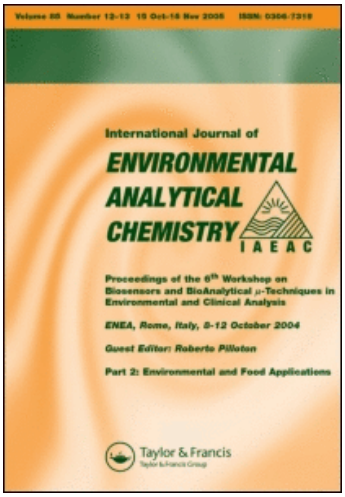

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Selenium speciation in anaerobic granular sludge

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Selenium speciation in anaerobic granular sludge

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Chromatographic (IC-CD, GC-FID) and spectroscopic (XRD) techniques that allow the specific determination of several selenium species present or formed during bioremediation processes of selenate contaminated drinking, ground, or wastewaters have been established. The developed techniques are shown to be applicable in determining selenium species in the range of target concentrations for emissions and are thus appropriate to characterize bioremediation processes. The applied techniques offer advantages regarding short analytical times without loss of satisfactory accurateness towards more sophisticated methods. By means of IC-CD, selenate and selenite can be detected specifically to concentrations far below allowance levels for metal finishing industries ($20\,\mu g\,L^{-1}$ selenate, $40\,\mu g\,L^{-1}$ selenite) within 16.0 min. Thus, the removal of selenate from the liquid phase by anaerobic granular sludge was quantified by IC-CD, and the concomitantly formed red precipitates were analysed by XRD. Hexagonal elemental selenium was found to be present in the XRD analysis. Even if the precipitate contained iron and zinc, as shown by ICP-OES, no iron- or zinc-containing selenium minerals were detected by XRD. The GC-FID method described determines dimethylselenide and dimethyldiselenide at a detection limit of 1 ng and 2 ng, respectively, without further chemical derivatization within 7.4 min. Thus, the GC-FID offers adequate detection of two major volatile organic selenium species potentially formed during bioremediation of selenium contaminated waters.

Keywords: Selenate; Selenite; Elemental selenium; Dimethylselenide; Dimethyldiselenide; UASB

1. Introduction

The importance of selenium in environmental research is related to the fact that this element shows only a marginal range between the nutritious requirement (as an essential element) and toxic effects upon exposure. According to Mayland [1], the nutritional requirement of animals for selenium lies in the range of 0.1–0.3 $mg\,kg^{-1}$ nutriment determined as total selenium, while chronic selenosis is caused by a concentration of 3–15 $mg\,kg^{-1}$. A concentration exceeding 25 $mg\,kg^{-1}$ in plant material can, upon consumption, result in death by acute stenosis.

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The toxicity of selenium compounds greatly depends on its speciation. For instance, the LD_{50} for oral administration in rats are much lower for selenate (2.5 mg Se kg^{-1} body weight) than for selenite (4.8–7.0 mg Se kg^{-1} body weight) or for elemental selenium (6700 mg Se kg^{-1} body weight) according to the US National Institute of Occupational Safety and Health [2]. Therefore, it is necessary to investigate the speciation of selenium during removal processes treating drinking, irrigation, or wastewaters. However, the great variety of environmentally important compounds poses a special challenge to selenium analysis, as selenium occurs in different oxidation states and in both organic and inorganic forms [3, 4].

As there is a legal need to reduce selenium emissions [5–7], different selenium removal processes have been developed [8] to remove selenium compounds from ground, irrigation and wastewaters. During the process of *dissimilatory metal reduction*, soluble oxy-anions of metals serve as electron acceptors for electrons originating from the degradation of organic compounds, H_2, or elemental sulphur [9]. Through this metal reduction, anaerobic bacteria conserve energy. It is distinguished from *assimilatory reduction*, where the reduction of metals is associated with cell synthesis [10]. In general, anaerobic treatment processes offer advantages compared with aerobic treatment. These advantages are a lower sludge production, energy savings because of dispensable aeration, cost savings in nutrient supply, smaller reactor volumes and the possibility of energy recovery by methane production [11]. One well-established reactor type in anaerobic treatment is the upflow anaerobic sludge blanket (UASB) reactor. It shows potential for the treatment of selenium-contaminated wastewaters, as special bioconversions can occur [12] in the bacterial granules formed in these reactors [13].

In contrast to adsorptive or chemical precipitation methods for selenium removal [8], application of a UASB reactor also allows for the treatment of the organic fraction of contaminated waters. Therefore, granular sludge originating from a full-scale UASB reactor was tested towards its remediation capacity for selenate under anaerobic conditions. The optimization of the selenate removal process should not only consider reaching a maximum formation of elemental selenium, which forms a solid phase and thus can be removed from contaminated waters, but also aim to prevent the formation of other toxic selenium species (i.e. selenite, hydrogen selenide, or selenoamino acids). Consequently, selenium should be determined not only as total selenium but also species-specific where possible. As selenium can be present in the liquid, solid, and gas phase, analytical methods for each of these phases were developed in this paper. It should be stated that this study is focused not on lowering detection limits by applying hyphenated methods [14], but on the establishment of species-specific methods, which have a relatively simple instrumental requirement but are still appropriate to characterize selenium bioremediation processes.

