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Microbial toxicity of ionic species leached from the II–VI semiconductor materials, cadmium telluride (CdTe) and cadmium selenide (CdSe)

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Abstract

This work investigated the microbial toxicity of soluble species that can potentially be leached from the II–VI semiconductor materials, cadmium telluride and cadmium selenide. The soluble ions tested included: cadmium, selenite, selenate, tellurite, and tellurate. Their toxicity towards the acetoclastic and hydrogen-consuming trophic groups in a methanogenic consortium as well as towards a bioluminescent marine bacterium, *Aliivibrio fischeri* (Microtox® test), was assessed. The acetoclastic methanogenic activity was the most affected as evidenced by the low 50% inhibiting concentrations (IC_{50}) values obtained of 8.6 mg L^{-1} for both cadmium and tellurite, 10.2 mg L^{-1} for tellurate, and 24.1 mg L^{-1} for selenite. Both tellurium oxyanions caused a strong inhibition of acetoclastic methanogenesis at low concentrations, each additional increment in concentration provided progressively less inhibition increase. In the case of the hydrogenotrophic methanogenesis, cadmium followed by selenite caused the greatest inhibition with IC_{50} values of 2.9 and 18.0 mg L^{-1}, respectively. Tellurite caused a moderate effect as evidenced by a 36.8% inhibition of the methanogenic activity at the highest concentration tested, and a very mild effect of tellurate was observed. Microtox® analyses showed a noteworthy inhibition of cadmium, selenite, and tellurite with 50% loss in bioluminescence after 30 min of exposure of 5.5, 171.1, and 458.6 mg L^{-1}, respectively. These results suggest that the leaching of cadmium, tellurium and selenium ions from semiconductor materials can potentially cause microbial toxicity.

Graphical Abstract

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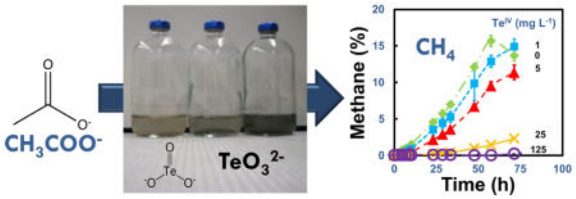
Supplementary information

Additional figures and analytical methods are described in the Supplementary Information section. Supplementary data associated with this article can be found in the online version, at @@@@



Keywords

II–VI semiconductors; chalcogens; methanogenic toxicity; Microtox®; microbial toxicity; *Aliivibrio fischeri*

1. Introduction

Cadmium telluride (CdTe) and cadmium selenide (CdSe) are two semiconductors of the II–VI group. Due to their remarkable optical and electrical properties, both compounds are extensively used in the manufacture of electronic devices. CdSe is a good light absorber which possesses excellent photoelectrical characteristics (Feng et al., 2010); therefore, it is used in the production of light emitting diodes (LEDs), photo-electronics, and transistors (Chate et al., 2013). The use of CdSe in the production of high efficiency hybrid solar cells has been investigated (Feng et al., 2010;Chate et al., 2013;Huynh et al., 2002). Likewise, CdTe is used in the production of optoelectronic devices, gamma ray detectors (Bicknell et al., 1987), and laser windows (Punitha et al., 2015). Most importantly, CdTe is used in the photovoltaic industry in the production of thin film solar cells. CdTe photovoltaic devices were ranked as the third most common type of photovoltaic solar panels commercially available in 2013 (Esterly, 2013).

Increasing concerns have arisen due to the implications of the potential release of hazardous substances from CdTe and CdSe containing devices. Firstly, it is expected that the manufacture of electronics based on these semiconductors will grow over time as technology moves forward which implies an increasing release of these compounds in the environment. Specifically, the production of solar panels is expected to increase since solar energy is one the fastest growing market shares of renewable energy (Century, 2015). Despite the remarkable efforts that are being made to recycle electronic waste, it is possible that an important fraction of the decommissioned solar panels in the future will end up discarded in municipal mixed landfills as a result of a lack of regulations related to the disposal of electronic waste in multiple countries. Secondly, there is evidence that toxic compounds might be leached from electronics based on leaching experiments using deionized water as the extraction fluid (Lithner et al., 2012). More importantly, recent works have demonstrated that soluble ions of Cd, Se, and Te, such as divalent cadmium (Cd^{II}), selenite (SeO_3^{2-} (Se^{IV})), selenate (SeO_4^{2-}, (Se^{VI})), tellurite (TeO_3^{2-} (Te^{IV})), and tellurate (TeO_4^{2-} (Te^{VI})) can leach out from CdSe and CdTe under conditions similar to those commonly found in landfills (Zeng et al., 2015). Thirdly, Cd and selenium (Se) are highly toxic elements which are included in the United States Environmental Protection Agency (USEPA) list of regulated drinking water contaminants, and the disposal of Se and Cd containing waste in municipal solid waste landfills is also regulated. The maximum contaminant levels (MCL)

established for Cd and Se are 0.005 mg L^{-1} and 0.05 mg L^{-1}, respectively, and the toxicity characteristic leaching procedure (TCLP) limit established for Se and Cd is 1 mg L^{-1}. Furthermore, Cd, Se and Te soluble fractions are highly toxic to some microorganisms (Trevors et al., 1986; Macken et al., 2009; Yu et al., 1997; Taylor, 1999). Given these considerations, the presence of soluble ions derived from CdTe and CdSe in the environment might negatively impact several important processes, such as, the anaerobic biodegradation of organic matter and the activity of aquatic organisms.

