbial reduction of uranium (U), technetium (Tc), neptunium (Np) and plutonium (Pu) with *S. oneidenis* MR-1²⁸. As it was reported, the addition of 10 µM of riboflavin, enhanced the rate of the microbial reduction of Tc(VII) to Tc(IV), Pu(VI) to Pu(III) and Np(V) to Np(IV) to a lesser extent, but riboflavin did not have any impact on the reduction of U(VI) to U(IV). Interestingly, the range of redox mediator concentrations in all three cases is very similar 160-16 µM in the case of Te(VI) and Te(IV), 130-13 µM in the case of Pt(IV) and 10 µM in the cases of Tc(VII), Pu(VI) and Np(V).

Methanogenic toxicity assays

Finally, the inhibition of acetoclastic methanogenic activity by Pt(IV) was studied. The evolution of the methane production as a function of Pt(IV) concentration is represented in Figure 9. A sharp decrease of the methane production rate was observed with increasing initial Pt(IV) concentrations, indicating that Pt(IV) causes a concentration dependent

inhibition to acetoclastic methanogens in the sludge. The methanogenic activity was completely inhibited at concentrations of 10 mg L⁻¹ or higher. The Pt concentration causing 50% of methanogenic inhibition (I₅₀) was approximately 3 mg L⁻¹. The time course of the acetoclastic methanogenic assay is shown in Figure S4. The graph shows that at concentrations of 10 mg L⁻¹ or higher, methane production ceased after 25 h.

This is the first time that the effect of Pt(IV) on the methanogenic activity of an anaerobic microbial consortium has been investigated. Thus there is no bench mark in the literature to compare the observed methanogenic toxicity of Pt(IV). However, the methanogenic toxicity of Pd(II) has been reported¹⁴. In that research, acetoclastic methanogens were highly inhibited at very low concentrations. The concentration of Pd(II) causing 50% of methanogenic inhibition was 0.96 mg L⁻¹ and nearly complete inhibition (92%) was observed at Pd concentrations of 3 mg L⁻¹. Thus, Pt(IV) and Pd(II) have comparable methanogenic toxicities.

Acetogenesis requires a H₂-consuming process to make it bioenergetically favorable (G° for ethanol acetogenesis is +9.5 kJ/mole ethanol)¹⁹. The inhibition of methanogenesis by Pt(IV) might knock out the H₂- consuming partner in the consortium that would be needed to make the reaction favorable. Nonetheless that role may be taken over by the chemical consumption of H₂ by Pt(IV).

CONCLUSIONS

This study demonstrated the ability of a microbial consortium in anaerobic granular sludge to reduce Pt(IV) to Pt(0) NPs as confirmed by XRD and TEM analyses. Both chemical and biological mechanisms of reduction were observed. Formate, H₂ and ethanol supported rapid chemical, both chemical and biological, or biological reduction of Pt(IV), respectively. A hypothesis of ethanol acetogenesis forming H₂ that subsequently causes chemical reduction of Pt(IV) was put forth indicating an integrated biological/chemical process. The rate of Pt(IV) reduction followed first order kinetics. Redox mediators such as riboflavin greatly improved Pt(IV) reduction rates. Methanogenic activity in the anaerobic was inhibited by a few mg L⁻¹ of Pt(IV).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Time course of Pt(IV) concentration by a methanogenic granular sludge using ethanol as electron donor. Legend: () abiotic control, (▲) endogenous control, (■) complete inoculated treatment with ethanol supplied at 0.368 g COD L⁻¹ and (X) heat killed cells control. The measured initial Pt(IV) concentrations were: 23.45 mg L⁻¹ in the abiotic control, 24.10 mg L⁻¹ in the endogenous control, 23.92 mg L⁻¹ in the complete inoculated treatment and 21.08 mg L⁻¹ in the heat killed cells control.

Figure 2. Maximum rates for biological reduction (□) and chemical reduction (■) of Pt(IV) obtained in treatments with different electron donors. The time course of Pt(IV) reduction is shown in Figure S1 in the supplementary information.

Figure 3.
Integrated biological and chemical mechanism for Pt(IV) reduction by ethanol acetogenesis.

Figure 4.

Results of XRD analyses. Panel A: samples (1 complete treatment with formate, (2) complete treatment with ethanol and (3) complete treatment with H₂. Panel B: analyses of samples (4) complete treatment with pyruvate, (5) complete treatment with lactate, (6) complete treatment with acetate, and (7) endogenous control. (†) Representative peaks for Pt(0).

Figure 5. TEM images showing the formation of Pt(0) nanoparticles produced by the microorganisms under anaerobic conditions in the presence of Pt(IV). Panels A and B: images of the intracellular NPs formed. Panels C and D: platinum NPs agglomerated to form bigger NPs with sizes going up to 198.7 nm.

Figure 6.

Influence of Pt(IV) initial concentration in the initial reduction kinetics. The time course of aqueous Pt(IV) concentrations are shown in Figure S2.

Figure 7. Maximum rates for biological reduction (□) and chemical reduction (■) obtained in treatments with different redox mediators with a Pt:redox mediator molar ratio of 1:1. The time course of Pt(IV) concentrations are shown in Figure S3.

Figure 8. Maximum rates of Pt(IV) reduction by an anaerobic granular sludge using H₂ as electron donor and different Pt:Riboflavin molar ratios.

Figure 9.

Maximum methanogenic activities as a function of the platinum concentration. Activities were calculated from the data points of the first 21 to 47 h of the total experiment time. The methane production time course can be seen in Figure S4.