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

Supporting Information

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Chemicals

Te^{IV} as Na_2TeO_3, Te^{VI} as $K_2TeO_4 \cdot 0.5\ H_2O$ (the stoichiometry of the molecular water was based on comparing the Te concentration of the salt with that of a Te standard), 9,10-anthraquinone-2,6- disulfonic acid (AQDS) disodium salt (≥ 98% purity), riboflavin (RF), and hydroxocobalamin, acetate salt were purchased from Sigma-Aldrich (St. Louis, MO, USA), 2-hydroxy-1,4-napthoquinone (lawsone) was obtained from TCI (Tokio Chemical Industry Co, LTD, Tokio, JP), ammonium pyrrolidinedithiocarbamate (APCD) was acquired from Fluka analytical (Sigma-Aldrich, St. Louis, MO, USA), and the Te standard (1000 ppm in 3% HNO_3) was purchased from RICCA Chemical Company (Arlington, TX, USA).

Mineral Basal Medium Used in the Batch Assays

The basal medium used in the batch bioassays consisted of the following macronutrients (in mg L^{-1} final medium concentration): K_2HPO_4 (25); $CaCl_2 \cdot 2\ H_2O$ (10); $MgCl_2 \cdot 6\ H_2O$ (100); NH_4Cl (7); and yeast extract (YE) (1). The medium also contained the following trace elements (in μg L^{-1} final medium concentration): H_3BO_3 (10); $FeCl_2 \cdot 4\ H_2O$ (400); $ZnCl_2$ (10); $MnCl_2 \cdot 4\ H_2O$ (10); $(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$ (18); $AlCl_3 \cdot 6\ H_2O$ (18); $CoCl_2 \cdot 6\ H_2O$ (400); $NiCl_2 \cdot 6\ H_2O$ (10); $CuCl_2 \cdot 2\ H_2O$ (6); $NaSeO_3 \cdot 5\ H_2O$ (20); EDTA (200); and resazurin (40). The pH of the medium was adjusted to 7.0-7.2 and then 2.5 g L^{-1} of sodium bicarbonate ($NaHCO_3$) was added to buffer the pH of the media. Te^{IV} and Te^{VI} were provided in the form of Na_2TeO_3 and K_2TeO_4 0.5 H_2O; respectively.

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

An aliquot taken from the filtrate obtained in the filtration step, 1 mL of an ammonium pyrrolidinedithiocarbamate (APDC) stock solution to reach a final concentration of 0.05% (m/v),

along with 0.5 mL of HNO_3 (70% wt. for a final concentration of 0.3 M) were added to 25 mL volumetric flasks. The mixtures were allowed to settle for ten minutes before passing them through the solid phase extraction cartridges (SampliQ C18 ODS, Agilent, Santa Clara, CA, USA), in order to allow for the formation of the Te^{IV}-APDC chelate. Before passing the sample through the SPE cartridges, the columns were preconditioned by passing 3 mL of methanol (HPLC grade) through, at a volumetric flow rate of one mL min^{-1} to remove impurities after that, 3 mL HNO_3 (2% v/v) were used to equilibrate the pH of the cartridges. A VisiprepTM SPE vacuum manifold (Supelco, Sigma-Aldrich, St. Louis, MO, USA) was used to load 12 cartridges at the same time. Three mL of the mixture sample-APDC-HNO_3 was then passed through the cartridges at a rate of 1 mL min^{-1}, Te^{IV}-APDC chelate got retained in the cartridges while Te^{VI} was collected in the effluent of the columns. The effluent was then analyzed with an ICP-OES, as mentioned above, for Te^{VI} and the amount of Te^{IV} was obtained as the difference between the concentration of total dissolved tellurium and that of Te^{VI}.

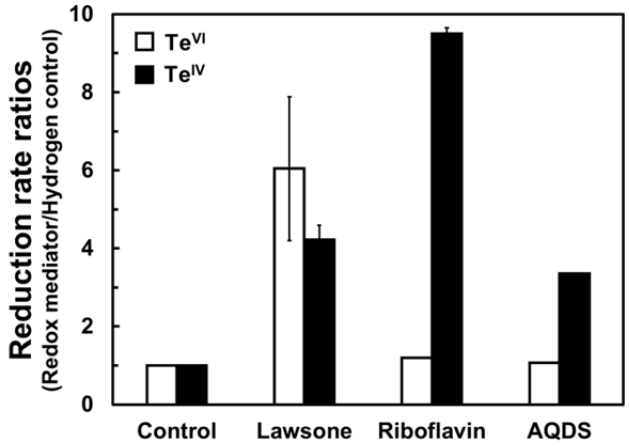

Figure S1. Comparison of the relative rates of tellurium reduction using different redox mediators (RMs) at a 1:1 molar ratio (Te:RM) and H_2 as an electron donor (hydrogen control), in comparison to the rate of reduction in the presence of H_2 without added RM.

