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Recovery of Elemental Tellurium Nanoparticles by the Reduction of Tellurium Oxyanions in a Methanogenic Microbial Consortium

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Abstract

This research focuses on the microbial recovery of elemental tellurium (Te^0) from aqueous streams containing soluble tellurium oxyanions, tellurate (Te^{VI}) and tellurite (Te^{IV}). An anaerobic mixed microbial culture occurring in methanogenic granular sludge was able to biocatalyze the reduction of both Te oxyanions to produce Te^0 nanoparticles (NPs) in sulfur-free medium. Te^{IV} reduction was 7-fold faster than that of Te^{VI}, such that Te^{IV} did not accumulate to a great extent during Te^{VI} reduction. Endogenous substrates in the granular sludge provided the electron equivalents required to reduce Te oxyanions; however, the reduction rates were modestly increased with an exogenous electron donor such as H_2. The effect of four redox mediators (anthraquinone-2,6-disulfonate, hydroxocobalamin, riboflavin, and lawsone) was also tested. Riboflavin increased the rate of Te^{IV} reduction by 11-fold and also enhanced the fraction Te recovered as extracellular Te^0 NPs from 21% to 64%. Lawsone increased the rate of Te^{VI} reduction by 5-fold and the fraction of Te recovered as extracellular material increased from 49% to 83%. The redox mediators and electron donors also impacted the morphologies and localization of Te^0 NPs, suggesting that NP production can be tailored for a particular application.

Keywords

Tellurium; nanoparticles; redox mediator; lawsone; riboflavin; hydroxocobalamin; AQDS; methanogenic granular sludge

INTRODUCTION

Tellurium (Te) is a metalloid which belongs to group 16 of the periodic table. It can be found in the environment in different oxidation states as tellurate TeO_4^{2-} ((+6), Te^{VI}), tellurite TeO_3^{2-} ((+4), Te^{IV}), or elemental Te^0 (0), which is a brownish-black or silver-shiny solid[1], and telluride (−2)[2]. The average Te concentration in the earth's crust is estimated

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ASSOCIATED CONTENT

Supporting Information

More information is available showing the description of materials and methods used in this work, a comparison of the rates of reduction of both oxyanions under different experimental conditions, the distribution of Te^{IV} between the phases of the experimental systems, TEM and EDS analysis, determination of background S^{2-} concentration in the granular sludge, as well as, a description of the statistical analysis performed to the rates of reduction data obtained for both Te oxyanions. This material is available free of charge on the ACS Publications website at DOI: @@@

to be around 0.027 ppm which is comparable to those of silver and gold[3]. In the lithosphere, Te is present in copper ores and also forms minerals with gold and silver e.g. calaverite ($AuTe_2$) and sylvanite ($AgAuTe_4$)[4, 5]. Despite the low solubility of Te^{VI}, it has been found to be the predominant form of Te in the hydrosphere[6, 7]. The oxyanions Te^{IV} and Te^{VI} are highly toxic to most microorganisms; however, Te^{IV} is more toxic than Te^{VI} [1]. Inhibitory effects have been observed in *Escherichia coli* at concentrations of Te^{IV} as low as 1 μg L^{-1} [8]. To date, Te is commercially obtained from the anode slimes in the process of electrolytic recovery of copper via chemical and pyrometallurgical processes[5].

Tellurium has broad industrial applications ranging from tarnishing metals to improving optoelectronic and thermal properties of steel and glass. Extensive research has been conducted in the development of new materials like Te-based fluorescent quantum dots which are capable to function as probes in biological detection[2, 5, 9]. In the transition to clean energy technology, Te has been widely used to produce CdTe thin film solar cells. This kind of photovoltaic device represents the third most common type of solar panels commercially available[10]. Due to the scarcity of Te, its supply may run out soon[11], compromising its applications and the development of new technologies.

The Department of Energy and the European Union are very concerned regarding a potential shortage in the supply of some strategic and critical elements, such as Te, which are pivotal for the development of advanced technologies[12–14]. Thus, the development of new technologies for the recovery of Te from mining waste streams and from its end-use applications is imperative to ensure its availability[12–14]. Biotechnological processes represent an eco-friendly and cost-effective option to recover critical elements from mine waste streams since microorganisms have proven to be able to reduce a wide range of oxidized elements to their insoluble zero-valent forms (Au^0, Se^0, Pt^0)[15–17]. Particularly, the bio-reduction of Te^{VI}, Te^{IV}, selenate (Se^{VI}), and selenite (Se^{IV}) might be used to recover Te^0 and Se^0 from mining residues, where they are found associated with copper ores[18–20], and from Te containing products to overcome the future tellurium supply risk, and mitigate toxicity concerns[21–23].

Several microorganisms have proven to be able to reduce Te^{VI} and Te^{IV} into its elemental form Te^0, e.g. *Bacillus selenitireducens* and *Sulfurospirillum barnesii* are able to grow using Te^{IV} and Te^{VI} as electron acceptors, respectively[24], and *Desulfovibrio desulfuricans*, a sulfate reducing bacteria (SRB), is able to reduce Te^{IV} and precipitate Te^0 cometabolically without sustaining growth in the process[25]. The actual reduction pathways of Te oxyanions are still not well understood. However, the formation of extracellular and intracellular black deposits of Te^0 nanoparticles (NPs) with different shapes, according to the microorganism and the electron donor used has been reported[24–27]. Elucidating the mechanisms underlying this process, as well as the factors that affect the rates of reduction and formation of Te^0 is critical for the development of practical biotechnological applications. The formation of extracellular material is highly desirable in a Te^0 recovery process to avoid additional downstream processes aimed at lysing cells to release the NPs.

The mechanism of Te reduction can also be affected by the presence of redox mediators (electron shuttles) which are compounds known to mediate biological redox reactions.

Several quinone analogs such as, 2-hydroxy-1,4- napthoquinone (lawsone) and antroquinone-2,6-disulfonate (AQDS) have been reported to enhance the reduction rate of metals, such as palladium (Pd), and to promote the formation of extracellular NPs[28]. Particularly, lawsone has been proven to be an effective accelerator of the reduction of Te^{IV} which also promoted the formation of extracellular Te^0 NPs[29, 30].