2. Experimental

2.1 *Instrumentation*

The ion-chromatographic system consisted of a Dionex DX 600 (Bavel, The Netherlands), equipped with an IonPac AS19 column (4 × 250 mm), an EG40 online Eluent Generator (potassium hydroxide), an ASRS Ultra II suppressor and a conductivity detector (IC-CD). An IonPac AS17 column (2 × 250 mm) was used in the

same system as well. The applied flows were 1.1 mL min^{-1} (AS19) and 0.25 mL min^{-1} (AS17). Sample loops of 25 μL and 5 μL, respectively, were used.

Gas-chromatographic analyses were performed on a Hewlett-Packard 5890 II series gas chromatograph with a flame ionization detection (GC-FID) system. The column used for separation was a Chrompack CP-PoraBOND Q with an internal diameter of 0.53 mm, a length of 25 m, and a film thickness of 10 μm (Varian, Middleburg, The Netherlands). Liquid samples of 1 μL were injected using splitless injection mode (purge on at 1.5 min). Helium was used as carrier gas at an inlet head pressure of 80 kPa. The FID detector was operated at 235°C at a hydrogen flow of 42 mL min^{-1} and airflow of 276 mL min^{-1}.

A Varian Vista-MPX simultaneous inductively coupled plasma optical emission spectroscopy (ICP-OES) system with a CCD detector was used with a plasma flow of 15 L min^{-1}, auxiliary flow of 1.5 L min^{-1}, and a nebulizer flow of 0.9 L min^{-1}. The generator power was 1300 W.

X-ray diffraction spectroscopy (XRD) was conducted with a PANalytical Expert Pro System (Almelo, The Netherlands) by using nickel-filtered CuKα radiation (tube operating at 40 kV and 40 mA). The data were collected using an automated divergence slit (5 mm irradiated length) and a 0.2 mm receiving slit.

2.2 *Chemicals*

Sodium selenate (SigmaUltra® grade), sodium selenite (99%), and elemental selenium (99.999%) were purchased from Sigma Aldrich (Steinheim, Germany). The anion standard for the IC measurement was provided by Dionex (Bavel, The Netherlands). A 1000 mg L^{-1} selenate and selenite stock solution was prepared by dissolving the respective sodium salts in Millipore Milli-Q water. This stock solution was diluted and mixed with the anion standard. Subsequently, the accuracy of the selenate and selenite standards was verified via ICP-OES.

For the ICP-OES measurements, calibration standards were prepared by stepwise dilution of multielement ICP standard solutions with the addition of appropriate amounts of selenium and sulphur ICP standards (Merck, Darmstadt, Germany).

Dimethylselenide (DMSe) and dimethyldiselenide (DMDSe) were purchased from Sigma-Aldrich as well. Standard solutions of the latter compounds were prepared by dilution in standard-solutions in HPLC-grade methanol (LabScale, Dublin, Ireland). All other chemicals used were of analytical grade.

2.3 *Source of biomass and experimental setup*

Anaerobic granular sludge originating from a full-scale UASB reactor treating paper mill wastewater (Industriewater Eerbeek B.V., Eerbeek, The Netherlands) was tested towards its selenate removal capacity in both batch and continous experiments. Both the physico-chemical [15] and microbiological [16] characteristics of this sludge have been well studied.

In batch tests, 0.5 g of wet sludge and 50 mL of mineral medium were put into 120 mL glass serum bottles, the bottles were sealed with a butyl rubber stopper and flushed with nitrogen. The bottles were placed onto a horizontal shaker (75 rotations min^{-1}) at 30°C. The mineral medium was prepared in Millipore Milli-Q

