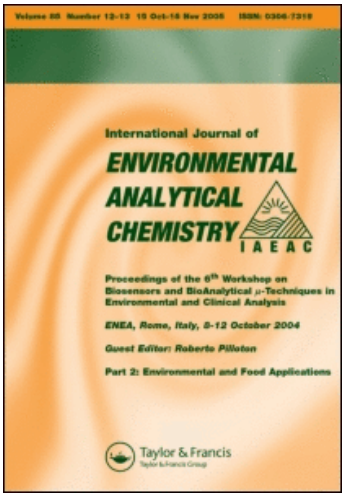

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Bio-accumulation of As(III) and As(V) species from water samples by two strains of *Aspergillus niger* using hydride generation atomic absorption spectrometry

Mária Žemberyová[a]; Adriana Shearman[b]; Alexandra Šimonovičová[c]; Ingrid Hagarová[a]
[a] Faculty of Natural Sciences, Department of Analytical Chemistry, Comenius University, 842 15 Bratislava, Slovakia [b] Water Research Institute, 842 19 Bratislava, Slovakia [c] Faculty of Natural Sciences, Department of Soil Science, Comenius University, 842 15 Bratislava, Slovakia

Online publication date: 22 September 2010

To cite this Article Žemberyová, Mária , Shearman, Adriana , Šimonovičová, Alexandra and Hagarová, Ingrid(2009) 'Bio-accumulation of As(III) and As(V) species from water samples by two strains of *Aspergillus niger* using hydride generation atomic absorption spectrometry', International Journal of Environmental Analytical Chemistry, 89: 8, 569 — 581

To link to this Article: DOI: 10.1080/03067310802716107

URL: http://dx.doi.org/10.1080/03067310802716107

Intern. J. Environ. Anal. Chem.
Vol. 89, Nos. 8–12, 15 July–15 October 2009, 569–581

Taylor & Francis
Taylor & Francis Group

Bio-accumulation of As(III) and As(V) species from water samples by two strains of *Aspergillus niger* using hydride generation atomic absorption spectrometry

Mária Žemberyová[a]*, Adriana Shearman[b], Alexandra Šimonovičová[c] and Ingrid Hagarová[a]

[a]*Faculty of Natural Sciences, Department of Analytical Chemistry, Comenius University, Mlynská dolina CH-2, 842 15 Bratislava, Slovakia;* [b]*Water Research Institute, Nábrežie arm. gen. L. Svobodu 5, 842 19 Bratislava, Slovakia;* [c]*Faculty of Natural Sciences, Department of Soil Science, Comenius University, Mlynská dolina B-2, 842 15 Bratislava, Slovakia*

(*Received 16 July 2008; final version received 30 December 2008*)

The bio-accumulation ability of two strains of *Aspergillus niger* was studied in various synthetic media containing inorganic single-species As(III) and As(V) solutions and their admixtures and mining water with high As content in order to study the extent of bio-accumulation of the As species by the fungi. The AN1 *A. niger* strain, which served as a reference, was isolated from the Eutric Fluvisol soil (pH H_2O/KCl 7.7/7.4) originating from an uncontaminated area near Gabčíkovo (Southwest Slovakia). The AN3 *A. niger* strain was isolated from the bottom sediment with a natural content of As 363 mg kg^{-1} (pH H_2O/KCl 5.27/4.8) collected from the Blatina stream running from abandoned antimony mines near Pezinok (West Slovakia). Samples of fungi biomass following bio-accumulation experiments were acid-decomposed in an autoclave under elevated temperature and pressure and used for total inorganic arsenic (As) determination. After 24 h of bio-accumulation in a solution containing 10 μg L^{-1} As(III), the AN1 strain showed to be more efficient transforming 73% of As(III), in comparison with the AN3 strain by which 38% of As(III) was bio-transformed. On the other hand, the AN3 strain demonstrated greater capacity to retain in its mycelia 17% of As from a solution containing 10 μg L^{-1} As(V) as compared with less than 10% of As(V) accumulated in the mycelia of the AN1 strain. Continuous hydride generation atomic absorption spectrometry (HG-AAS) was used for simple, rapid, sensitive and accurate determinations of total inorganic As and As(III). The accuracy of the method for the determination of As(III) was evaluated by analysing spiked synthetic and natural river waters. Recoveries of 96–102% of spikes were obtained. Limits of detection (3σ-criterion) for total inorganic As determination and As(III) determination were 0.22 and 0.28 μg L^{-1}, respectively.