The present work is aimed at evaluating the potential of an anaerobic granular sludge from a methanogenic bioreactor to reduce Te^{VI} and Te^{IV} to Te^0 NPs under different experimental conditions. A granular sludge was selected since microorganisms inside the granules are expected to be less directly exposed to toxic metal(loids) compared to planktonic cells[31]. All the experiments in this work were conducted using sulfur (S)-free medium to avoid any possibility of chemical reduction of Te^{VI} and Te^{IV} by biogenic sulfide (S^{2-}) generated by sulate reducing bacteria (SBR) commonly found in anaerobic granular sludge. The background sludge-derived endogenous S^{2-} levels were ≤ 0.0089 mM. The effect of background sludge-derived S^{2-} present in the anaerobic sludge on the reduction of Te^{IV} was investigated and was found to be negligible (see Supporting Information). The effect of two sources of electron donors, acetate and hydrogen (H_2), the impact of four different redox mediators on the reduction of both Te oxyanions, the effect of the redox mediators on the yield of extracellular Te^0 NP formation as well as, changes in Te speciation were also investigated.

MATERIALS AND METHODS

Biomass Source

An anaerobic granular sludge obtained from a full scale up-flow anaerobic sludge blanket (UASB) reactor at Mahou's (beer brewery in Guadalajara, Spain) wastewater treatment plant, was used as the source of inoculum. This biomass contained 0.0792 g volatile suspended solids (VSS) g^{-1} wet wt. The maximum methanogenic activities of the sludge were 565.8±63.8 mg COD-CH_4 g VSS^{-1} day^{-1} and 570.9±25.9 mg COD-CH_4 g VSS^{-1} day^{-1} for the assays utilizing acetate and hydrogen as substrate, respectively. The sludge was stored at 4°C.

Batch Assays

Batch experiments were conducted in 160 mL serum bottles (Wheaton, Millville, NJ, USA), amended with granular sludge, 100 mL of a liquid mixture -containing Te^{IV} or Te^{VI}, mineral basal medium and different electron donors and redox mediators (according of the purpose of the assay)- and 60 mL of headspace. The mineral basal medium used in the assays is described in the supporting information. Aliquots (5 mL) of a 400 mg L^{-1} Te^{IV} or Te^{VI} stock solution were provided for a final concentration of 20 mg L^{-1} (0.157 mM) as Te. The anaerobic granular sludge was added to the serum bottles to reach a final concentration of 1.5 g VSS L^{-1}.

Different electron donors were added to the media as follows: 3.12 mM sodium acetate (stoichiometric excesses of 26.6- and 40-fold in the case of Te^{VI} and Te^{IV}, respectively; based on e^- equivalents of electron donor), or hydrogen (H_2) supplied at 10.7 mmol $H_{2\ gas}$

L^{-1}_{liq} (stoichiometric excesses of 20- and 34-fold in the case of Te^{VI} and Te^{IV}, respectively). The flasks were flushed with a gas mixture of N_2/CO_2 (80:20, v/v), the mixture was bubbled through the liquid phase of the opened flasks for 3 min, and then after closing the bottles with a butyl rubber septum and an aluminum seal, the N_2/CO_2 mixture was passed through the headspace of the bottles for an additional 4 min using an inlet and outlet needle inserted at the top of the stoppers, to eliminate the remaining O_2 and ensure anaerobic conditions in the experiments. After O_2 was eliminated from the flasks, H_2 was provided to the appropriate bottles as a gas mixture of H_2:CO_2 (80:20 v/v) with an overpressure of 8 psi (0.54 atm) by inverting them and injecting the gas directly to the liquid phase, in order to attain the desired concentration (10.7 mmol $H_{2\ gas}$ L^{-1}_{liq}).

The impact of the presence of several redox mediators (RM) at two different concentration levels (estimated to obtain Te:RM molar ratios of 1:1 and 10:1) was tested using H_2 as electron donor. The proper amounts of one of the following compounds: 9,10 anthraquinone-2,6-disulfonic acid (AQDS), riboflavin (RF), hydroxycobalamin (HCB_{12}) or 2-hydroxy-1,4-napthoquinone (lawsone) were supplied to the batch reactors. Concentrated stock solutions of the four compounds were prepared and then, an aliquot was provided to each flask to reach final concentrations of 57.42 and 5.74 mg L^{-1} in the case of AQDS, 58.52 and 5.85 mg L^{-1} for RF, 211.03 and 21.10 mg L^{-1} for HCB_{12} and of 27.30 and 2.73 mg L^{-1} for lawsone, to achieve the desired Te:RM molar ratios.

Several controls were prepared to account for the biological, electron donor and redox mediator contribution to the reduction of Te oxyanions. Un-inoculated (sterile) bottles with or without e^- donor and sterile bottles containing heat killed inoculum amended with or depleted of external electron donors were used. The heat-killed inoculum was prepared by subjecting the sludge in medium (lacking $NaHCO_3$ and yeast extract (YE)) to three autoclaving cycles at 121 °C for 1 h and the bottles were allowed to cool down for 24 h between cycles. Any water lost based on weight difference during the autoclaving was replaced using sterile water and $NaHCO_3$ and YE were provided along with the corresponding Te species and electron donor to get the desired level in 100 mL of medium.

Due to the large volume of sample required to perform the corresponding analyses required to verify the distribution of Te between the liquid and solid phases, the same batch set up preparation procedure was followed as described above, but this time the experiments were conducted in 590 mL serum bottles (Wheaton, Millville, NJ, USA).

All the experiments were carried out as duplicates and incubated in the dark at 30°C on a 105 rpm orbital shaker. Samples of the liquid phases were periodically withdrawn to study Te^{IV} and Te^{VI} reduction as follows: flasks were shaken in order to suspend any colloidal material and then allowed to settle for 1.5 min to sediment the coarser rapidly settleable biological material before carefully withdrawing a sample of the liquid phase, containing dispersed colloidal material, with a syringe. Afterwards, the samples were processed according to the purpose of the analysis.