The aim of this work was to investigate the toxicity of the soluble species potentially released from CdSe and CdTe (Cd^{II}, Se^{IV}, Se^{VI}, Te^{IV} and Te^{VI}) towards an anaerobic methanogenic consortium to assess microorganisms involved in anaerobic digestion processes and the Microtox® tests that measure the bioluminescence of the marine bacterium, *Aliivibrio fischeri*, which is commonly used to assess aquatic toxicity.

2. Materials and methods

2.1. Chemicals

Cd^{II} as $Cd(OH)_2$ (≥ 99.99% purity), Te^{IV} as Na_2TeO_3, and Te^{VI} as $K_2TeO_4 \bullet xH_2O$ (x = 0.5) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The stoichiometry of the molecular water of $K_2TeO_4 \cdot xH_2O$ was determined by comparing the Te concentration of a TeO_4^{2-} salt solution against a Te standard (described below). Se^{IV} as Na_2SeO_3 was acquired from MP Biomedicals (Santa Ana, CA, USA), Se^{VI} as Na_2SeO_4 (≥ 98% purity) was obtained from ACROS Organics (Geel, Belgium), and the Te standard (1000 mg L^{-1}) was purchased from RICCA Chemical Company (Arlington, TX, USA). All the Microtox® reagents were obtained from ModernWater (Cambridge, UK).

2.2. Inoculum source

An anaerobic granular sludge was obtained and used as the source of inoculum from a full-scale upflow anaerobic sludge blanket reactor treating effluent at Mahou beer brewery (in Guadalajara, Spain). This granular sludge contained 0.0792 g volatile suspended solids (VSS) g^{-1} wet wt. The maximum methanogenic activities of the sludge were 566.8±64 and 571±26 mg chemical oxygen demand-methane (COD-CH_4) g VSS^{-1} $day^{-1}$for the assays utilizing acetate and hydrogen as substrate, respectively. The granular sludge was stored at 4°C prior to use. The marine bacterium *Aliivibrio fischeri* (lyophilized culture of *A. fischeri* NRRL-B-11177), was obtained from ModernWater (Cambridge, UK).

2.3. Methanogenic toxicity bioassays

2.3.1. Mineral media—The basal medium (M1) used in the experiments assessing the toxicity of the chemical compounds towards acetate-consuming microorganisms contained (in mg L^{-1} in final medium): CH_3COONa (2563), K_2HPO_4 (250), $CaCl_2 \bullet 2H_2O$ (10), $MgSO_4 \bullet 7H_2O$ (100), $MgCl_2 \bullet 6H_2O$ (100), NH_4Cl (280), yeast extract (100), and 1 mL L^{-1} of a trace elements stock solution described below. The pH was subsequently adjusted to 7.0–7.2, and the medium was finally amended with $NaHCO_3$ to a concentration of 4 g L^{-1}. The basal medium (M2) used in the assays designed to study the toxicity of the chemical compounds to hydrogen-consuming microorganisms consisted of (in mg L^{-1} in final

medium): NH_4Cl (280), K_2HPO_4 (250), $CaCl_2•2H_2O$ (10), $MgCl_2•6H_2O$ (183), yeast extract (100) and 1 mL L^{-1} of a trace elements stock solution. After adjusting the pH to 7.0–7.2, $NaHCO_3$ was added to a final concentration of 3 g L^{-1}. H_2 was added via the gas phase (as described below). The trace elements stock solution for both media consisted of (in mg L^{-1}): H_3BO_3 (50), $FeCl_2•4H_2O$ (2000), $ZnCl_2$ (50), $(NH_4)_6Mo_7O_{24}•4H_2O$ (50), $AlCl_3•6H_2O$ (90), $CoCl_2•6H_2O$ (2000), $NiCl_2•6H_2O$ (50), $CuCl_2•2H_2O$ (30), $NaSeO_3•5H_2O$ (100), EDTA (1000), resazurin (200), and 1 mL of HCl (37% wt). Cd^{II} and Te^{VI} stock solutions were prepared in demineralized (DI) and acidified water (10 mM HCl), the rest of the salts were diluted in DI water.

2.3.2. Batch bioassays—Methanogenic inhibition assays were carried out in 160 mL glass serum flasks (Wheaton, Milville, NJ, USA). The experimental set up consisted of 25 mL of liquid and 135 mL of gas (headspace) in the bottles. Firstly, 20 mL of 1.25× concentrated mineral medium M1 or M2 (according to assay purpose) along with 1.5g of VSS L^{-1} of sludge inoculum were added to the serum flasks. After closing the bottles with rubber septa and aluminum crimp seals, the headspaces of the bottles were flushed with N_2:CO_2 (80:20, v/v) for four minutes to eliminate oxygen. Either sodium acetate (originally amended in the 20 mL of the 1.25× concentrated mineral medium M1 to give a final concentration of 2.5 g COD L^{-1} in the 25 mL of liquid) or hydrogen (H_2) were used as substrates depending on the experiment. H_2 was supplied afterwards with a H_2/CO_2 gas mixture (80:20, v/v) to reach an overpressure of 0.5 atm to the corresponding bottles (final concentration of 1.83 g COD as H_2 in headspace L^{-1}_{liq} in 20 mL of 1.25× medium M2, and 140 mL of headspace). The flasks were preincubated overnight in an orbital shaker at 100 rpm in a climate controlled room at 30°C for the adaptation of the sludge to the media.