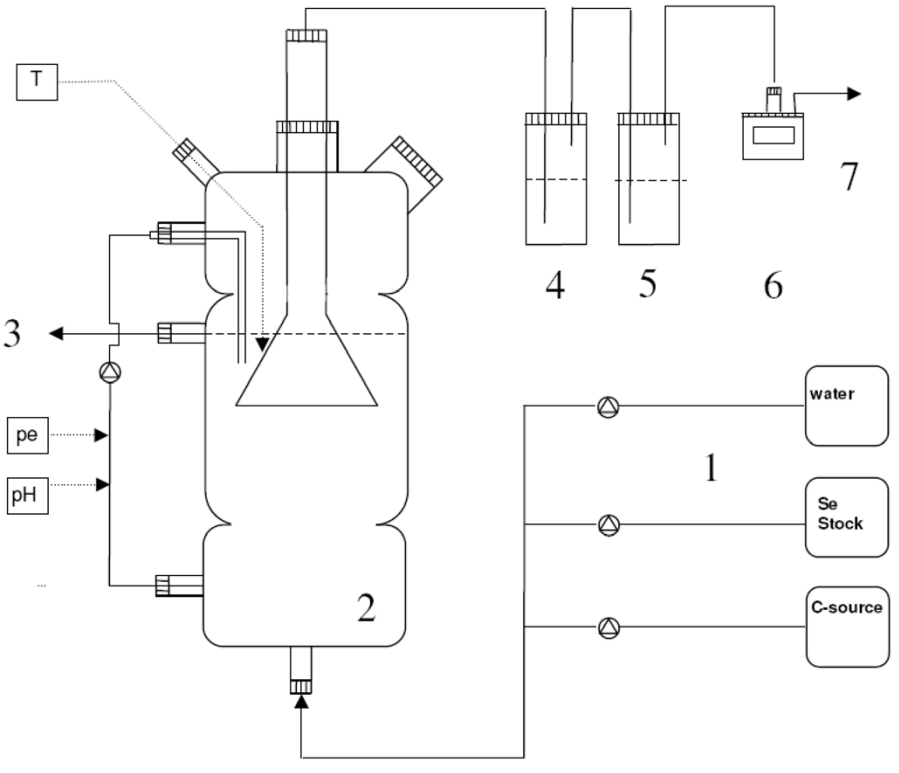

Figure 1. Schematic overview of an UASB reactor used for the simultaneous treatment of sulphate and selenate. (1) Feed solutions, (2) UASB reactor, (3) effluent, (4) ethylene glycol trap, (5) sodium hydroxide trap, (6) gas meter, and (7) gas outlet.

water after Stams *et al.* [17], but using a 40 mmol L^{-1} (mM) PO_4^{3-} buffer at pH 7.0 ± 0.1. Lactate (20 mM) was used as electron donor. To monitor the bioconversions during incubation, liquid and gas phase samples were taken anaerobically (N_2 atmosphere) at regular time intervals. The solid phase was sampled upon termination of the experiments. All batch experiments were done in triplicate.

The UASB reactor (0.46 L working volume) was inoculated with the same anaerobic granular sludge, operated at pH 7.0, a superficial upflow velocity of 1 m h^{-1} and a hydraulic retention time of 6 h. The design and operation of the UASB reactor (figure 1) are described in detail by Omil *et al.* [18]. Lactate was used as electron donor at an organic loading rate of 5 g COD L^{-1} day^{-1}. The selenate influent concentration was 10 μM. In order to trap volatile organic selenium compounds, the biogas was bubbled through ethylene glycol [19].

3. Results

3.1 *Specific determination of selenate and selenite by IC-CD*

Under the prerequisite of short analytical times but achieving separation of a high number of major anions, a method has been developed, separating eight anions

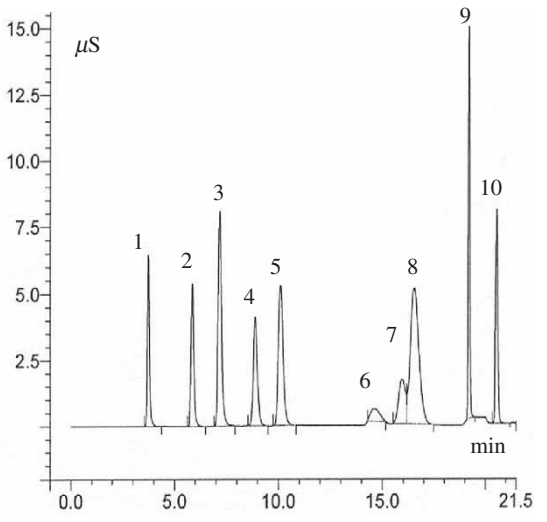

Figure 2. IC-chromatogram showing separation of an anion standard by means of an IonPac AS19 column (mg L^{-1}): (1) F^- 1.62, (2) Cl^- 2.4, (3) NO_2^- 7.9, (4) Br^- 8.04, (5) NO_3^- 8.10, (6) CO_3^{2-} not quantified, (7) SeO_3^{2-} 11.52, (8) SO_4^{2-} 11.99, (9) SeO_4^{2-} 9.79, and (10) PO_4^{3-} 12.33.

Table 1. IC-gradient applied for colums AS19 and AS17.

Time (min)	Concentration eluent AS19 (mM KOH)	Time (min)	Concentration eluent AS17 (mM KOH)
0	15	0	10
16	15	5.5	10
16	50	10	50
19.5	50	14	50
19.5	15	14	10
21.5	End	16	End

(fluoride, chloride, nitrite, bromide, nitrate, carbonate, sulphate and phosphate) from selenite and selenate in less than 22 min using an IonPac AS19 anion-exchange column. The chromatogram for separation of the anion standard can be seen in figure 2, and the applied gradients in table 1. The detection limits are 0.05 mg L^{-1} for selenate (0.028 mg L^{-1} selenium) and 0.25 mg L^{-1} for selenite (0.138 mg L^{-1} selenium). The method was used to show that anaerobic granular sludge is able to remove selenate from the liquid phase in the presence of nitrate/nitrite (initial nitrate concentration 5 mM, figure 3).