Keywords: total inorganic arsenic; arsenite; speciation; water; *Aspergillus niger*; hydride generation atomic absorption spectrometry

1. Introduction

The toxicity of arsenic (As) varies widely, ranging from highly hazardous inorganic arsenicals (arsine, arsenite As(III) and arsenate As(V)) to relatively harmless organic

*Corresponding author. Email: zemberyova@fns.uniba.sk

ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310802716107
http://www.informaworld.com

species (monomethylarsonate, MMA and dimethylarsenate, DMA). Indeed, some organoarsenicals, such as arsenobetaine and arsenocholine, are effectively non-toxic towards living organisms [1]. Among these As compounds, of particular interest is arsenite, which is 10 times more toxic than arsenate and 70 times more toxic than the methylated species, DMA and MMA [2]. These facts indicate why it would be of priority interest to develop a method for selective determination of As(III).

For the routine determination of trace As concentrations, hydride generation atomic absorption spectrometry (HG-AAS) is probably the technique of choice. This technique accounts for the generation of volatile As hydrides (arsines) using, as a rule, $NaBH_4$ as an efficient reducing agent. Since the formation of arsine is affected by the oxidation state, with As(V) reacting more slowly than As(III), pre-reduction of As(V) to As(III) (usually with KI and ascorbic acid admixture) is performed prior to the total As determination. Furthermore, the reaction between $NaBH_4$ and an As ion in a solution is sensitive to pH and it appears that, for the reaction to proceed rapidly, the target species must not be present in solution as a negatively-charged species. The most abundant As species in waters are the inorganic species arsenate and arsenite. Therefore, it means that arsenite and arsenate must be fully protonated if they are to be converted to arsine. As pKa_1 for 'As' acid is 2.3, the reaction must be carried out at very low pH. Arsenious acid, on the other hand, is protonated under most conditions ($pKa_1 = 9.2$) and will react with $NaBH_4$ under conditions which are only mildly acidic [3]. Hence, simple speciation analyses, such as the differentiation of arsenite and arsenate, can be performed by careful control of reaction conditions.

This theory was applied for optimisation of the analytical conditions for the reliable determination of As(III) in synthetic solutions and various water samples by continuous HG-AAS [4,5]. The concentration of inorganic As(V) was calculated as the difference between total inorganic As and As(III).

Since As contamination of water can cause a serious health and environmental problem, different techniques have been developed and tested for the removal of this element [6]. These techniques, with or without a pre-oxidation step, can basically be divided into co-precipitation, electrochemical methods, ion-exchange processes, membrane filtration and sorption methods. Sorption methods are simple to perform and are inexpensive [7]. In addition to synthetic materials, natural sorption materials can be used in this process some of the latter have been investigated by several groups [8,9]. It is well known [10] that heterotrophic organisms, especially microscopic fungi, have a remarkable ability to utilise toxic ions of various metals and radio-nuclides, thus decreasing their content in the environment they live in. This effect is realised *via* specific receptor channels and pathways in which suitable metal transferases are involved leading to the modification of some genes and resulting in the accumulation of these ions in the intracellular milieu and organells of these organisms. When this process takes place on the cell membrane, it can be considered as adsorptive, because no biological activity is present. Otherwise, when live cells are used, the metallic species are first adsorbed on a cell membrane; after passing through this membrane, they are absorbed into this structure [11]. The groups responsible for the adsorption processes are found from bio-molecules (proteins, polysaccharides and cellulose), which contain sulphates, carboxylates, phosphates [12], etc., in their structures. The application of fungi in resolving the problem of water contaminated with toxic compounds is receiving increasing interest because fungi are ubiquitous in the natural environment and are often the dominant organism in many soils [13,14].

In order to contribute to solving the problem of As contamination of surface water, the aim of this work was to examine the ability of the *Aspergillus niger* strain isolated from a stream sediment with elevated As content to accumulate different species of As and to compare it with the ability of a control strain of *A. niger* originating from an uncontaminated area.

To achieve this, procedures for the speciation of inorganic forms of As in various media, such as single- and mixed-species inorganic As model solutions, a synthetic river water (SRW) sample, natural river water (NRW) and mining water (MW), were optimised.

2. Experimental

2.1 *Equipment*

Solaar 939 atomic absorption spectrometer equipped with continuous VP 90 hydride system (Ati Unicam, Great Britain) was used for the determination of total As and As(III) under the following conditions: hollow cathode lamp current 12 mA; wavelength λ_{As} 193.7 nm; slit width 0.5 nm; D_2 background correction; argon flow-rate: 150 mL min^{-1}; sample flow-rate: 7.5 mL min^{-1}; reductant ($NaBH_4$) flow-rate 4 mL min^{-1}; signal stabilisation 40 s; baseline delay 30 s; sample measurement 10 s. Diluted hydrochloric acid (10% v/v) was used as a baseline and carrier medium.