After the pre incubation period, the bottles were amended with 5 mL of different amounts of the correspondent stock solutions of test chemicals and DI water to reach the desired initial concentration of the compound of interest, and controls were set up using 5 mL of DI water lacking additions of the inhibitory test chemicals. Table S1 summarizes the experimental concentration ranges utilized in the different toxicity bioassays. Subsequently, all bottles were flushed again with the mixture N_2/CO_2 (80:20, v/v), and H_2 was provided to the appropriate (with H_2 as the substrate) bottles using H_2/CO_2 gas mixture (80:20, v/v). The final added substrate concentrations were 2.0 g COD L^{-1} as acetate or 1.41 g COD as H_2 gas L^{-1}_{liq} after completing the dilution corresponding to 25 mL medium and 135 mL of headspace. The controls were carried out in triplicate and the treatments were performed in duplicate.

All the assays were incubated at 30±2°C in an orbital shaker at 100 rpm. In order to monitor the production of methane, gas samples of 100 μL were collected from the headspace of the bottles and analyzed (as described below) every two hours during the first eight to ten hours of incubation, and after that, two or three times per day until the maximum theoretical methane production was reached. The maximum specific methanogenic activity for each concentration tested was obtained from the slope of the cumulative methane produced as a function of time. These values were normalized with respect to the maximum specific activity of the corresponding uninhibited control. The time period used to calculate the maximum activity for each initial concentration was a discrete interval shared by all

treatments in each experiment. The time interval used in each case is shown in Table S1 in supplementary information (SI). The normalized methanogenic activities (NMA) were calculated as follows:

$$NMA(\%) = \frac{Maximum\ rate\ of\ CH_4\ production\ at\ each\ concentration\ tested}{Maximum\ rate\ of\ CH_4\ of\ the\ control} \times 100$$

The initial concentrations of the compounds that caused a 20%, 50% and 80% decrease in the methanogenic activity compared to the uninhibited control (IC_{20}, IC_{50} and IC_{80}) were estimated as described elsewhere (Tapia-Rodriguez et al., 2012).

2.4. Microtox®

The acute toxic effect of Cd^{II}, Te^{IV}, Te^{VI}, Se^{IV} and Se^{VI} on the bioluminescent marine bacteria *A. fischeri* was assessed using a Microtox® Model 500 analyzer (Strategic Diagnostics, Inc. SDIX, Newark, DE, USA). Microbial inhibition was measured at 25°C in triplicate experiments. Table S1 shows the range of concentration used for each chemical compound. The concentrations causing 50% decrease in the bacterial luminescence (IC_{50}), compared to the toxicant-free control, after 5, 15, and 30 min of exposure were obtained as previously described (Bulich and Isenberg, 1981).

2.5. Analytical methods

2.5.1. Methane determination—Methane content in the gas phase of the serum flasks was analyzed by gas chromatography using a HP 5890 Series II system (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector and a Stabilwax-DA fused silica capillary column (30 m length x 0.53 mm ID, Restek Corporation, Bellefonte, PA, USA). Gas samples of 100 μL were injected using Helium as the carrier gas at a flow rate of 85 mL min^{-1}. The temperatures of the oven, injection port and detector were 140, 180 and 250°C, respectively.

3. Results

3.1. Methanogenic inhibition

Figure 1 shows the time course of methane production in the assays evaluating acetoclastic methanogenesis in response to being exposed to a gradient of Cd^{II}, Te^{IV}, Te^{VI} and Se^{IV} concentrations. The control assays rapidly produced methane reaching the theoretically maximum expected concentration of 14.4% CH_4 in the headspace within approximately 60 h. As the concentrations of the potential II/VI semiconductor ions, Cd^{II}, Te^{IV}, Te^{VI} and Se^{IV}, were increased, the slopes of the methane production curves clearly decreased as a function of the increasing ion concentration. This relationship was consistent up to a point when the toxicity was too severe. At concentrations of 12 to 16 mg L^{-1} Cd^{II} and 36 mg L^{-1} Se^{IV} (Figures 1A and D), there was initially some methane production, but the production subsequently slowed down and stopped during the assay. Higher concentrations (90 mg L^{-1} Se^{IV} and 125 mg L^{-1} Te^{IV} in Figures 1B and D) caused almost complete inhibition from the very start of the assays. Te^{VI} was also found to be inhibitory at low concentrations; however, only partial inhibition was observed even at the highest concentration used in the assays

(Figure 1C). The methane production rate even recovered with an extended incubation time (Figure S1) in the SI section.

The toxicity of Se^{VI} was also tested, but it was not effective in causing inhibition to acetoclastic methanogenic activity (data not shown). Partial inhibition was only observed at environmentally irrelevant concentrations of 4.2 g L^{-1} Se^{VI} (data not shown).

Figure S2 in SI shows the time course of methane production assays evaluating hydrogenotrophic methanogensis in response to being exposed to a gradient of Cd^{II} and Se^{IV} concentrations. The control assays rapidly produced methane reaching the theoretically maximum expected concentration of 10.2% CH_4 in the headspace after approximately 60 h. As the concentrations of the potential II/VI semiconductor ions, Cd^{II}, and Se^{IV}, were increased, the slopes of the methane production curves decreased as a function of the increasing ion concentration (similar to the acetoclastic methanogenic assay). The inhibitory response was particularly severe between 2 and 5 mg L^{-1} (Figure S2A in SI). As the concentration reached 12 mg L^{-1} Cd^{II} or 40 mg L^{-1} Se^{IV} (Figure S2B in SI), the inhibition impact was so severe that the methane production which occurred in the beginning of the assay came to a total standstill before the incubation was complete. Te^{VI} was tested and had no significant toxic effect at the highest concentration used in this experiment (500 mg Te^{VI} L^{-1}, see Figure S3 in SI),. High concentrations of Se^{VI} (4.2 g Se^{VI} L^{-1}, data not shown) caused only partial inhibiton. For Te^{IV}, the inhibitory response was particularly important at 12.5 mg Te^{IV} L^{-1} however, above that concentration, the toxicity response was maximally 37% and further increases in Te^{IV} concentration did not increase the toxicity further.