In order to improve the separation of sulphate and selenite, an IonPac AS17 column was tested and the gradient optimized with respect to analysis time (table 1). Indeed, baseline separation of selenite and sulphate was achieved (compare peaks 6 and 8 in figure 4 vs. the incompletely separated peaks 7 and 8 in figure 2). The resulting detection limits were 0.04 mg L^{-1} for selenite (0.025 mg L^{-1} selenium) and 0.02 mg L^{-1} (0.011 mg L^{-1} selenium) for selenate. The reproducibility for three subsequent injections of selenate and selenite standards is given in table 2. The applicability of the method was shown by an exemplary determination of anions in the influent (A) and the effluent (B) of a continuous UASB reactor treating sulphate and selenate simultaneously (figure 5).

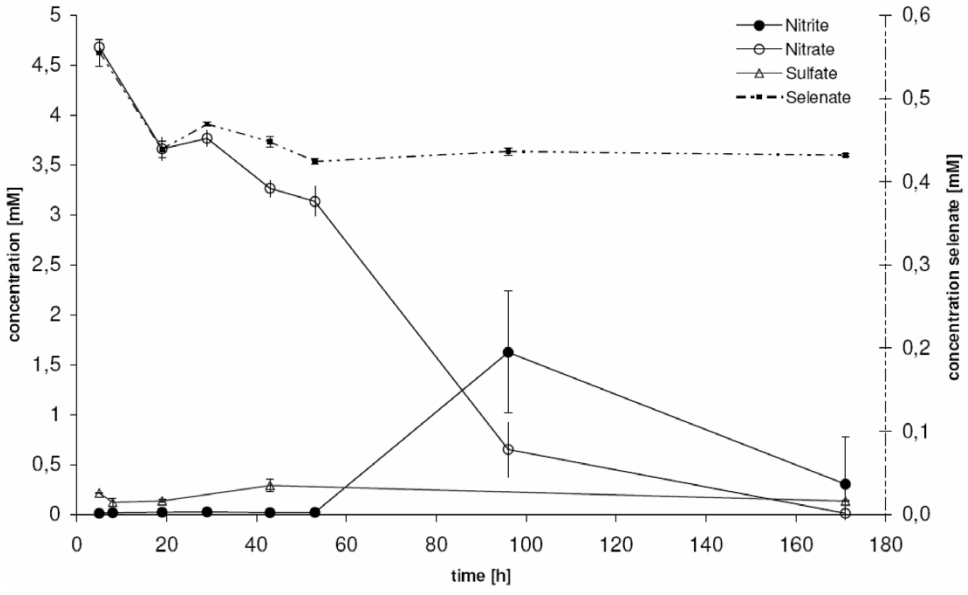

Figure 3. Bioconversion of 0.5 mM selenate and 5 mM nitrate by anaerobic granular sludge in batch incubation (performed in triplicate); nitrite, nitrate, and sulphate in [mM] (primary *y*-axis), selenate (mM) (secondary *y*-axis for better overview). Concentrations were determined via IC-CD with column IonPac AS19.

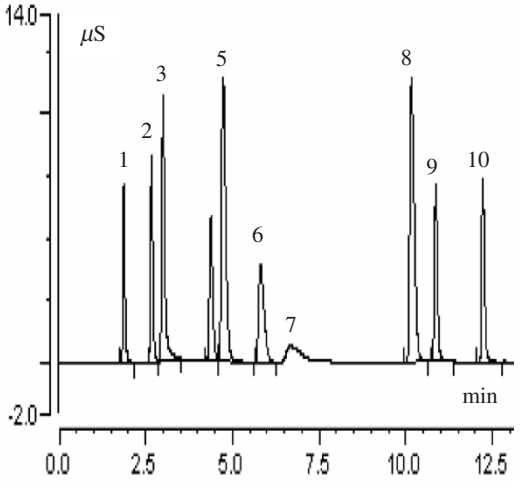

Figure 4. IC-chromatogram showing separation of an anion standard by means of an IonPac AS17 column (mg L^{-1}) using IonPac AS 17: (1) F^- 1.00, (2) Cl^- 1.51, (3) NO_2^- 5, (4) Br^- 4.99, (5) NO_3^- 5.00, (6) SeO_3^{2-} 10.00, (7) CO_3^{2-} not quantified, (8) SO_4^{2-} 7.45, (9) SeO_4^{2-} 5.00, (10) PO_4^{3-} 7.45.