The sample solutions and sodium tetrahydroborate carried by argon were mixed in a mixing coil to generate the covalent gaseous As trihydride (arsine, AsH_3, CAS 7784-42-1). Following separation in a gas–liquid separator, the gaseous hydride and excess hydrogen were carried in the stream of Ar into a flame-heated quartz cell of 5 cm effective length and 0.5 cm i.d. The procedure was automated by means of auto-sampler and control software.

KBCG drying box (Premed, Poland) was used to dry fungal mycelia at a temperature of 40°C for about 48 h (to a constant weight).

Stainless steel autoclave vessels (JZD Zahnašovice, Czech Republic) with teflon internal coating were used for the decomposition of dried mycelia.

All glassware was soaked in 10% (v/v) HNO_3 for a minimum of 12 h and washed with distilled water and finally rinsed with deionised water (Water Pro PS, Labconco, USA).

2.2 *Reagents and solutions*

All chemicals were of p.a. grade. A total of 1 g L^{-1} As (as H_3AsO_4) atomic absorption standard, As_2O_3, $KAsO_2$, concentrated HCl, concentrated HNO_3, $NaBH_4$, NaOH, KI, ascorbic acid, citric acid, $Na_2HPO_4 \cdot 12H_2O$, $CaCl_2 \cdot 2H_2O$, NaCl, $MgSO_4 \cdot 7H_2O$, KCl and $(NH_4)_2HPO_4$ were purchased from Merck, Darmstadt, Germany. Deionised water was used for dilution and rinsing. Solid Sabouraud Maltose Agar was obtained from HiMedia, Mumbai, India. Liquid Sabouraud media (glucose 40 g, mycological peptone 10 g, H_2O 1000 mL) was prepared in our laboratory.

2.2.1 *Arsenic solutions*

A total of 1 g L^{-1} As(III) stock solutions were prepared by dissolving 0.1949 g of $KAsO_2$ in 100 mL of deionised water or 0.1320 g of As_2O_3 first in 2 mL of 1.0 mol L^{-1} NaOH, then neutralised by 5 mL of 0.5 mol L^{-1} HCl and finally made up to 100 mL with deionised water. Both As(III) stock solutions were kept refrigerated at 4°C.

As(V) stock solution was a commercial atomic absorption standard solution containing $1.000 \pm 0.002\,g\,L^{-1}$ as H_3AsO_4 in $0.5\,mol\,L^{-1}$ HNO_3.

Calibration solutions of 1, 2, 5, 10, 20 and $50\,\mu g\,L^{-1}$As(III), single-species experimental media containing 10, 50 and $100\,\mu g\,L^{-1}$ As(III) used in bio-accumulation experiments and all other solutions used in optimisation of the determination of As(III) were prepared afresh prior to use by a serial dilution of the respective As stock solutions in deionised water.

Calibration solutions of 1, 2, 5, 10, 20 and $50\,\mu g\,L^{-1}$As(V), single-species experimental solutions containing 10, 50 and $100\,\mu g\,L^{-1}$ As(V) used in bio-accumulation experiments and all other solutions used in the optimisation of the determination of As(III) were prepared afresh prior to use by a serial dilution of the respective As stock solution with deionised water.

A mixed-species solution containing $10\,\mu g\,L^{-1}$ As(III) and $10\,\mu g\,L^{-1}$ As(V) used in bio-accumulation experiments and all mixed-species solutions used in the optimisation of As speciation were prepared by serial dilution of the respective As stock solutions with deionised water.

2.2.2 *Pre-reducing agent for total As determination*

A total of 20% KI and 10% ascorbic acid admixture solution was prepared by dissolving 50 g of KI and 25 g ascorbic acid in 250 mL of deionised water and served as a pre-reducing agent in the determination of total As. This solution was stable for at least a month when stored in an amber glass bottle in a refrigerator at 4°C until use.

2.2.3 *Reductant solutions*

A total of 1.0% (w/v) $NaBH_4$ in 1.0% (w/v) NaOH, used as a reducing agent for total As determination by HG-AAS, was prepared by dissolving 10 g of $NaBH_4$ and 10 g of NaOH in 1000 mL of deionised water. Reducing solutions containing 0.05% (w/v) $NaBH_4$ in 0.05% (w/v) NaOH; 0.1% (w/v) $NaBH_4$ in 0.1% (w/v) NaOH and 0.2% (w/v) $NaBH_4$ in 0.2% (w/v) NaOH were prepared by diluting 1.0% (w/v) $NaBH_4$ in 1.0% (w/v) NaOH solution and were used in optimisation of the determination of As (III). All these solutions were prepared afresh prior to use.