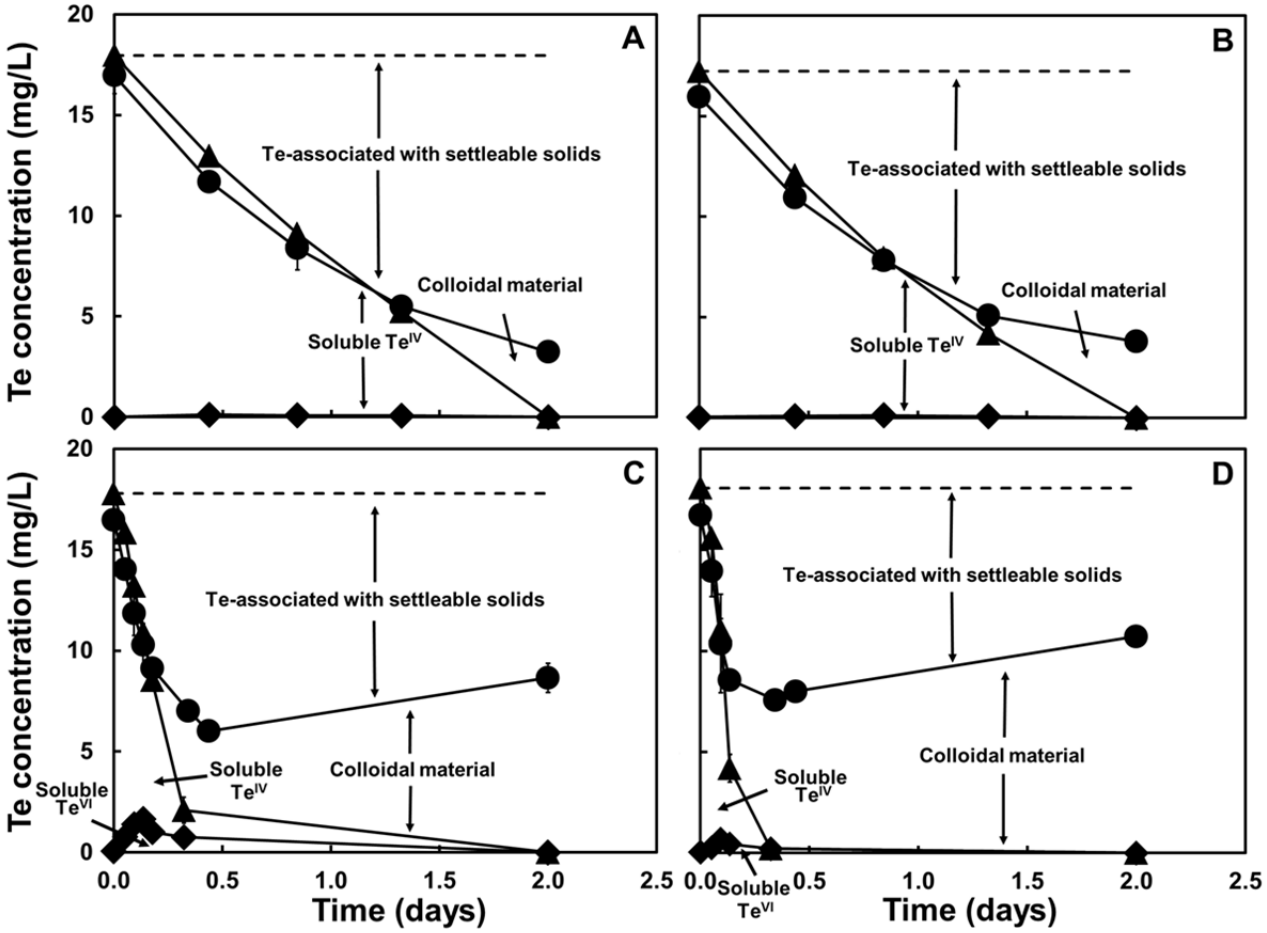

Figure S2. Distribution of Te^{IV} between the phases of the system using H_2 as exogenous electron donor and different redox mediators (RM). **Panel A,** sludge without external electron donor or RM. **Panel B,** sludge with H_2 as electron donor and no RM. **Panel C,** sludge amended with H_2 and lawsone as redox mediator at a molar ratio 1:1 (Te:RM). **Panel D,** sludge amended with H_2 and RF as RM 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^{VI}.