3.2 *Determination of elemental selenium using XRD and investigation of the precipitate by ICP-OES*

Batch incubations of granular sludge were able to convert selenate at high concentrations (1.8 g L^{-1} $selenium_{total}$) removing significant amounts of selenate (262 mg L^{-1}) during 10 days of incubation. Upon further incubation, a red precipitate

Table 2. Reproducibility of the IC method (AS17 column) for three subsequent injections of the selenate and selenite standard.

	Reproducibility with selenite or selenate (RSD, %, $n = 3$)						
	0.05	0.25	2.5	25	Calibration ($y = mx + b$)[a]		
Compound	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	m	b	r^2
Selenite	7.1	1.4	0.9	0.4	0.0745	–0.01	0.9997
Selenate	5.8	3.2	0.4	0.1	0.1406	–0.003	0.9999

[a] y = peak area (μS · min); x = concentration (mg L^{-1}).

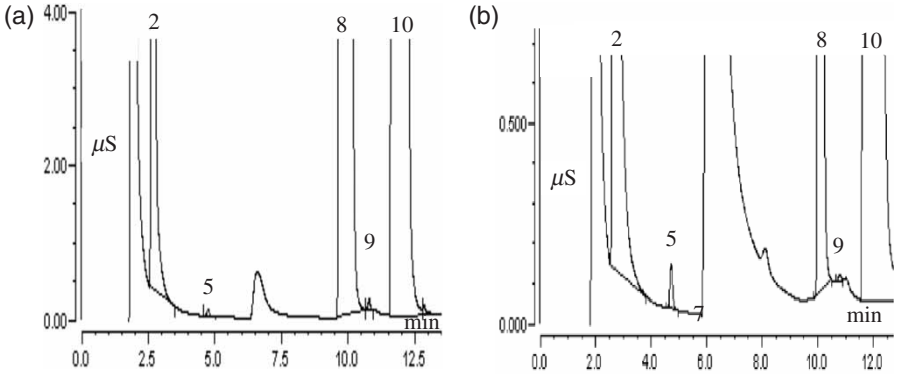

Figure 5. (a) Separation of anions present in the influent of a continuous reactor system treating selenate and sulphate (mg L^{-1}): (2) Cl^- 336.15, (5) NO_3^- 0.41, (8) SO_4^{2-} 383.64, (9) SeO_4^{2-} 1.03, (10) PO_4^{3-} 3011; and (b) anions in the effluent at (mg L^{-1}): (2) Cl^- 330.19, (5) NO_3^- 0.56, (8) SO_4^{2-} 52.45, (9) SeO_4^{2-} 0.15, (10) PO_4^{3-} 2883. Concentrations were determined via IC-CD with column IonPac AS17.

was formed (see figure 6a and b). Subsequent to separation by centrifugation, the precipitate was washed twice with oxygen-free Millipore water under N_2 atmosphere. The formation of elemental selenium within this precipitate was investigated using XRD (figure 7 and table 3).

Peaks number 5, 10, and 12 match with the 100, 101, and 102 plane of elemental selenium with a hexagonal crystalline structure in d-spacing, with minor differences on the second decimal place. Furthermore, the peak at an angle of $2\theta = 23.50^\circ$ yields the highest relative intensity, $2\theta = 29.63^\circ$ shows the second highest and $2\theta = 43.83^\circ$ the lowest relative intensity. This is in accordance with the standard for elemental selenium. Further selenium planes could not be identified, as the baseline noise was too high.

In order to limit the number of minerals to be compared with standard diffractograms, the precipitate was studied further. Elemental analysis by ICP-OES was conducted after microwave-assisted aqua regia destruction of the precipitate. Dry weights [%] of the investigated elements contained in 2 mg of the precipitate are shown in table 4.

Table 4 shows that iron and zinc are the only potential elements present in the precipitate in significant amounts that could form selenium minerals [20]. A comparison with the highest intensity peaks of the standard diffractograms of dzharkenite ($FeSe_2$), achavalite (FeSe), mandarinoite ($Fe_2^{3+}Se_3O_9 \times 6H_2O$), stilleite (ZnSe) and sophiite ($Zn_2(SeO_3)Cl_2$) did not result in any matches with the sample investigated (maximum difference of 0.01 Å). Only peak number 6 is in accordance with the