2.2.4 *Samples*

Synthetic river water spiked with $10\,\mu g\,L^{-1}$ of As(V) was prepared by dissolving 294 mg of $CaCl_2 \cdot 2H_2O$; 216 mg of NaCl; 862 mg of $MgSO_4 \cdot 7H_2O$; 9.7 mg of KCl; 7.3 mg of $(NH_4)_2HPO_4$ in 1000 mL of deionised water [15].

This sample was used in the interference study as part of the optimisation of the determination of As (III).

Natural river water from the Hron with a natural content of approximately $8\,\mu g\,L^{-1}$ As was collected near the village of Kamenica, Southwest Slovakia. This water was used in the recovery test in optimisation of the determination of As(III).

Mining water containing approximately $46\,\mu g\,L^{-1}$ As originated from the Blatnica stream running from former antimony mines near the town of Pezinok (West Slovakia). MW was used in experiments with bio-accumulation of As by fungi.

The unfiltered natural water samples were transferred into 1000 mL acid-cleaned polyethylene bottles and stored at 4°C.

2.2.5 *Quality assurance and control*

Slovak Reference Material 'Trace Elements in Water' No. 12-3-10 (Slovak Institute of Metrology, Bratislava, Slovak Republic) with certified concentration of total inorganic As $21 \pm 10\,\mu g\,L^{-1}$ (expanded uncertainty, coverage factor $k = 2$) was analysed to assure quality control.

2.3 *Sample preparation*

2.3.1 *Determination of total As*

About 2 mL of the concentrated HCl was added to 10 mL of the sample, blank or standard solution and mixed thoroughly. In cases of digests after decomposition of fungi mycelia with HNO_3, 100 µL of 40% of urea was added in order to reduce interferences from NO_x. About 1.6 mL of the pre-reducing agent (Section 2.2.2) was added and mixed thoroughly again. The solutions were left for 45 min at laboratory temperature (20 ± 3°C). A total of 1% $NaBH_4$ in 1% NaOH solution was used for hydride generation according to Sections 2.1 and 2.2.3.

2.3.2 *Determination of As(III)*

About 4.45 g of $Na_2HPO_4 \cdot 12H_2O$ and 1.7 g of citric acid were added to 10 mL of a solution intended for the determination of As(III) and mixed thoroughly until the solid chemicals were completely dissolved. As(III) was determined by hydride generation technique under conditions described in Sections 2.1. and 2.2.3.

2.3.3 *Cultivation and experiments with* A. niger *fungi*

The AN1 *A. niger* strain was isolated from Eutric Fluvisol soil (pH H_2O/KCl 7.7/7.4) collected from uncontaminated area near Gabčíkovo (South Slovakia); this strain served as a control sample. The AN3 *A. niger* strain was isolated from bottom sediment with a natural content of As 363 mg kg^{-1} and Sb 93 mg kg^{-1} (pH H_2O/KCl 5.27/4.8) taken from the Blatina stream running from Kolársky hill near Pezinok (West Slovakia) noted for its past mining activity.

Both strains were cultivated on the solid Sabouraud maltose agar medium at a temperature of 25°C for 14 days. Conidia were flushed out with 5 mL of deionised water, poured into 45 mL of liquid Sabouraud medium in a 250 mL Erlenmayer flask and allowed to grow at 25°C for 14 days. The sporulating fungi mycelia were filtered off, rinsed with deionised water and transferred into flasks filled with 50 mL of experimental media containing As(V), As(III), admixture of As(V) and As(III) and MW for treatment for 24 and 48 h. All experiments were carried out in triplicate.

In order to determine the proportion of As bio-accumulated by the fungi, the mycelium was removed from the solution studied, rinsed with deionised water and dried in a drying box at 40°C for 48 h to a constant weight. The As solutions by which the fungi were treated also underwent determination of total inorganic As or As(III) species. The whole biomass of dried mycelium was weighed into a teflon autoclave vessel, 2 mL of deionised water and 4 mL of concentrated HNO_3 were added. The vessels were covered with a teflon lid, placed in a drying box set to 140°C and decomposition of the mycelia proceeded for 4 h. Subsequently, the vessels were cooled to laboratory temperature and the

digests were quantitatively transferred into 50 mL glass volumetric flasks and filled with deionised water to the mark. A blank sample was prepared under the same conditions as the samples, using 50 mL of deionised water as a bio-accumulation medium. The digests were used for total As determination according to Sections 2.1 and 2.3.1.