Figure 2 shows the normalized maximum activity (NMA) of the methanogenic anaerobic sludge as a function of the initial concentrations of the compounds utilized in this study, relative to the maximum activity of the control without added toxic compounds. Figure 2A presents the most relevant results for the assays where the acetoclastic activity was studied. Cd^{II}, Te^{IV}, Te^{VI} and Se^{IV} all caused concentration related toxicity responses to acetoclastic methanogenic activity in the granular sludge. In all cases, severe inhibitions (> 80%) were evident at concentrations less than 50 mg L^{-1}. There were however noticeable differences in the response pattern.

The toxicity response curve to Cd^{II} concentrations was the steepest of all the ions tested. Cd^{II} concentrations as low as 12 mg L^{-1} caused around 80% inhibition. However, the inhibition response to Cd^{II} was less steep from 0 to 4 mg L^{-1} most likely due to precipitation of Cd^{II} with biogenic sulfide formed from the 39.0 mg L^{-1} of sulfate (SO_4^{2-}) in the medium, M1. The Te oxyanions provided a sharp response to the methanogenic acetoclastic activity at low concentrations causing greater than 25% inhibition at the lowest concentration tested of 1 mg L^{-1} and 1.7 mg L^{-1} for Te^{IV} and Te^{VI}, respectively. However, each additional increment in concentration provided progressively less toxicity response (pattern of “diminishing returns”). In order to achieve 80% or more inhibition, relatively high concentrations of the Te oxyanions (20.5 and 39.7 mg L^{-1} for Te^{IV} and Te^{VI}, respectively) were required. This concentration was 3.3 × higher than that needed by Cd^{II} for a similar level of inhibition. The response pattern for Se^{IV} was intermediate between that of Cd^{II} and the Te oxyanions.

Figure 2B presents the most relevant results for the hydrogenotrophic methanogenic experiments. As was observed with the acetoclastic methanogenic activity, the hydrogenotrophic microorganisms were inhibited by Cd^{II} in a steep linear fashion with incrementing concentrations of Cd^{II} such hat already at 5 mg L^{-1} greater than 80% inhibition was observed. The medium in the hydrogenotrophic methanogenic assay did not contain SO_4^{2-} (M2), and thus the steep inhibition response to Cd^{II} started with the lowest concentration tested of 1 mg L^{-1} because the medium was free of biogenic sulfide that could have precipitated Cd^{II}. However, the other ions did not show a strict concentration related response over the full range of concentrations tested. In the case of both Te^{IV} and Se^{IV}, the hydrogenotrophic methanogens had an inhibitory response that became saturated at a given concentration and additional increases in concentration did not result in higher toxicity responses. For Te^{IV}, the response became saturated at concentrations of 50 mg L^{-1} and higher at an inhibition of 27.7%, For Se^{IV}, the response became saturated at 70 mg L^{-1} or higher at a maximum inhibition of 76.2%. Te^{VI} caused no noteworthy toxic response up to 200 mg L^{-1}.

The concentrations of the compounds tested in this work which inhibited the acetoclastic and hydrogenotrophic methanogenic activity by 20%, 50% and 80% are summarized in Table 1. Cd^{II} and both Te oxyanions were highly inhibitory for the acetoclastic community with IC_{50} values of approximately 10 or less mg L^{-1}. Se^{IV} was also highly toxic but 2 to 3 fold less toxic compared to Cd^{II} and Te oxyanions. Se^{VI} was found to be completely non-toxic even at concentrations as high as 6 g L^{-1}. Only Cd^{II} and Se^{IV} were highly toxic to hydrogenotrophic methanogens with IC_{50} values ranging from approximately 3 to 18 mg L^{-1}.

3.2. Acute toxicity towards Aliivibrio fischeri

Important toxic effects were observed when the bioluminescent marine bacterium *A. fischeri* was exposed to Cd^{II}, Se^{IV} and Te^{IV}. Toxicity was evident as the bioluminescence of the bacteria decreased with time (5 to 30 min of exposure) and with increasing toxic compound concentrations. Figure S4 shows the inhibitory effect on the bioluminescent activity of *A. fischeri* after being exposed to various concentrations of the most toxic compounds, Cd^{II} (Panel A) and Se^{IV} (Panel B); while Table 2 reports the IC_{50} values for the five compounds assessed in this work for all time intervals. The high toxicity of Cd^{II} is evident from the IC_{50} values of only a few mg L^{-1} while moderate toxicity was observed for Se^{IV} and Te^{IV} with IC_{50} values of several hundred mg L^{-1}. No effect was evident when Te^{VI} was tested in the whole range of concentrations, and Se^{VI} caused partial inhibition when the marine bacteria were exposed to high concentrations of approximately 32.5 g L^{-1} (not environmentally relevant, data not shown) of this oxyanion.

4. Discussion

4.1. Main findings

The toxic impact of Cd^{II}, Te^{IV}, Te^{VI}, Se^{IV} and Se^{VI} on the acetatoclastic and hydrogenotrophic microorganisms in the methanogenic community of an anaerobic granular sludge was investigated. Our results indicate that both trophic groups were highly inhibited

by the presence of Cd^{II} and Se^{IV}. Te^{IV} and Te^{VI} caused important inhibition of the acetoclastic trophic group, while they caused medium and mild inhibitory effects on the hydrogenotrophic methanogens, respectively. Both trophic groups tolerated exposure to extremely high non-environmentally relevant concentrations of Se^{VI}.