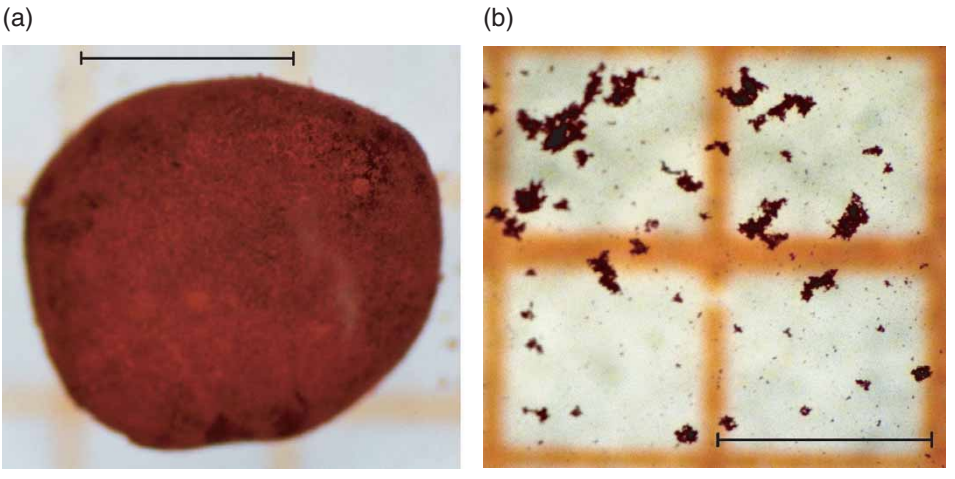

Figure 6. Reflected-light microscopic picture of an anaerobic sludge granule originating from batch culture treating selenate solutions (1.8 g L^{-1} selenium) covered with red precipitate (a) and precipitate floating in mineral medium (b); bars correspond to 1 mm.

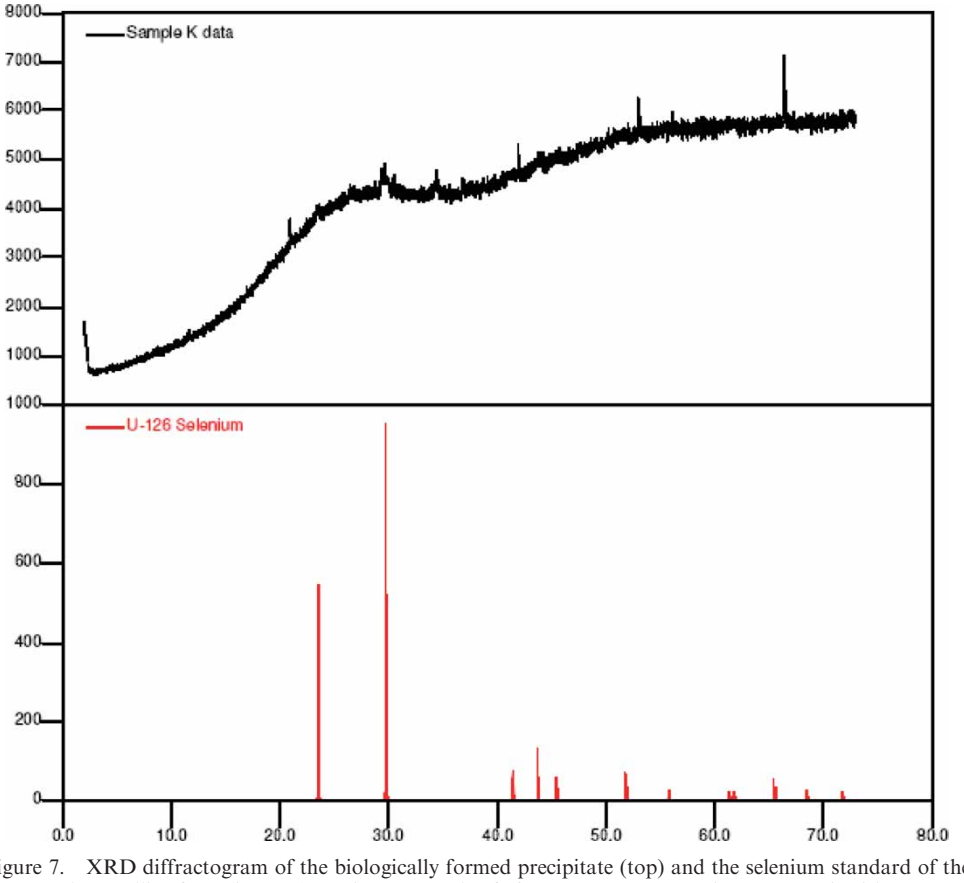

Figure 7. XRD diffractogram of the biologically formed precipitate (top) and the selenium standard of the hexagonal crystalline form (bottom). Peaks at an angle of $2\theta = 23.50°$, 29.63°, and 43.83° match with 100, 101, and 102 planes of hexagonal selenium.

Table 3. Elemental composition of precipitate (2 mg dry weight) formed by anaerobic granular sludge (pH 7.0, 20 mM SeO_4^{2-}, 20 mM lactate) after microwave-assisted aqua regia destruction determined via ICP-OES.

Element	Dry weight (%)
Se	49.85
Ca	3.38
Na	1.94
Mg	0.88
Fe	0.27
K	0.13
Zn	0.02
Ag, Cd, Co, Cu, Mn, Ni, S	< 0.007

Table 4. Peaks identified in XRD diffractogram, sorted by relative intensity (with peaks indicating hexagonal elemental selenium in bold).