Total Soluble Te

Liquid samples, obtained as described above, were taken to measure changes in the soluble tellurium concentration. Samples were transferred to centrifugal filters (Amicon® ultra-4 3K, EMD Millipore, Billerica, MA, USA) and immediately centrifuged (Centrifuge 5804, Eppendorf, Enfield, CT, USA) at 4,500 rpm for 25 min. After this step, the filtrate was transferred to a 2% v/v HNO_3 solution. The acidified samples were analyzed for Te using an inductively coupled plasma-optical emission spectroscopy instrument (ICP-OES Optima 2100 DV, Perkin-Elmer TM, Shelton, CT) at a wavelength of 214.281 nm. The detection limit of tellurium was 10 μg L^{-1}.

Te speciation in Liquid Samples (Te^{IV} and Te^{VI})

An adaptation of the method described elsewhere[32] for the solid phase extraction technique (SPE) was used to perform speciation studies to the liquid phase of the reactors. A brief description of the method used in this work is presented in the Supporting Information.

Determination of Te^0 Nanoparticles

Samples of the liquid phase containing colloidal material were digested using 9 mL of concentrated HNO_3 (70% wt) and 3 mL of concentrated HCl (37% wt) according to EPA standard procedures[33]. Digested samples were diluted in demineralized (DI) water to reach a HNO_3 concentration of 2% v/v and were analyzed for Te using an ICP-OES as described above. The amount of dispersed Te^0 NPs was then calculated as the difference between the total Te from the digestions and the total dissolved Te.

Transmission Electron Microscopy (TEM)

Carbon coated 300 mesh grids were floated carbon side down on droplets of NPs suspended in isopropyl alcohol for two minutes. Excess liquid was removed by holding a piece of non-ash filter paper against one edge of the grid and allowed to air dry. Grids were viewed in a Tecnai Spirit Biotwin operated at 100 kV. Eight bit tilt images were captured via an AMT 4M pixel camera. Measurements were taken at eucentric height using FEI TIA software.

Quantification of Volatile Te

The amount of Te volatilized into the gas phase of the flasks used to determine the effect of different electron donors on the reduction of Te^{VI} was quantified as follows. A mixture N_2/CO_2 (80:20 v/v) was passed through the headspace of the bottles using an inlet needle and outlet conduction adapted at the top of the stoppers to purge the volatile Te standing in the gas phase. The outlet conduction was placed inside a 40 mL HNO_3 (1N) trap and the gas mixture was bubbled in the acid solution for 40 min to dissolve any amount of Te coming out of the flasks. The HNO_3-Te mixture was analyzed for Te using an ICP-OES as described above.

RESULTS AND DISCUSSION

Effect of Added Electron-donors Sources on Te Oxyanion Reduction

The effect of adding exogenous H_2 as an electron donor on the reduction of Te oxyanions by anaerobic granular sludge is shown in Figures 1A and 1B for Te^{VI} and Te^{IV}, respectively. The figures show that the presence of anaerobic granular sludge was required to catalyze Te oxyanions reduction. No loss of either oxyanion was observed in controls lacking the granular sludge (Figure 1). Additionally heat-killed granular sludge (autoclaved) did not catalyze the conversion of the Te oxyanions (data not shown). Significant reduction of both oxyanions occurred in the presence of the live anaerobic granular sludge. This suggests a biologically mediated reaction. Addition of H_2 caused a modest but significant stimulation in Te oxyanion reduction. In the case of Te^{VI}, the impact was to lower the lag-phase prior to Te^{VI}-reduction commencing (Figure 1A). In the case of Te^{IV}, the rate of Te^{IV}-reduction was increased slightly. Acetate was also tested as an electron donor to stimulate Te oxyanion reduction but it had no significant effect compared to the endogenous reduction rate (results not shown). The fact that electron donors had either no effect or at best modest effects on the rate of Te oxyanion reduction, clearly suggests endogenous substrates in the sludge were the main source of electron donor.

Anaerobic granular sludges like that used in this study were found to reduce the oxidized forms of the metalloid, arsenic (arsenate, As^V) and the actinide, uranium (hexavalent uranium, U^{VI}) to their reduced biotransformation products, arsenite and uraninite[34, 35]. The ability of anaerobic bacteria to respire and reduce selenium (Se), an element closely related to Te since both belong to the Group 16 of the periodic table, using acetate or hydrogen as the external source of electrons has been previously reported[36]. Also, two different anaerobic granular sludges, obtained from waste water treatment plants, showed the ability to reduce selenate (Se^{VI}), an oxidized form of selenium (Se), to its elemental form Se^0 [37]. The behavior with respect to electron donors with As^V and U^{VI} was very similar to that observed in this study. Arsenate and U^{VI} were readily reduced by the sludge without added electron donor meanwhile, a modest reduction of Se^{VI} was observed in the systems depleted of external electron donor. Addition of acetate to the systems amended with As^V and U^{VI} had no or very minor impacts and H_2 had a significant but modest effect. Taken collectively, H_2, an interspecies electron donor, is effective in accelerating the reduction of these oxidized inorganic elements; whereas acetate is a poor electron donating substrates for reducing oxidized contaminants by the mixed microbial community. The sludge itself contains significant endogenous substrates that drive the reduction reactions. Based on methane production from biomass decay of anaerobic granules, the level of endogenous substrate corresponds to 60 to 166 mg chemical oxygen demand g^{-1} VSS[34, 35]. In this study 1.5 g VSS L^{-1} was utilized that corresponds to 90 to 249 mg COD L^{-1} (11 to 31 e^- meq L^{-1}) of endogenous substrates in the culture. Based on 20 mg L^{-1} Te used in the study, only 0.63 and 0.94 e^- meq L^{-1} were actually needed to reduce either Te^{IV} or Te^{VI} to Te^0, respectively. Thus clearly the reservoir of endogenous substrates in the anaerobic granular sludge was in large excess of that needed to drive the observed reduction.

Several electron donors, including H_2 and acetate, have been proven to support the biological reduction of Te^{VI} and Te^{IV} to Te^0 under similar conditions to those used in this study. The effect of H_2 and acetate was very similar to that observed in this work. Addition of H_2 improved the removal of Te^{IV} in a system amended with sediment slurry under anaerobic conditions[38] and acetate did not provide the required electron equivalents to reduce Te^{VI} in the presence of the strain ER-Te-48[26]. Neither H_2 nor acetate was able to support growth of *Bacillus beveridgei* when Te^{VI} and Te^{IV} were supplied as electron acceptors, respectively[38]. Other electron donors, such as, lactate[24, 38], formate[25] and glycerol[39] were found to serve as effective electron sources for the reduction of both tellurium oxyanions.