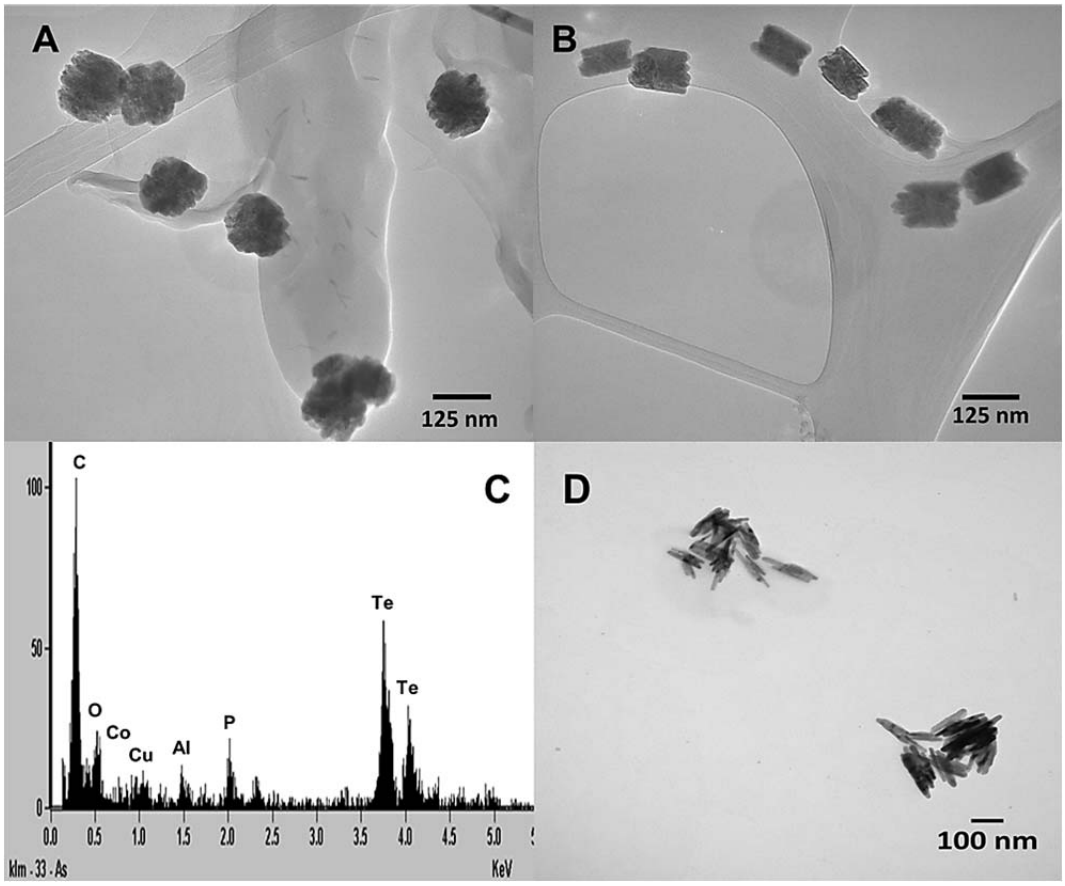

Figure S3. TEM and EDS images of the Te^0 nanoparticles formed outside the anaerobic granules when Te^{VI} was amended as Te source. **Panel A,** Spherical shape obtained in the endogenous control. **Panel B,** Bundles of needle-like NPs produced in the systems amended with H_2 as electron donor. **Panel C,** EDS spectra for the NPs produced in the endogenous control. **Panel D,** Clusters of disorderly arranged needle-like NPs produced in the systems supplied with H_2 as electron donor and lawsone (Te:RM 1:1 molar ratio).