3. Results and discussion

3.1 *Verification of As total determination*

In order to verify the determination of total inorganic As, single-species solutions of As(III) prepared from $KAsO_2$ and As_2O_3 and As(V) and mixed-species solutions with concentrations $2\,\mu g\,L^{-1}$ As(III) + $10\,\mu g\,L^{-1}$ As(V); $4\,\mu g\,L^{-1}$ As(III) + $10\,\mu g\,L^{-1}$ As(V); $6\,\mu g\,L^{-1}$ As(III) + $10\,\mu g\,L^{-1}$ As(V); $8\,\mu g\,L^{-1}$ As(III) + $10\,\mu g\,L^{-1}$ As(V); $10\,\mu g\,L^{-1}$ As(III) + $10\,\mu g\,L^{-1}$ As(V) were analysed according to the procedures described in Sections 2.1 and 2.3.1. Under these conditions, hydride formation was quantitative from both species; from As(III) regardless of the compound being used for its preparation. Recovery of these determinations varied in a range of 96–101%. The effect of other ions, usually present in natural surface waters, on the determination of total inorganic As was studied using SRW and NRW. Three different calibration curves of As(V) were prepared: in deionised water, SRW and RW. Since no significant differences in their slopes were found, it could be concluded that other ions usually present in natural surface waters did not affect the determination of As. Traceability of total inorganic As determination was demonstrated by the analyses of Slovak Reference Material No. 12-3-10 with certified value of $21.0 \pm 10\,\mu g\,L^{-1}$ As (expanded uncertainty with coverage factor $k = 2$). The verified procedure for the determination of total inorganic As provided the average value of $21.3 \pm 1.8\,\mu g\,L^{-1}$ As (standard deviation) for 12 determinations which was in a very satisfactory agreement with the certified concentration. LOD based on 3σ-criterion achieved $0.22\,\mu g\,L^{-1}$ of total inorganic As. The analytical sensitivity expressed as the slope of calibration curve was 0.0145.

3.2 *Optimisation of As(III) determination*

Many papers have been published on direct methods for As speciation based on different reaction media to achieve a selective hydride generation. Various buffer solutions, such as acetic acid/sodium acetate [4,5,16], citric acid/sodium citrate [4,5,17] citric acid/sodium hydroxide [18] and *tris*-HCl [19] have been utilised in order to prioritise volatilisation of arsenite from a solution also containing arsenate. Hydride generation coupled with atomic spectrometry techniques for the speciation analysis of As in environmental water samples was reviewed by Kumar and Riyazuddin [20]. The same authors have recently developed a simple procedure for the direct determination of As(III) and total inorganic As in water samples using continuous-flow HG-AAS without pre-reduction of As(V) [21].

In our experiments, Na_2HPO_4/citric acid buffer (pH 4.80) was used for sample preparation (2.3.2) and various concentrations of $NaBH_4$ reductant solution for selective hydride generation optimisation.

The analyses of single-species solutions with various concentrations of As(V), As(III) prepared from $KAsO_2$ and As_2O_3, and mixed-species solutions with various As(III) to As(V) concentration ratios and 1% $NaBH_4$ reductant solution revealed that hydride-generation reaction was sufficiently rapid only with As(III) single-species solutions,

irrespective of the compound ($KAsO_2$ and As_2O_3) used for As(III) solutions preparation. Under these conditions, no hydride was generated from As(V) single-species solutions. However, in mixed-species solutions hydride formation from As(V) was negligible only when its concentration was less than $2\,\mu g\,L^{-1}$. With higher concentrations of As(V) an increase in analytical signal was observed due to a partial reduction of As(V) to As(III) caused by 1% $NaBH_4$ solution which contributed to a recovery of As(III) up to 500% in a solution containing $2\,\mu g\,L^{-1}$ As(III) in the presence of 50-times higher content of As(V). In a system with inorganic As(V), hydride-generation proceeds as a two-step reaction which is strongly dependent upon pH as demonstrated by, e.g. Aggett and Aspell [22] in their early work aimed at this topic. In the first step, As(V) is reduced to As(III) which is followed by the evolution of gaseous hydride generated from As(III) in the second step. $NaBH_4$ serves as a reducing agent in the first step and as a hydrogen donor in the second step. In order to suppress the rate of conversion of As(V) to As(III) in mixed-species solutions, the concentration of $NaBH_4$ reductant solution was optimised. In addition to 1% $NaBH_4$ in 1% NaOH, three different concentrations were tested: 0.2% $NaBH_4$ in 0.2% NaOH, 0.1% $NaBH_4$ in 0.1% NaOH and 0.05% $NaBH_4$ in 0.05% NaOH, and solutions containing $2\,\mu g\,L^{-1}$ As (III) in the presence of up to 50 times higher concentration of As(V), the recoveries of As(III) were 500, 161, 115, 114%, respectively, and the sensitivities expressed as the slope of calibration curve were 0.009, 0.0119, 0.0118 and 0.0069, respectively. Selective and quantitative determination of inorganic As(III) in a mixture containing $2\,\mu g\,L^{-1}$ As(III) with $100\,\mu g\,L^{-1}$ As(V) was achieved only with 0.1% $NaBH_4$ in 0.1% NaOH and with 0.05% in 0.05% NaOH reduction solutions, though with a very poor analytical sensitivity with the latter.