Even though the anaerobic granular sludge originated from a UASB treating brewery wastewater that was presumably not contaminated with Te, it is remarkable that both Te^{IV} and Te^{VI} were reduced by the sludge with no lag phase or with only a few days of lag phase. This indicates that the biological system capable of reducing Te oxyanions was intrinsic and probably did not require any special enrichment of Te-oxyanion respiring organisms. Instead fortuitous cometabolic reduction of Te oxyanions is implicated. A similar intrinsic behavior was observed with U^{VI} reduction[35].

Comparison Te^{IV} and Te^{VI} Reduction Rates

A remarkable difference in the rate of Te oxyanions reduction was observed (Figure 1) depending on its oxidation state. This anaerobic granular sludge was able to reduce Te^{IV} species notably faster than Te^{VI} oxyanions. The maximum rate of Te^{IV} reduction was approximately seven-fold faster than that observed with Te^{VI} in both, the endogenous and in the system amended with H_2 as electron donor (in both cases, the differences between the rates of Te reduction are statistically significant $p \leq 0.005$, see Supporting Information for details of the statistical analysis). A summary of the reduction rates obtained for both oxyanions is presented in Table 1. According to these results, it is evident that the reduction of Te^{VI} to Te^{IV} is the rate limiting step in the precipitation of Te^0. Even though, the redox potential of the pair $HTeO_4^-/HTeO_3^-$ ($E^{0\prime} = 0.399$ V) indicates that Te^{VI} would be a better electron acceptor than Te^{IV} (pair $HTeO_3^-/Te^0$; $E^{0\prime} = 0.196$ V), using $E^{0\prime}$(pH 7) calculated from E^0 values[40], the huge difference in the behavior of both reactions must be linked to kinetic factors. Previously there were no reports available in which the rates of reduction of these two oxyanions are compared when utilizing the same source of inoculum.

Impact of Redox Mediators (RM) on the Reduction of Te Oxyanions

RM are organic substances which are known to shuttle electrons from cells to oxidized compounds[41]. Humic substances and their quinone analogs, flavins and cobalamins have been shown to be effective in shuttling electrons from biological reactions to stimulate the reduction of nitroaromatics, polyhalogenated compounds, azo dyes and inorganic compounds, such as, selenium and palladium[41–43].

Figure 1A shows the time course of soluble Te concentration as a function of RM addition to assays with Te^{VI}. The rate of Te^{VI} reduction was slightly to greatly increased in the presence of 10:1 to 1:1 Te:lawsone molar ratios, respectively (Figure 1A). In the case of

Te^{IV}, RF greatly enhanced Te^{IV} reduction already at a Te:RF ratio10:1 (Figure 1B). Even faster Te^{IV} reduction were observable at a Te:RF ratio of 1:1. Lawsone was the most effective RM for Te^{VI}, enhancing the rate by 1.6- to 5.2-fold at Te:lawsone ratios of 10:1 and 1:1, respectively (Table 1, Figure S1 in Supporting Information). The only other RM having a stimulatory impact was HCB_{12} causing a rate increase of 1.6-fold at the equimolar concentration but only after a lag phase of five days. However at the lower concentration, HCB_{12} lowered the rate of Te^{VI} reduction.

Three of the four RM's stimulated Te^{IV}-reduction (Table 1). At a Te:RM ratio of 10:1 lawsone, AQDS and RF increased the reduction rate of Te^{IV} by 2.0, 2.2 and 3.6-fold; respectively; whereas at a Te:RM ratio of 1:1 the rate increase was 4.2, 3.8, and 10.8-fold respectively (Figure S1, Supporting Information). Thus for Te^{IV}, RF was the best RM; however, lawsone and AQDS were also effective RMs (in all cases, the differences in the Te^{IV} reduction rate between the treatment using RM and the control lacking RM are statistically significant $p \leq 0.005$). Table 1 presents the maximum specific reduction rates obtained by amending cultures containing Te^{VI} and Te^{IV}; respectively, with the four different RMs tested at two different concentrations.

Previously research was only conducted on the use of RM (lawsone, AQDS, menadione)[30] to enhance Te^{IV} reduction. To the best of our knowledge, this is the first report of the effect of a RM in the reduction of Te^{VI} oxyanions. The findings of this research are in agreement with the information reported for the reduction of Te^{IV} using lawsone as RM. Lawsone almost doubled the Te^{IV} reduction rate when pyruvate was used as carbon source for the photosynthetic bacterium *Rhodobacter capsulatus*; however, stimulation was reported to be independent of the lawsone concentration[29]. In a second study, lawsone increased the Te^{IV} reduction rate of the bacterium *E. coli* by 10-fold when glucose was used as carbon source[30] and the rate of reduction was dependent of the lawsone concentration. The molar ratios Te:RM used[30] were very similar to the one reported for our study with anaerobic granular sludge (1:0.1–0.6). Our study shows that lawsone is the only effective RM for Te^{VI} reduction. Lawsone, RF and AQDS were effective in stimulating on Te^{IV} reduction rates; however, RF was the most effective RM for Te^{IV}. RF has previously been successfully used to catalyze the reduction of several azo dyes[44, 45], chloroform[46], and ferric iron (Fe^{III})[47]. Likewse AQDS was also previously shown to be effective in shuttling electrons for azo dye reduction[41] and Fe^{3+} reduction[48]. In one previous study, AQDS failed to enhance Te^{IV} reduction by *E. coli* when glucose was added as the carbon source[30], which clearly contrasted our findings here with a mixed anaerobic consortium.

According to the standard redox potentials ($E^{0'}$ for pH 7) of the chemical species involved in these reductions, $2H^+/H_2$ $E^{0'}$=−0.414 V[49]; RF $E^{0'}$= −0.208 V[50]; lawsone $E^{0'}$= −0.145 V[29]; $HTeO_4^-/HTeO_3^-$ $E^{0'}$= 0.399 V; $HTeO_3^-/Te^0$ $E^{0'}$ = 0.196 V (calculated from E^0 values[40]) the two RM compounds would be potentially effective electron shuttles, since their redox potentials are between those of the electron donor and electron acceptor reactions (H_2 oxidation and Te oxyanions reduction). The failure of HCB_{12} to act as a RM might be explained by the highly negative redox potential of HCB_{12} (E^0= −0.530V[51]) that was outside of the range. However, their effectiveness as electron shuttles is also dependent of the energy of activation of their reduction and oxidation[41].