Peak no.	d-spacing (Å)	Relative intensity (%)	Angle (°2θ)	Peak height (counts/s)	Background (counts/s)	d-spacing in hexagonal selenium	Relative intensity in hexagonal selenium
1	1.40755	100	66.35671	544.89	2291.53		
2	1.72646	53.9	52.99552	293.67	2204.07		
3	1.40405	48.37	66.54374	263.54	2291.15		
4	4.23787	41.48	20.9447	226	1282.12		
5	**3.01206**	**32.45**	**29.63395**	**176.8**	**1723.96**	**3.00**	**100**
6	2.60293	29.19	34.42635	159.04	1707.53		
7	1.72238	28.97	53.13066	157.84	2206.15		
8	2.14948	25.75	41.99864	140.34	1873.51		
9	2.43621	16.02	36.86405	87.3	1733.13		
10	**3.78129**	**15.55**	**23.50777**	**84.75**	**1494.41**	**3.78**	**55**
11	2.9256	15.47	30.53066	84.28	1723.07		
12	**2.06361**	**10.28**	**43.83455**	**55.99**	**1942.68**	**2.07**	**35**
13	7.57447	8.08	11.67349	44.03	544.3		

highest-intensity peak of ferroselite ($FeSe_2$) (Table 4). Since the second highest peak belonging to a d-spacing of 2.49 Å of ferroselite (85% relative intensity) is completely missing in the sample, ferroselite could not be shown to be present in the sample.

3.3 *Optimization of a GC-FID method for DMSe, DMDSe determination*

A Chrompack CP-PoraBOND Q column was used for separation of DMSe and DMDSe. No interference with the methanol peak was observed up to a temperature of 140°C, resulting in a retention time of 3.54 min for DMSe. For the resolution and detection of DMDSe, the temperature was subsequently increased at a maximal rate of 70°C min^{-1} to 240°C. To obtain a stable baseline, this temperature was not elevated any further. The result was a short analytical time of 7.4 min in total (figure 8), with detection limits of 1 ng (total amount) injected for DMSe and 2 ng for DMDSe, respectively. These values are slightly lower than the values reported by Hunter *et al.* [21] (1–5 ng for DMSe and 5 ng for DMDSe).

Gas-phase aliquods (50 mL) of the batch cultures were collected via a syringe and carefully bubbled through 2 mL of methanol using a glass frit in order to

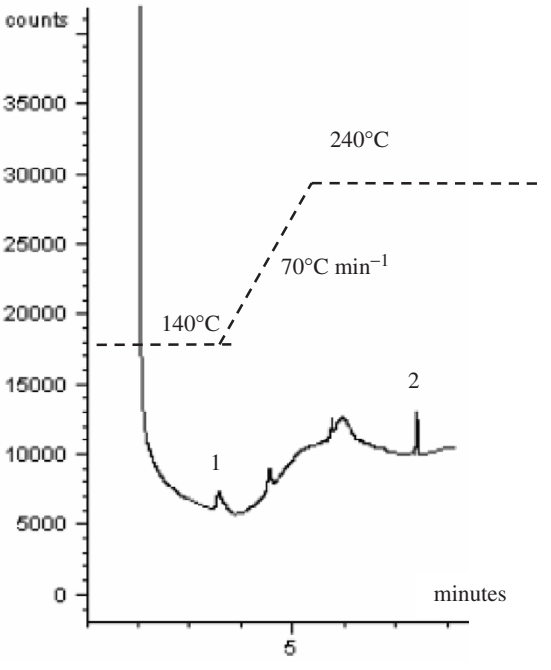

Figure 8. Separation of (1) DMSe (3.54 min) and (2) DMDSe (7.37 min) by GC-FID (injected amount 10 ng each). The dashed line represents the temperature gradient applied.

pre-concentrate organic selenium compounds. The methanol extract was injected into the GC, but neither DMSe nor DMDSe could be detected. The ethylene glycol trap of the continuous bioreactor (figure 1) was sampled after 30 days of biological selenate treatment by diluting different amounts of ethylene glycol in methanol under the addition of an internal standard. Neither DMSe nor DMDSe were detected using the developed method.

4. Discussion

4.1 *Liquid-phase analysis*

Among the techniques currently applied for species-specific determination of selenate and selenite, hydride generation coupled to atomic absorption spectroscopy (HG-AAS) [22] or ICP (HG-ICP) is commonly used [23]. As the hydride formation is selective for selenite, a quantitative reduction of selenate to selenite is required. This is disadvantageous, because of possible losses or incomplete chemical conversion between the selenium species. In contrast to this, ion chromatography does not require species interconversion and has little sample preparation. Using the IonPac AS17 column as described in this work (table 1), the analysis is completed in 16.0 min (figure 4). Additionally, ion chromatography offers the possibility to determine a variety of other anions simultaneously, which is important to monitor remediation processes in bioreactors. Utilizing the IC-CD method developed made it possible to follow up selenate and selenite species specifically to concentrations in the low mg L^{-1} range

both in batch incubations (figure 3) and in a continuous reactor system (figure 1 and 5A and B).