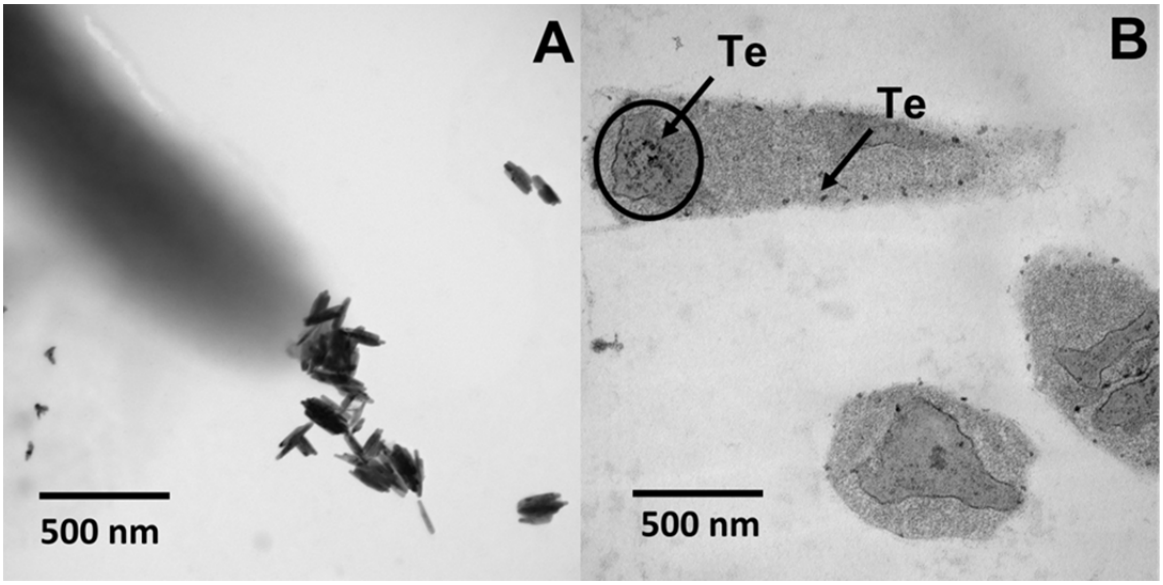

Figure S4. Formation of extracellular and intracellular Te^0 nanoparticles produced by the anaerobic granules using Te^{IV} as the initial source of Te with H_2 as external source of electrons, in the presence or absence of riboflavin. **Panel A,** Te^0 NPs formed extracellularly when the bioassays were amended with H_2 as electron donor and riboflavin as RM. **Panel B,** Te^0 NPs formed intracellularly when the systems were amended with H_2 as electron donor with no RM.

Description of the Statistical Analysis Performed to the Rates of Reduction Data

In order to investigate if the differences observed between the rates of reduction of both Te oxyanions in the systems amended with RM and those of the systems lacking a redox mediating compound are statistically significant, we performed right-tailed hypothesis tests using the t-student distribution as the test statistics with a significance level α=0.05.

1- Null Hypothesis:

Ho:= μ_1-$\mu_2 \leq 0$

where:

μ1 = rate of Te reduction in the system containing RM and H_2 as electron donor

μ2 = rate of Te reduction in the control lacking RM with H_2 as electron donor

2- Alternative Hypothesis:

Ha:= μ_1-$\mu_2 \geq 0$

3- Test Statistics (t)

$$t = \frac{\overline{x_1} - \overline{x_2}}{s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

$$s^2 = \frac{\sum(x_1 - \overline{x_1})^2 + \sum(x_2 - \overline{x_2})^2}{n_1 + n_2 - 2}$$

where:

$\overline{x_1}$ = mean of the Te reduction rate in the assay containing RM and H_2 as electron donor (sample)

$\overline{x_2}$= mean of the Te reduction rate in the control lacking RM with H_2 as electron donor (sample)

s = standard error

n_1 = size of the sample obtained from the systems containing RM

n_2 = size of the sample obtained from the systems lacking RM

4- Compare the Test Statistic with t-critical Value Giving a Significance Level of α=0.05

If the test statistic was larger than the t-critical value then, the null hypothesis was rejected and the alternative was accepted.

In all the hypothesis tests performed with our data, the estimated p-values (obtained with the test statistics) were smaller than 0.005, which indicates that there is a strong statistical

significance in the observed differences between the reduction rates obtained in the different systems.