Distribution and Speciation of Tellurium

Batch experiments were conducted in 590 mL glass flasks to study the distribution and speciation of the total Te between the solid and liquid phases of the systems as a function of time. The monoprotonated oxyanions, $HTeO_3^-$ (Te^{IV}) and/or $HTeO_4^-$ (Te^{VI}), are expected to be the predominant species of Te in the liquid phase at pH 7 based on the pKa values of 5.45 and 7.74 for H_2TeO_3, and 6.17 and 10.38 for H_2TeO_4[52]. Meanwhile, in the solid phase Te^0 NPs were found in the colloidal fraction (the material that did not settle with the coarser material after 1.5 min), as well as, internalized Te^0 NPs and monoprotonated Te species adsorbed onto the positively charged material of the granules in the settleable solids fraction. Figures 2 and S2 (Supporting Information) depict the changes in soluble Te^{VI} and Te^{IV} concentration, the formation of colloidal Te^0 suspended in the liquid media, and the Te associated with settleable solids. Both figures represent the mass balances of Te in the systems as a function of the incubation times.

During the reduction of soluble Te^{VI} to Te^0, no accumulation of Te^{IV} was detected, in the liquid media. A significant fraction of colloidal Te^0 remained dispersed in the liquid media at the end of the incubation periods in the systems amended with Te^{VI}; whereas, the colloidal fraction in systems amended with Te^{IV} was much lower by comparison. However, the most relevant contribution of this work to the study of the recovery of Te^0 NPs is the noteworthy effect of the addition of a RM to the biological systems. A remarkable increase in the amount of Te^0 NPs formed extracellularly to the cells was found. In order to corroborate that the total Te amended to the systems was distributed only between the liquid and the solid phase, the gas phase of selected bottles was analyzed at the end of the incubation period for Te content as described before. These findings are discussed in more detail in the following paragraphs.

The first line of evidence for the reduction of both Te-oxyanions and the formation of Te^0 NPs is the elimination of dissolved Te^{VI} and Te^{IV} oxyanions during the biological active incubations. Te^{IV} was observed to not be a major intermediate during Te^{VI} reduction (Figure 2). Te^{IV} only briefly accumulated compared to the total Te in system at the beginning of the incubation period in treatments receiving H_2. These findings support the idea that the reduction of Te^{VI} to Te^{IV} is the limiting step in Te^{VI} reduction to Te^0. The observation of Te^{IV} as a transient intermediate was also noted during the growth of *S. barnesii* on Te^{VI} using lactate as external source of electrons[24]. In the biologically active cultures, the loss of dissolved Te oxyanions was concomitant with visually observable formation of a black or dark brown dispersed precipitates in the liquid media and associated with the granules. The most compelling evidence of the Te^0 NP is the TEM-EDS imaging of samples collected from the dispersed precipitates (Figures 3 and S3, in Supporting Information) from bioassays reducing Te^{VI} and Te^{IV} showing Te-containing particles with nano-dimensions (discussed in more detail below).

The formation of extracellular and intracellular Te^0 NPs produced via the reduction of Te^{IV} and Te^{VI} oxyanions has been observed by different microorganisms, in the presence of different electron donors. Internal deposits of Te^0 NPs were found in the cytoplasm of the phototrophic bacteria *R. capsulatus* when grown under either aerobic or anaerobic/

photosynthetic conditions using fructose as the carbon source, in the presence of Te^{IV}. The shape of the NPs was influenced by the growth conditions[27, 53]. Evidence of the formation of Te^0 crystallites bound to the periplasmic space or to the plasma membrane was found for gram negative bacteria such as, *Pseudomonas aeruginosa* and *Erwinia carotovora* VKM B-567 using glucose as the electron donor, and *E. coli* using lactate[54]. External precipitation of Te^0 NPs was evident in systems where the reduction of Te^{IV} and/or Te^{VI} was studied in the presence of the haloalkaliphilic bacteria: *Bacillus beveridgei*[38], *B. selenitireducens*, and *S. barnesii*[24], using lactate as the electron donor.

A remarkable increase in the formation of extracellular Te^0 NPs due to the presence of RM, such as, RF and lawsone was observed. These findings might contribute importantly to the development of efficient Te recovery bio-technologies. From the stand point of recovering Te^0 NPs, the formation of an extracellular dispersion of Te^0 NPs is preferable over intracellular Te^0 or Te oxyanions adsorbed to biomass that would be associated with the settleable solids. Direct recovery of Te^0 from the extracellular culture fluid would obviously negate the need for additional processing steps aimed at releasing the metalloid NPs from the sludge. Lawsone had a noteworthy impact on increasing the fraction of extracellular Te^0 NPs during the reduction of Te^{VI} (Figure 2) and, riboflavin (and to a lesser extent lawsone) followed the same trend during Te^{IV} reduction. After 35 d in the bioassays with Te^{VI}, 83% of the total Te was found as dispersed NPs outside the cells when lawsone was used; meanwhile, only 49% and 27.1% were found dispersed in the liquid media of the control lacking RM and, in the control lacking both H_2 and RM, respectively. Lawsone, also achieved a high fraction of extracellularly dispersed Te^0 NPs faster during Te^{VI}-reduction, requiring less than 5 days compared to 20 days when the bioassay lacked this RM. The remainder of Te was associated with settleable solids. After 2 days in the bioassay with Te^{IV} (Figure S2), the formation of extracellular Te^0 was not as high as in the case of Te^{VI}; however, both RF and lawsone greatly enhanced the fraction of the extracellular dispersed Te^0 NPs. RF enhanced the fraction of extracellularly formed Te^0 NPs the most. The formation of extracellular Te^0 NPs was 64.1% and 52.5% of Te when RF and lawsone were used as the RMs, respectively; meanwhile, only 24% and 19% of the Te was recovered as extracellular Te^0 NPs in the treatments lacking RM and lacking both H_2 and RM, respectively. As was observed with Te^{VI}-reduction, the RMs, achieved a high fraction of extracellularly dispersed Te faster during Te^{IV} reduction, requiring only 0.3 days compared to 2 days when the bioassay lacked the RM. The effectiveness of lawsone to enhance the extracellular precipitation of Te^0 and Se^0 from Te^{IV} and Se^{IV} oxyanions has also been reported for *E. coli* in systems amended with glucose as an electron donor and Te/Se:RM molar ratios of 10:1 through 1.7:1[30] and for the photosynthetic bacteria *R. capsulatus* using pyruvate as the carbon source and a Te:RM molar ratio 5:1[29]; the increase of extracellular material when the RM was added to the experimental system was assessed based on qualitative observations. An increase in the formation of extracellular NPs of Pd^0 from Pd^{II} by *Geobacter sulfurreducens* was also reported when acetate and AQDS were supplied to the systems as electron donor and RM, respectively[43]. The present study is the first attempt to quantify the importance of RM on enhancing the extracellular fraction of Te.