Since no reference material was available for As(III), recovery studies were carried out with NRW and MW samples. These samples, in which As occurred almost exclusively as As(V) species, were spiked with 2, 4, 6, 8 and $10\,\mu g\,L^{-1}$ As(III). For both water samples, the recovery of As(III) varied within a range of 97–102%. Examples of As concentration determined in the SRW spiked with $10\,\mu g\,L^{-1}$ As(V), and NRW and MW spiked with $10\,\mu g\,L^{-1}$As(III) are also shown in Table 1. As expected, no detectable concentration of As(III) species was found in the well-aerated surface water from the Hron (NRW); in the MW only a negligible concentration of $1.22\,\mu g\,L^{-1}$ As(III) was determined. The LOD based on 3σ-criterion for As(III) determination was $0.28\,\mu g\,L^{-1}$. The analytical sensitivity expressed as the slope of calibration curve was 0.0118.

Table 1. Arsenic concentrations (mean ± SD); $n = 6$ determined in water samples according to Section 2.3.1.

Sample	As(III) added ($\mu g\,L^{-1}$)	As(III) determined ($\mu g\,L^{-1}$)	Total inorganic As determined ($\mu g\,L^{-1}$)
SRW	–	–	9.7 ± 0.4
NRW	–	–	8.5 ± 0.3
NRW	10	9.9 ± 0.3	18.1 ± 1.3
MW	–	1.2 ± 0.2	45.7 ± 2.4
MW	10	11.3 ± 1.1	56.4 ± 2.5

Notes: SRW-Synthetic river water spiked with $10\,\mu g\,L^{-1}$ As(V). NRV-Natural river water spiked with $10\,\mu g\,L^{-1}$ As(III). MW-Mining water spiked with $10\,\mu g\,L^{-1}$ As(III).

3.3 *Bio-transformation of As species by* A. niger

The relationship between different microorganisms and As is widely demonstrated [23–27] with As compounds being accumulated, oxidised or methylated by various bacteria and fungi. In our experiments, the bio-transformation of As compounds was tested with two *A. niger* strains, AN1 and AN3. *Aspergillus niger* was selected as an experimental fungus due to its easy manipulation, cosmopolitan character and the fact that the AN3 strain was isolated from an As-contaminated area near Pezinok. The AN1 strain, which originated from an uncontaminated area near Gabčíkovo, was not affected by the presence of the toxic element under study and hence served as a reference. Following cultivation in a liquid Sabouraud medium, this fungus was fully developed and its small and dense mycelium created well-differentiated heads (Figure 1). The AN3 strain was isolated from a stream sediment originating from an area with an old ecological and environmental burden (antimony mines near Pezinok). The processes of weathering, solubilisation and oxidation of metallic ferrous rocks enriched with Fe, Sb, Fe–As, As–Sb sulphides present in this area resulted in the formation of soluble forms of heavy metal ions and toxic elements