The inhibitory response to Cd^{II} was the most dramatic of all the ions tested. There was a less steep inhibitory response to this ion at concentrations below 4 mg Cd^{II} L^{-1} in the case of the acetoclastic compared to that of the hydrogenotrophic trophic group which could be attributed to the sequestration of soluble Cd^{II} by the biogenic sulfide (Mori et al., 2000) formed from the sulfate in the M1 medium.

One of the chalcogen elements studied, Se, is a well-known trace element required by microorganisms (Heider and Bock, 1993). Selenium is involved in the prosthetic group of key enzymes such as hydrogenases in *Clostridia, Escherichia coli* and methanogens (Ljungdahl, 2009; Yamamoto et al., 1983; Sorgenfrei et al., 1997). Selenium is also part of a common amino acid, selenocysteine in microorganisms (Peters et al., 2004). Se is added in small amounts to wastewaters to stimulate the production of methane during anaerobic digestion (Lenz et al., 2008; Munk and Lebuhn, 2014). Se^{VI} was for all practical purposes completely non-toxic, and this may be related to the preferred form of selenium as a nutrient. While Se^{IV} was toxic, it was not as toxic to methanogens at low concentrations compared to Cd^{II} and Te^{IV} as evidenced by higher IC_{20} values.

To the best of our knowledge, the methanogenic toxicity of the closely related chalcogen, Te, has never been reported before. Te oxyanions caused an unusual response towards the methanogenic consortium. The acetoclastic methanogens were very sensitive to inhibition by these oxyanions. In contrast, the hydrogenotrophic-methanogens were only mildly inhibited by Te^{IV} and were highly tolerant to Te^{VI}. Both Te species caused a sharp toxic response at the lowest concentrations tested; however, a response of diminishing returns at higher concentrations was observed. This may be due to a capacity of methanogens to express an alternative enzyme system, in response to an inhibition. Induction of such a system would require higher concentrations of tellurium oxyanions. In a previous work, we found that both Te oxyanions might be enzymatically reduced to insoluble Te^0 (Ramos-Ruiz et al., 2016). Batch experiments were conducted to study the biological reduction of the oxyanions using an anaerobic granular sludge like the one of this work. The systems were supplied with 20 mg L^{-1} of Te^{IV} or Te^{IV}, and acetate or H_2 as the external source of electrons. The concentration of soluble Te in the media decreased over time until no Te was detected in the liquid phase. At the same time, a black precipitate was found to be formed associated to the granular sludge. In contrast, the concentration of soluble Te remained unchanged in the controls lacking anaerobic granular sludge during the whole experiment. A decrease in the concentration of soluble Te in the medium caused by the biologically-mediated reduction of Te^{IV} and Te^{VI} to insoluble Te^0 might have enhanced the tolerance to Te.

The toxic effects of Cd^{II}, Te^{IV}, Te^{VI}, Se^{IV} and Se^{VI} on the marine bacteria *A. fischeri*, was also assessed. Of the six compounds tested in this work, Cd^{II} was the most toxic to *A. fischeri*, Se^{IV} caused the second most important effect; while, Te^{IV} caused only a partial inhibitory effect.

4.2. Comparison to literature data and possible mechanisms

Previous studies have evaluated the inhibitory impact of Cd^{II} to methanogens in anaerobic granular sludge utilizing different assays substrates such as glucose (Altas, 2009) and volatile fatty acid mixtures (Lin and Chen, 1999). In these studies, the IC_{50} values of Cd^{II} to methanogenic activity ranged from 36 to 450 mg L^{-1} which are 1 to 2 orders of magnitude less inhibitory than that observed in this study. The difference with our results might be explained by sulfate amendment in the medium in one of the studies (Altas, 2009). This level of sulfate was approximately 4 times higher than the concentration used in our acetoclastic assay and that might have been reduced to sulfide by sulfate reducing bacteria forming CdS(s). The formation of insoluble cadmium sulfide (CdS(s)) is expected in a sulfide containing medium as can be inferred from its low K_{sp} value (stability constant) of $10^{-28.85}$ (Benjamin, 2002). The formation of sulfide under the same experimental conditions has been demonstrated in a previous study from our research group (Gonzalez-Estrella et al., 2015). The potential yield of biogenic sulfide was in a 1.4 fold stoichiometric excess compared to the maximum amount of Cd^{II} (128 mg L^{-1}) used in their methanogenic toxicity assays which might have potentially limited the availability of the soluble Cd to inhibit the methanogenic consortium. However, this hypothesis cannot explain the large differences in Cd^{II} in our study with the second study (Lin and Chen, 1999) because the maximum biogenic S production (based on sulfur added to media) could only have precipitated at most 18.5% of the Cd^{II} at the highest concentration tested (1000 mg L^{-1}). The large differences in the inhibitory concentrations reported might be potentially explained by several differences in the sludge characteristics such as, the content of extracellular polymeric substances (EPS) or the distribution of the microorganisms in the granules. A pure methanogen culture *Methanobacterium thermoautotrophicum* KHT-2 (Mori et al., 2000) was moderately tolerant of Cd^{II} compared to the methanogens of our anaerobic sludge. No inhibition of methane production was observed when a pure culture of the methanogen *Methanobacterium thermoautotrophicum* KHT-2 was exposed to 11.2 mg Cd^{II} L^{-1} while the methanogenic activity in the acetoclastic- and hydrogenotrophic-trophic groups of our anaerobic sludge were completely inhibited when exposed to concentrations of 12 mg Cd^{II} L^{-1} (Mori et al., 2000). Nonetheless, it should be noted that strong inhibition of *M. thermoautotrophicum* KHT-2 was observed at a concentration of 56.2 mg L^{-1} (Mori et al., 2000). These results might be explained by a high tolerance of *M. thermoautotrophicum* KHT-2 towards Cd^{II} since no sulfide was present in the medium used in the bioassays, or alternatively Cd^{II} complexation with organic constituents in the medium may have occurred, resulting in total/partial removal of free Cd^{II} from the medium (yeast extract and peptone collectively accounted for 4 g L^{-1}).