Role of Sulfide in Biomass on Te^{IV} Reduction

In order to determine the amount of sulfide released from the biomass to the S-free medium at different times (covering a typical incubation period used in the Te^{IV} reduction experiments of 0-5 days) and its potential effect on the reduction of Te in our systems, we performed batch experiments using only 100 mL of the mineral medium (described in page S2) and 1.5 g VSS L^{-1} of the granular sludge. We incubated the bottles under the same experimental conditions used in the manuscript to determine the initial concentration of sulfide in the medium after 2 and 5 days of incubation. The dissolved sulfide content was 0, 8.9 x 10^{-3} ± 6.7 x10^{-4} mM S^{2-}, and 7.7 x 10^{-3} ± 9.6 x 10^{-4} mM S^{2-} after 0, 2, and 5 days of incubation, respectively. According to the Te^{IV} reduction reaction using S^{2-} as the electron donor (eq. 1), two moles of S^{2-} would be required to reduce one mole of Te^{IV}. Therefore, the amount of S^{2-} found in the medium will be able to reduce only between 2.5 to 2.9% of the total Te^{IV} (0.156 mM) amended to the system.

$$2H^+ + 2H_2S + TeO_3^{2-} \rightarrow Te^0 + 2S^0 + 3H_2O \qquad (1)$$

H_2S can potentially be recycled from S^0, thus to account for the effect of a possible rapid reduction of S^0 to S^{2-} by the elemental sulfur-reducing bacteria (commonly found in anaerobic granular sludge) on the reduction of Te^{IV}, a typical batch experiment was carried out using 100 mL of mineral media, 10.7 mM H_2 L_{liq}, 1.5 g VSS L^{-1}, 0.156 mM Te^{IV}, and two different amounts of S^{2-} (0.007 and 0.035 mM). The sum of the added S^{2-} and the background S^{2-} concentration expected after 5 days (7.7 x 10^{-3} mM) exceeded 1.9- and 5.5-fold the background

S^{2-} concentration after 5 days of incubation. The time course of Te^{IV} reduction is shown in Figure S5. No important effect was observed except for a small increase in the Te^{IV} reduction rate in the assay supplied with 0.035 mM S^{2-}. These results provide conclusive evidence that the background S^{2-} production by the biomass did not contribute in a significant fashion to the rate of Te^{IV} reduction. It is also important to note that according to the $E^{0,}$ of the couple $HTeO^{3-}/Te^{0}$ (0.196 V), the reduction of Te^{IV} is highly bioenergetically favorable over sulfate reduction (-0.217 V).

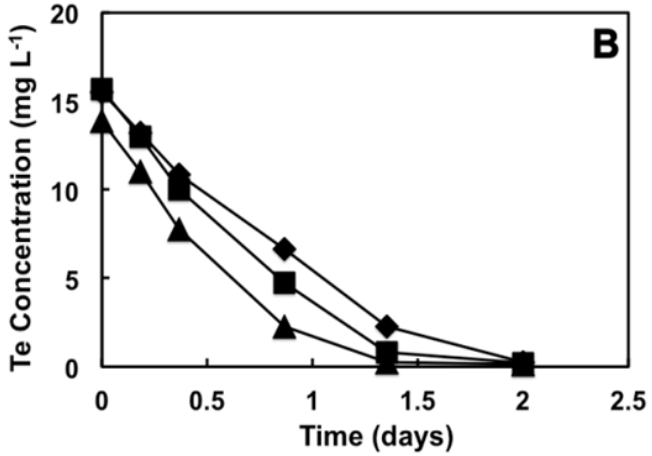

Figure S5. Time course of Te^{IV} reduction in batch assays amended with low levels of sulfide (S^{2-}). Biological reduction of Te^{IV} (0.156 mM as Te) in assays supplied with H_2 as the electron donating substrate, 1.5 g VSS L^{-1} of granular sludge, and two different levels of S^{2-} selected to provide a total concentration of S^{2-} that is approximately 1.9- to 5.5-fold times higher than the background of sludge-derived S^{2-} in sulfur-free mineral medium after 5 days of incubation. Legends: (♦) control incubated in S-free mineral medium, (■) assay supplied with 0.007 mM S^{2-}, (▲) assay supplied with 0.035 mM S^{2-}.