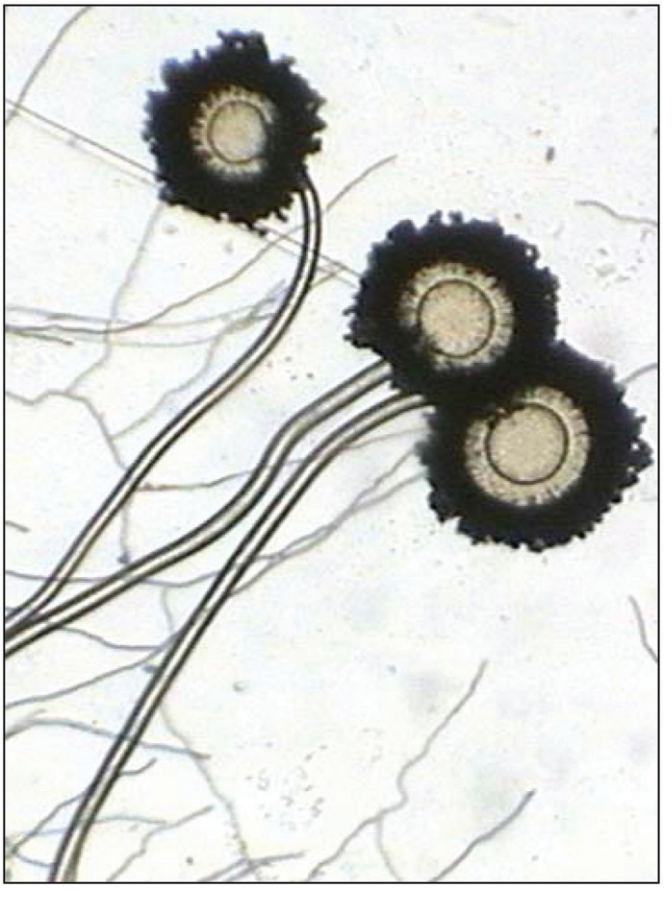

Figure 1. The AN1 strain of *A. niger* isolated from soil from an uncontaminated area near Gabčíkovo (Southwest Slovakia), a reference strain.

(e.g. Pb, Al, Sb, As), thus leading to the contamination of soils, water and stream sediment. A response of the AN3 strain to stress caused by the toxic elements is apparent from Figure 2, which shows morphological changes in the fungus (malformations, i.e. reduction and suppression of the apparatus, development of irregularly-swollen aerial hyphae, etc.) [28]. Morphological changes of microscopic fungi have their background in molecular and genetic changes induced by As and other ions of toxic elements [29]. From a genetic point of view, the AN3 strain which showed features of damage as a response to the toxic environment it was isolated from can be considered as a mutant. This is also supported by changes in its protein spectra which result from the simultaneous inhibition and increased activity of certain genes which are activated by respective transcriptases under stress conditions [30].

Mycelia of both AN1 and AN3 strains of *A. niger* were subjected to treatment with various As species, the concentrations of which in the experimental media were selected based on the requirements of the respective Slovak legislative documents (maximum permitted concentration in drinking water $10\,\mu g\,L^{-1}$ As and surface water $50\,\mu g\,L^{-1}$ As).

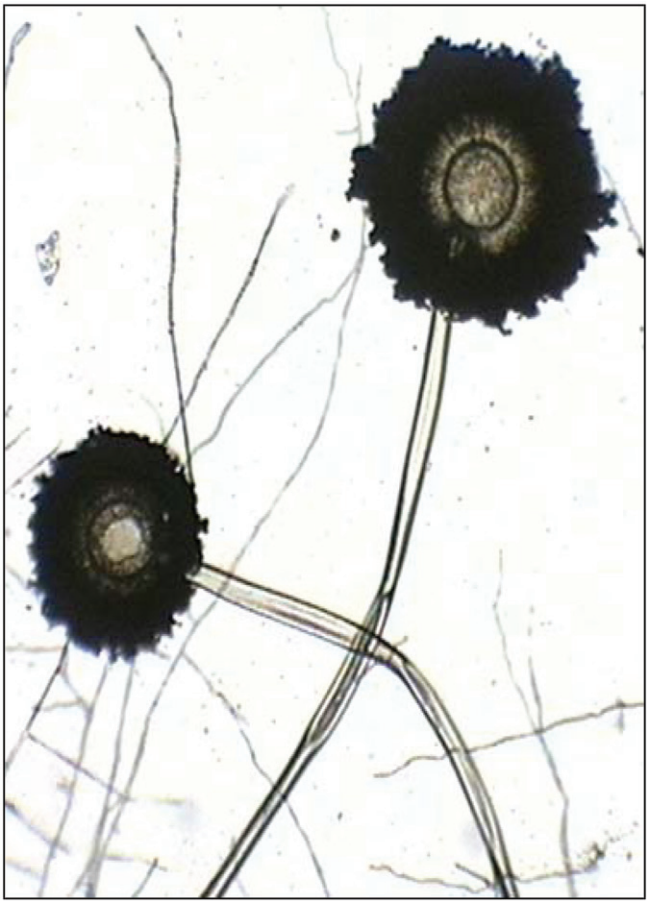

Figure 2. The AN3 strain of *A. niger* isolated from stream sediment with elevated content of As originating from abandoned antimony mines in the vicinity of Pezinok (West Slovakia).