The toxic effects of Cd^{II} on the marine bacterium, *A. fischeri,* have been previously reported (Villaescusa et al., 1996). In the case of Cd^{II}, similar IC_{50} values to those obtained in this work were found. For the three commonly used exposure time periods (5, 15 and 30 min) the IC_{50} values ranged from 10.1 to 33.5 mg Cd L^{-1}. Due to the tendency of Cd to form stable complexes with chloride ions, the toxicity of Cd has been associated to the presence of the cadmium complexes formed with the NaCl in the Microtox® osmotic adjusting solution and not to the presence of Cd^{II} alone, which might also be true for the methanogens in the anaerobic sludge since our basal medium was amended with a considerable amount of

chloride (Cl^-) of ~ 200 mg L^{-1}. The species of Cd present in the Microtox® test have been determined from thermodynamic data at the Microtox® experimental conditions and are reported to be $CdCl^+$, $CdCl_2$ and $CdCl_3^-$ (Villaescusa et al., 1996).

The results observed in this work correlate well with studies on the toxicity of Se^{IV} and Se^{VI} towards the acetoclastic and hydrogen- consuming microorganisms in anaerobic granular sludge (Lenz et al., 2008). The reported IC_{50} values of 73.0 mg L^{-1} and 55.5 mg L^{-1} for the acetoclastic and hydrogenotrophic activities for Se^{IV}, respectively, are in the same order of magnitude of those reported in this work. The IC_{50} values for Se^{VI} were 1283 mg L^{-1} and 3518 mg L^{-1} for the acetate- and hydrogen-consuming groups, respectively (Lenz et al., 2008). Our results also demonstrated a high tolerance of the methanogenic communities to Se^{VI}. Likewise Te, both Se oxyanions can be reduced to insoluble Se^0 by microbial means (Astratinei et al., 2006; Borghese et al., 2014). The lower bioavailability of soluble Se resulting from the formation of insoluble Se^0 might have improved the tolerance to Se.

The toxic effects of both Se oxyanions on the marine bacteria *A. fischeri* have also been assessed previously (Yu et al., 1997). Se^{IV} caused higher inhibitory effects compared to Se^{VI} (Yu et al., 1997), which is in agreement with the results from this work. However, the IC_{50} values estimated in the present study for each Se oxyanion were higher than those previously reported.

This study reports for the first time on the methanogenic toxicity of Te oxyanions. Previously methanogens were implicated in the formation of Te methylated species (Meyer et al., 2008). Also previously, the toxicity of the Te oxyanions to bacteria was studied (Turner et al., 2012). The valence of Te oxyanions, as well as, the localization of the bacteria (planktonic cells or inside biofilms) had important impacts on the observed toxicity. In the case of planktonic cells of *E. coli* and *Staphylococcus aureus*, Te^{VI} was 10× and 3.7× less toxic than Te^{IV}, respectively (based on the minimal inhibitory concentration (MIC) ratios MIC_{TeVI}/MIC_{TeIV}) (Turner et al., 2012). In the case of bacteria in biofilms, Te^{VI} was 3× and 1.8× less toxic than Te^{IV}, respectively; based on the MIC required to prevent regrowth of the bacteria from a treated biofilm (Turner et al., 2012). The latter results are in agreement with our results, the acetoclastic activity of the methanogenic consortium studied here was 1.2× less affected by Te^{VI} based on the IC_{50} values than by Te^{IV} (Table 1A). Due to the lower toxicity of both oxyanions to the hydrogen consuming over the acetoclastic trophic group, it was not possible to estimate the IC_{50} values for Te^{IV} and Te^{VI} in the ranges of concentrations used in this work for the hydrogenotrophic methanogens (Table 1B); however, the methane production was more affected by Te^{IV} than by Te^{VI} (Figure 2B). These findings showed that the toxic effects of the Te oxyanions are also dependent of the trophic group in the methanogenic consortium.

The difference in the toxicity of the Te oxyanions towards both methanogenic trophic groups might be explained by some of the different mechanisms of toxicity resistance that have been widely discussed in the literature, such as the reduction of Te soluble species by enzymatic or non-enzymatic means, Te volatilization or the presence of Te resistance determinant genes ($Te1^R$) (Chasteen et al., 2009). Even though the formation of methylated Te species by methanogens has been previously reported (Meyer et al., 2008), the results of

this work indicated that volatilization of Te was not significant in these systems since less than 0.00002% of the total Te amended to the systems was found in the gas phase of the bottles used to determine Te volatilization at the end of the experiment (see SI).