Even though several microorganisms have been found to produce Te volatile species when provided with Te^{IV} [55–57], the amount of Te in the gas phase of the systems amended with Te^{VI} was assessed in this work and found to be insignificant (~ 0.14%) compared to the total Te provided to the liquid phase (data not shown). This fact supports our assumption that Te is highly converted to insoluble Te^0 since at the end of the incubations no soluble Te was measured in the liquid phase nor was significant Te measured in the gas phase at the end of the incubation period.

TEM-EDS Evidence

The presence of Te^0 NPs in the extracellular environment was corroborated with TEM-EDS evidence in the bioassays reducing Te^{VI}. In the endogenous control, irregular spherical nanoparticles (~ 120 nm in diameter) are evident and they appear to be built of clusters of smaller size rod-shaped particles can be observed in Figure S3A. The extracellular NPs observed in the systems supplied with H_2 (Figure S3B) occur as more ordered bundles formed by agglomerated rods of ~ 120 nm in length and the width of the rods range from 10 to 20 nm. Clusters of disorderly oriented rod-shaped NPs can be observed in Figure S3D. The extracellular material precipitated in the systems supplied with lawsone as RM occurred as agglomerated rods of ~ 100 nm in length and the width of the rods varies between 10 and 20 nm. The difference between the orderly shaped bundles from Figure S3B and the clusters coming from disorderly oriented rods in Figure S3D, might be explained by electrostatic interactions between the individual rods. The images presented in Figure S3 suggest that the shape of the Te^0 NP clusters depends on the presence or absence of an added electron donor and RM. Energy dispersive X-ray spectrometry (EDS) analysis confirmed the dominant composition of Te in the NPs produced in both systems. Figure S3C depicts only the spectra obtained for the endogenous control since it is very similar to that obtained for the system using H_2 as electron donor. A surprisingly difference in Te^0 NPs shape has been observed previously when comparing the growth of *S. barnesii* and *B. selenitireducens* on Te^{IV} using lactate as carbon source. Nanospheres were observed as the end-product of Te^{IV} reduction by *S. barnesii* and nanorods, similar to those of this work, were found in the culture with *B. selenitireducens*[24]. The morphology of the NPs obtained in the bioassays reducing Te^{IV} with lawsone or RF was very similar to those precipitated in the systems given Te^{VI} (Figure S4, Supporting Information).

Even though the reduction mechanisms of Te^{VI} and Te^{IV} are not fully elucidated yet, the images presented in Figures 3 and S4, corroborate that the reduction and precipitation of Te oxyanions in this anaerobic sludge occurs both, intracellularly and extracellularly. Figure 3A depicts the nucleation of needle-like NP structures highly associated with the cells in the system supplied with Te^{VI}, H_2 and no RM. The bundles observed in Figure S3B (obtained in the same system) might have been formed once the individual rods were released from the cells to the extracellular environment due to electrostatic interactions. In addition to cell associated Te^0 NPs, Figures 3C and 3D clearly show evidence of clusters of needle-like rods in the extracellular environment of the systems amended with H_2 and lawsone. Evidence of the formation of intracellular Te^0 NPs is shown in Figure 3B. Clusters of Te^0 shards and individual NPs can be observed in the cytoplasm of the cells coming from the systems where the reduction of Te^{VI} was assessed using H_2 as electron donor with no RM. Agglomerations