Because of the lower diameter compared with the IonPac AS19, the AS17 column shows a three- to fourfold increase in sensitivity according to the supplier's manual (Dionex, Bavel, The Netherlands). Hence, with the detection limits achieved using anion standards, it is possible to monitor selenate and selenite down to the $\mu g\,L^{-1}$ range (table 2). The achieved detection limits are much lower (90-fold for selenate and 40-fold for selenite, respectively) than the allowance levels for liquid effluents in metal finishing industries of $1\,mg\,L^{-1}$ $selenium_{total}$ already applied in some European countries (e.g. Germany) [6]. Hence, the IC methods developed have been shown to be sufficient in determining selenium species in concentrations related to bioremediation processes. One future application for this IC method might be its adoption to other water-soluble oxyanions that can pose an environmental hazard, e.g. molybdate, tungstate, chromate, or uranate.

4.2 *Solid-phase analysis*

Anaerobic granular sludge exposed to high concentrations of selenate ($1.8\,g\,L^{-1}$ $selenium_{total}$) formed a red precipitate during the removal of water-soluble selenate (figure 6). As described in the literature [24], this can be due to amorphous selenium (red in powder form) or crystalline monoclinic selenium (deep red). Crystalline hexagonal selenium—the most stable variety—has a metallic grey colour; amorphous selenium is black in vitreous form [24]. The total metal analysis via ICP-OES showed that 49.8% (dry weight) of the formed precipitate is selenium, with only minor fractions consisting of iron and zinc (table 4). Thus, the formation of stable metal selenides with silver, cadmium, cobalt, and copper in significant amounts can be excluded by ICP-OES, limiting the number of possible compounds formed. Hockin and Gadd [25] described a mechanism, where sulphide and selenite—a common intermediate during the reduction of selenate—co-precipitate to elemental sulphur and selenium. As sulphur was not found in the precipitate, and selenite was not detected by IC-CD at any sampling time, this mechanism is probably not taking place under the described conditions.

In the subsequent XRD analysis, it was indicated that part of the precipitate is elemental selenium with a hexagonal crystalline structure. The resulting diffractogram (figure 7 and table 3) showed a high baseline noise and small signal for crystalline selenium. This can be due to the small amount of precipitate investigated or the low content of crystalline selenium in the sample. However, the d-spacing characteristic for hexagonal selenium could be identified in the diffractogram with differences of 0.01 Å to the selenium standard. As insoluble metal selenides can be formed under reducing conditions [26], standard diffractograms of six minerals containing iron and zinc were compared with the sample (see section 3.2). The standard peaks could not be matched to the peaks obtained in the precipitate. However, the high baseline noise makes it impossible to exclude their formation completely. Both the formation of these metal selenides and the crystalline forms of selenium need further investigation. Higher amounts of precipitate (several milligrams) would be appropriate to further confirm the formation of hexagonal selenium. Such higher amounts of precipitate can be produced by a continuous bioreactor equipped with a settler. It should be noted

that the degree of re-solubilization or chemical oxidation of the mineral by ambient O_2 in the settler is a restriction to be assessed.

As XRD analysis is not suitable for the confirmation of elemental selenium with a high number of samples, one future goal is to find applicable methods that give an indication of selenium-binding forms in the solid phase. Combining fractionation methods like sequential extraction schemes with the determination of 'acid volatile selenium' could be very well suited. Unlike the 'acid volatile sulphur' fractionation, which is commonly used to characterize sulphur-binding forms [27], its analogue 'acid volatile selenium' still needs to be developed and validated using reference materials.

4.3 *Gas-phase analysis*

Dimethylselenide and dimethyldiselenide represent two major volatile organic selenium species in the environment [28]. The formation of methylated compounds by plants and associated bacteria is used in large-scale bioremediation projects [29]. In the methods developed by Hunter *et al.* [21], interferences of dimethylselenide and dimethyldiselenide with the methanol injection peak were encountered, resulting in limitations for the detection limit. The authors used two single injections in order to analyse the latter compounds. In the present work, these problems have been overcome by the substitution of a porous layer open tubular column by a recently developed Chrompak Capillary CP-Pora BONDQ column and applying an appropriate temperature gradient (figure 8). Thus, the application range for the determination of DMSe and DMDSe was increased by the resulting lower detection limits. The method was applied both to batch cultures treating high selenate loads and in the continuous bioreactor system, but neither DMSe nor DMDSe could be detected. Nevertheless, the method represents a simple technique to analyse two main products of selenium biomethylation. In order to rule out the formation of these compounds, the detection limit can be lowered from the nanogram range to the picogram range by using a photo-ionization detector [21]. The adaptation of the GC-FID method to other organic selenium compounds, e.g. diethyldiselenide or diethylselenide [30, 31], completing the spectrum of possibly formed volatile organic selenium species, is possible.

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