The remarkably higher toxicity levels caused by both oxyanions to the acetate consuming group compared to those caused to the hydrogen consuming group might be attributed to several factors. First of all, evidence points out that the microbial reduction of the Te oxyanions to insoluble Te^0 is used as a mechanism to decrease their toxic effects (Turner et al., 2012; Chasteen et al., 2009; Moore and Kaplan, 1992) by lowering its bioavailability. H_2 is a superior electron donor for Te reduction according to the lower redox potential of the $2H^+/H_2$ pair ($E^{0\prime} = -0.414$ V (Madigan, 2009)) compared to that of CO_2/acetate ($E^{0\prime} = -0.28$ V (Madigan, 2009)). Since the redox potential $E^{0\prime}$ (pH 7) of the pairs $HTeO_4^-/HTeO_3^-$ and $HTeO_3^-/Te^0$ is 0.399 V and 0.196 V (Bouroushian, 2010), respectively, this suggests a more thermodynamically favorable formation of insoluble Te^0 species in the systems amended with H_2 than in those supplied with acetate as substrate. Furthermore, in recent experimental work, H_2 was shown to stimulate the microbial reduction of both Te oxyanions in methanogenic granular sludge; whereas acetate did not have a stimulatory effect (Ramos-Ruiz et al., 2016). Therefore, the decreased availability of soluble Te species might potentially lead to an apparent increased tolerance of the hydrogenotrophic methanogens to both oxyanions. The formation of a black precipitate, which is characteristic of the formation of Te^0 (Baesman et al., 2007; Ramos-Ruiz et al., 2016), was observed in the bioassays.

The higher inhibition of methane production in the systems amended with acetate might also be explained by a potential competition between the soluble tellurium species and acetate to enter the cells. Evidence points out that some microorganisms, such as the facultative phototroph *Rhodobacter capsulatus* transport Te^{IV} into the cells through an acetate permease (ActP) which is also responsible for acetate uptake (Borghese and Zannoni, 2010). Therefore, the presence of acetate might have induced the expression of the ActP which provided a mechanism of Te entry which was probably not the case with H_2 as substrate. The results of this work are in agreement with the general understanding that Te^{IV} is more toxic than Te^{VI} since higher inhibition of methane production was observed in both methanogenic trophic groups after being exposed to Te^{IV}.

To date, there have also not been any reports on the toxicity of either Te oxyanions towards *A. fischeri*; however, our results indicate that Te^{IV} is more toxic than Te^{VI} which is consistent with the general conclusion for most microorganisms (Chasteen et al., 2009; Turner et al., 2001; Turner et al., 2012).

5. Conclusion

Of all the potential ions that can potentially be leached from II-VI semiconductor materials, Cd^{II} caused the most important toxic effect towards the methanogens in the anaerobic granular sludge and to the marine bacterium *A. fischeri*. The observed inhibition of the acetoclastic methanogens in the granular sludge agrees with the basic understanding that the toxicity level of the chalcogens (Se and Te) to most microorganisms increases in the

following order: Se^{VI}, Se^{IV}, Te^{VI}, and Te^{IV} (Zannoni et al., 2008). In the case of the hydrogenotrophic methanogens, the inhibitory effect increased in the following sequence: Se^{VI}, Te^{VI}, Te^{IV} and Se^{IV}. The toxic effects of Te^{IV} and Se^{IV} to the marine bacterium, *A. fischeri*, were also observed to be important. This work demonstrates for the first time the toxic effects of the Te oxyanions towards a consortium of methanogens

From the findings of this work, it can be concluded that the presence of soluble ions leaching from II-VI semiconductor materials can potentially negatively affect anaerobic treatment processes such as the methanogenic phase in a landfill. Thus caution should be taken if decommissioned II-V semiconductors materials are disposed of in municipal landfills. Likewise anaerobic treatment processes may be impacted in wastewater treatment systems handling effluents from industrial facilities processing II-V semiconductor materials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Leached ionic species from II-VI semiconductor materials toxic to microorganisms
- Toxicity of tellurium oxyanions to methanogens reported for the first time
- Toxicity to acetoclastic methanogens increased as follows $Se^{IV} < Te^{VI} < Te^{IV} \approx Cd^{II}$
- Only Cd^{II} followed by Se^{IV} caused strong toxicity to hydrogenotrophic methanogens
- *Aliivibrio fischeri* was strongly inhibited by Cd^{II} and less by Se^{IV} and Te^{IV}

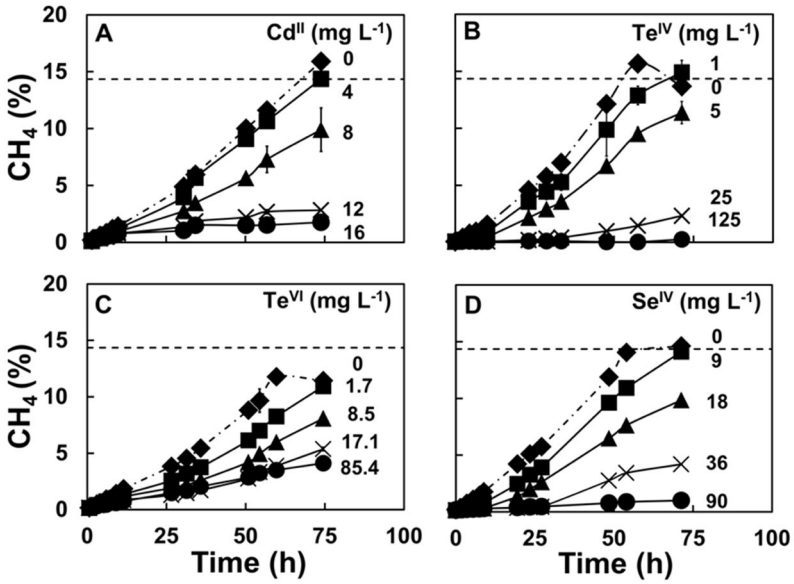

Fig 1.