of Te^0 NPs were also observed associated to the cell membranes in the systems amended with Te^{IV} (Figure S4). These findings correlate well with previous reports in which the reduction of Te^{IV} was studied. The formation of Te precipitates in the cytoplasm of *R. capsulatus* grown under anaerobic-photosynthetic conditions using pyruvate as the carbon source was observed[29] as well as, the accumulation of Te^0 nanorods in the periphery of *B. selenitireducens* cultured under anaerobic conditions using lactate as electron donor[24]. In both studies, the formation of extracellular precipitates was also confirmed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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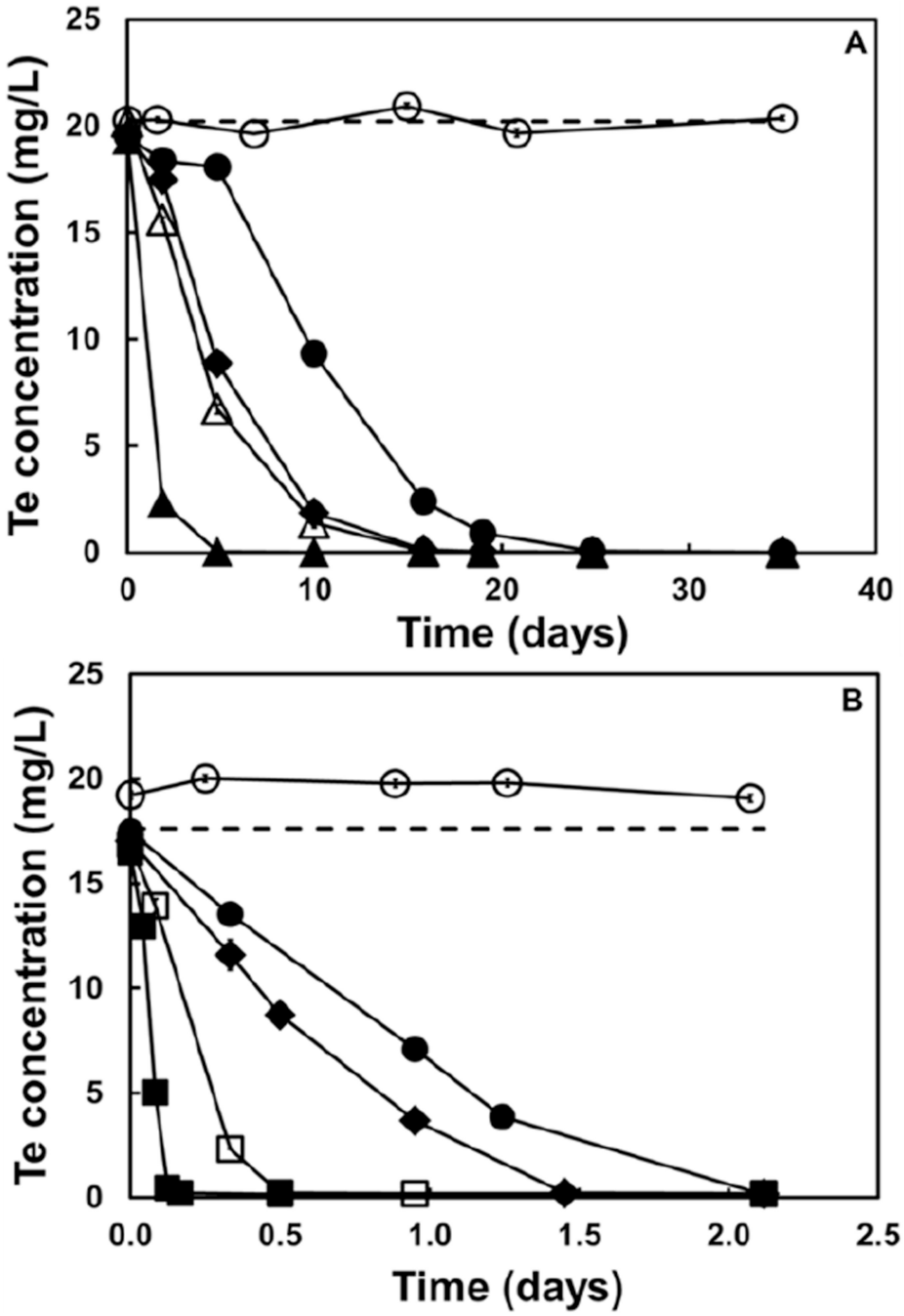

Figure 1.

Time course of the reduction of tellurium oxyanions in the presence of different redox mediating compounds using H_2 as electron donor. **Panel A**, tellurate (Te^{VI}) as the initial Te species. **Panel B**, tellurite (Te^{IV}) as the initial Te species (please note the large difference in time scale between panels). Legends: (- - -), initial concentration of Te; (○), sterile control; (●), live control without electron donor or RM; (◆), live assay with H_2 as electron donor without RM; (Δ), live assay with lawsone as RM at a molar ratio 10:1 (Te:RM); (▲), live assay with lawsone as RM at a molar ratio 1:1 (Te:RM); (□), live assay with riboflavin (RF)

as RM at a molar ratio 10:1 (Te:RM); (■), live assay with RF as RM at a molar ratio 1:1 (Te:RM).

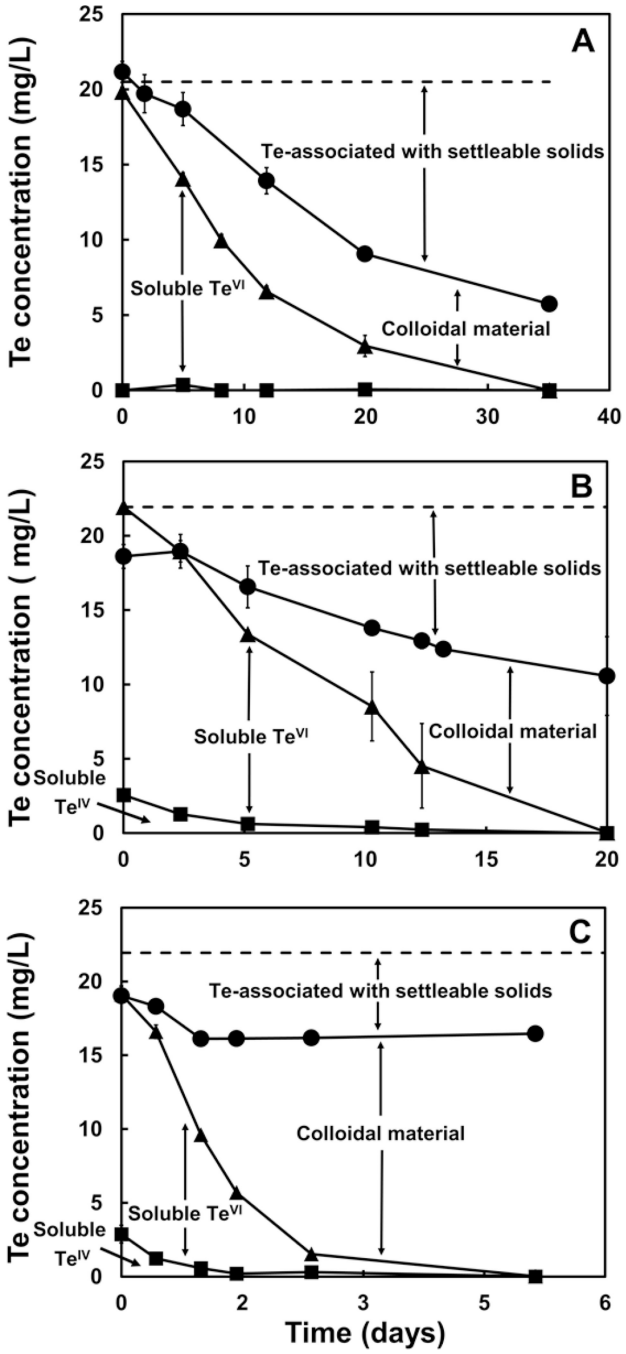

Figure 2.

Distribution of Te^{VI} between the phases of the inoculated system with H_2 as exogenous electron donor and lawsone as RM. **Panel A**, control lacking external electron donor and RM. **Panel B**, control with H_2 as electron donor and no RM. **Panel C**, system with H_2 as electron donor and lawsone at a molar ratio 1:1 (Te:RM). Legends: (- - -), initial concentration of Te in the liquid phase; (●) sum of colloidal and dissolved Te in the liquid phase; (▲) total dissolved Te; (■) dissolved Te^{IV}.

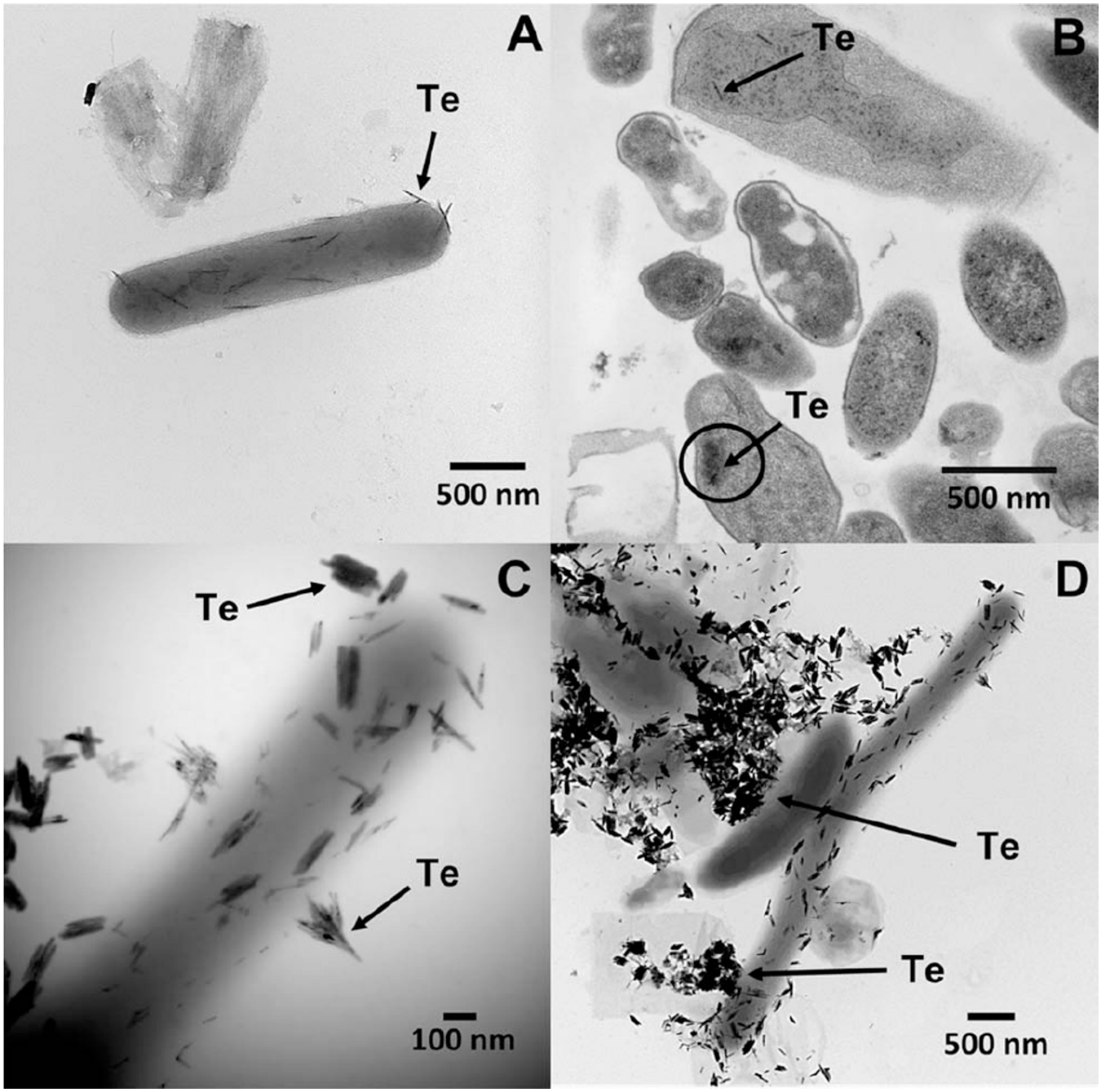

Figure 3.

TEM image showing the formation of extracellular and intracellular Te^0 NPs produced by the anaerobic granules using Te^{VI} as the initial source of Te with H_2 as exogenous source of electrons, in the presence or absence of lawsone. **Panels A** and **B**, Te^0 NPs formed extracellularly and intracellularly when the bioassays were amended with H_2 as electron donor with no RM, respectively. **Panels C** and **D**, Te^0 NPs formed when the bioassays were amended with lawsone as RM.

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Table 1

Specific Reduction Rates of Te^{VI} and Te^{IV} Under Different Experimental Conditions.

External electron donor	Redox Mediator	Te:RM molar ratio	Te^{VI} Specific rate (mg $gVSS^{-1}$ day^{-1})			Te^{IV} Specific rate (mg $gVSS^{-1}$ day^{-1})		
			Mean[e]	Std. deviation	r^2	Mean[e]	Std. deviation	r^2
None	None[a]	-	0.92	0.03	0.990	6.40	0.80	0.998
H_2	None[b]	-	1.18	0.08	0.986	8.30	1.10	0.994
	Lawsone	10:1	1.89	0.09	0.997	16.70	0.50	0.999
		1:1	6.08	0.1	1.000	35.20	0.60	0.998
	AQDS	10:1	1.07	NA[c]	0.984	18.3[d]	0.30	0.924
		1:1	0.93	NA[c]	0.987	31.30	0.20	0.995
	HCB_{12}	10:1	0.66	0.03	0.995	8.00	0.00	0.994
		1: 1	1.83[a]	0.03	1.000	8.70	0.20	0.976
	RF	10:1	1.24	0.15	0.999	29.50	0.20	0.992
		1:1	1.25	0.19	0.999	89.60	0.60	0.979

[a] A lag phase of five days was observed.

[b] A lag phase of two days was observed.

[c] NA=Not available

[d] A lag phase of 4 h was observed.

[e] The specific rates were estimated from the slopes of the time courses of Te oxyanions reduction using a linear regression of at least 3 experimental points, Due to the nature of the data, only 2 points were used in the system using Te^{VI} and lawsone at a Te:RM molar ratio of 1:1.