Time course of methane production, expressed as cumulative percentage of methane (% CH_4), in the headspace of acetoclastic methanogenic bioassays in the presence of increasing initial concentrations of Cd, Te and Se ions. **Legends:** (- - -) Theoretical methane production. **Panel A,** Cd^{II} (in mg L^{-1}): 0 (◆), 4 (■), 8 (▲), 12 (×), and 16 (●). **Panel B,** Te^{IV} (in mg L^{-1}): 0 (◆), 1 (■), 5 (▲), 25 (×), 125 (●). **Panel C**, Te^{VI} (in mg L^{-1}): 0 (◆), 1.7 (■), 8.5 (▲), 17.1 (×), 85.4 (●). **Panel D**, Se^{IV} (in mg L^{-1}): 0 (◆), 9 (■), 18 (▲), 36 (×), 90 (●).

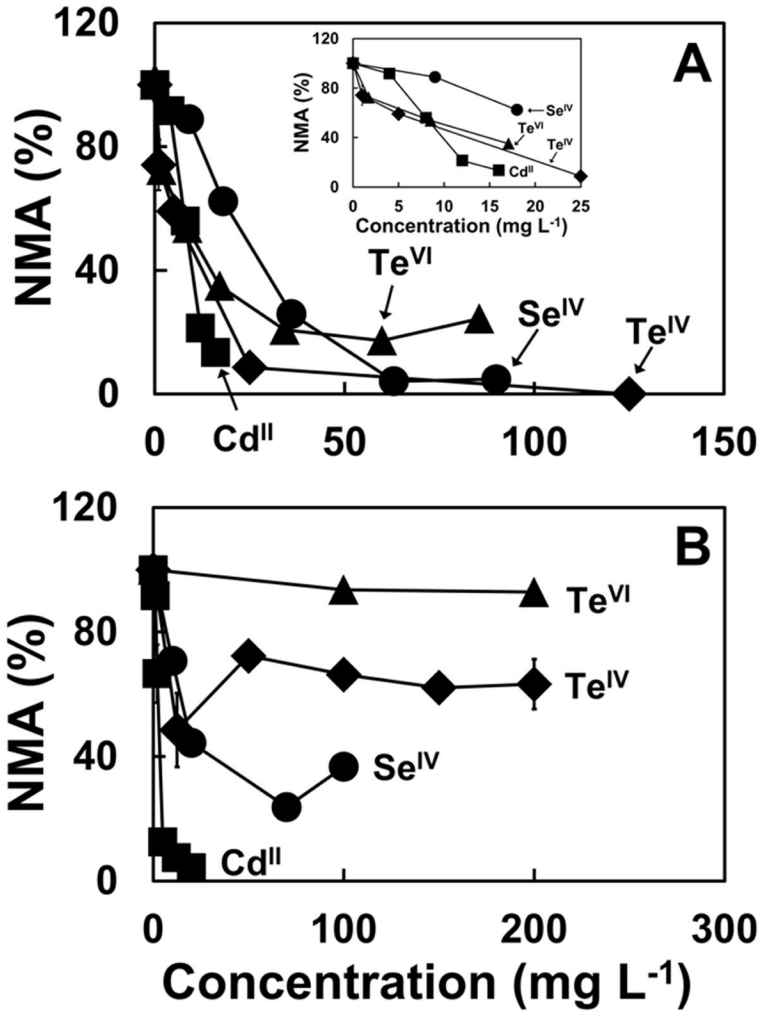

Fig 2.

Normalized specific methanogenic activity (NMA) as a percent of control activity in acetoclastic (**A**) and hydrogenotrophic (**B**) methanogenic experiments as a function of different initial concentrations of Cd, Te or Se ions. Legends: Cd^{II} (■), Te^{IV} (◆), Te^{VI} (▲), Se^{IV} (●).

Table 1

Summary of the IC_{20}, IC_{50}, IC_{80} values determined for the II-VI ionic species toward the acetoclastic and hydrogenotrophic methanogenic activities of anaerobic sludge.

A) Acetoclastic methanogenesis

Species	IC_{20}	Std dev	IC_{50}	Std dev	IC_{80}	Std dev
	mg L^{-1}					
Cd^{II}	5.3	0.2	8.6	1.0	12.7	0.3
Te^{IV}	1.3	0.8	8.6	0.9	20.5	0.3
Te^{VI}	2.4	1.8	10.2	0.8	38.1	6.7
Se^{IV}	11.8	1.5	24.1	0.1	43.3	0.7
Se^{VI}	3595	273	5514	146	≥ 6000.0	NA

B) Hydrogenotrophic methanogesis

Species	IC_{20}	Std dev	IC_{50}	Std dev	IC_{80}	Std dev
	mg L^{-1}					
Cd^{II}	**1.5**	**0.2**	**2.9**	**0.4**	**4.6**	**0.1**
Te^{IV}	5.0	1.2	≥ 200	NA	≥ 200	NA
Te^{VI}	≥ 500	NA	≥ 500	NA	≥ 500	NA
Se^{IV}	6.8	0.0	18.0	1.1	≥ 100	NA
Se^{VI}	≥ 6000	NA	≥ 6000	NA	≥ 6000	NA

NA = Not available

Table 2

Summary of the 50% inhibitory concentrations (IC_{50}) determined for the various II-VI ionic species toward *A. fischeri* after different times of exposure.

Species	IC_{50}					
	5 min	Std dev	15 min	Std dev	30 min	Std dev
	----------mg L^{-1}----------					
Cd^{II}	64.2	6.8	17	0.5	5.5	0.2
Te^{IV}	≥ 655	NA	≥ 655	NA	459	30
Te^{VI}	≥1082	NA	≥1082	NA	≥1082	NA
Se^{IV}	776	127	253	23.8	171	13
Se^{VI}	≥32491	NA	≥32491	NA	≥32491	NA

NA = Not available