The results of these experiments are given in Tables 2 and 3. The ability to accumulate As was not very high with either of the fungal strains and varied slightly according to the concentration of As in the experimental media (10, 50 and 100 $\mu g\,L^{-1}$, respectively). It seems that the AN3 strain showed approximately two-fold higher ability to retain As in

Table 2. Arsenic concentrations determined according to Section 2.3.1 (pre-reduction with KI and ascorbic acid admixture) in experiments with bio-accumulation by AN1 *A. niger* strain in experimental media containing As(V), As(III) + As(V) and in MW.

Arsenic concentration in $\mu g\,L^{-1}$ (mean ± SD); $n = 6$			
As in medium before accumulation	As remaining in medium after accumulation	As in mycelia	As in mycelia [%]
As(V)			
10	8.5 ± 0.4	0.9 ± 0.1	9
50	48.6 ± 2.6	1.3 ± 0.2	3
100	95.4 ± 4.2	4.3 ± 0.4	4
100[a]	93.4 ± 4.4	5.0 ± 0.5	5
As(III) + As(V)			
10 + 10	18.4 ± 0.9	1.0 ± 0.1	5
As in MW before accumulation	As in MW after accumulation	As in mycelia	As in mycelia [%]
45.7 ± 0.7	44.4 ± 1.1	1.4 ± 0.2	3

Notes: [a]Mycelium removed after 48 h; As remaining in experimental medium after accumulation of As = total inorganic As determined in media after mycelium removal; As accumulated in mycelia = total inorganic As determined in biomass of mycelia (bio-accumulated As).

Table 3. Arsenic concentrations determined according to Section 2.3.1 (pre-reduction with KI and ascorbic acid admixture) in experiments with bio-accumulation by AN3 *A. niger* strain in experimental media containing As(V), As(III) + As(V) and in MW.

Arsenic concentration in $\mu g\,L^{-1}$ (mean ± standard deviation); $n = 6$			
As in medium before accumulation of As	As remaining in medium after accumulation of As	As in mycelia	As in mycelia [%]
As(V)			
10	7.8 ± 3.5	1.7 ± 0.1	17
50	46.7 ± 2.4	2.2 ± 0.1	4
100	90.7 ± 4.1	7.3 ± 0.6	7
100[a]	86.1 ± 3.9	9.3 ± 0.6	9
As(III) + As(V)			
10 + 10	17.6 ± 1.3	1.7 ± 0.2	8
As in MW before accumulation	As remaining in MW after accumulation of As	As in mycelia	As in mycelia [%]
45.7 ± 0.7	40.9 ± 2.0	3.1 ± 0.3	7

Notes: [a]Mycelium removed after 48 h; As remaining in experimental medium after accumulation of As = total inorganic As determined in media after mycelium removal; As accumulated in mycelia = total inorganic As determined in biomass of mycelia (bio-accumulated As).

its cells and this ability was higher when As(III) was present in the experimental medium as compared with the AN1 strain.

In the case of the solutions containing exclusively As(III) species of a concentration of 10, 50 and 100 $\mu g\,L^{-1}$ (Tables 4 and 5), a similar pattern was observed as in the experiments previously described. The ability to bio-accumulate As(III) was again, higher with the AN3 strain and only a minor decrease in the amount of As accumulated in either *A. niger* strain under examination was observed after the 48 h treatment as compared with the 24 h treatment. What was different, however, was the fact that a certain proportion of As, following the treatment of the fungi by As(III), was absent from the experimental As(III) medium. It is expected that this amount of As was bio-transformed either to volatile methyl compounds which escaped during the treatment of the fungi as suggested

Table 4. Arsenic concentrations determined according to Section 2.3.2 (in buffer solution, pH = 4.80) in experiments with bio-accumulation by AN1 *A. niger* strain in experimental media containing As(III).

Arsenic concentration in $\mu g\,L^{-1}$ (mean ± SD); $n = 6$					
As in medium before accumulation	As remaining in medium after accumulation	As accumulated in mycelia	As accumulated in mycelia [%]	Bio-transformed As(III)	Bio-transformed As(III) in [%]
10	1.8 ± 0.2	0.9 ± 0.1	9	7.3	73
50	21 ± 1.1	1.8 ± 0.2	4	27.0	54
100	92.2 ± 3.3	3.8 ± 0.4	4	3.3	3
100[a]	78.8 ± 2.8	5.7 ± 0.7	6	15.5	16

Notes: [a]Mycelium removed after 48 h; As remaining in experimental medium after accumulation of As = As(III) determined in media after mycelium removal; As accumulated in mycelia = As(III) determined in mycelium (bio-accumulated As); Bio-transformed As(III) = As(III) absent from the total As(III) concentration in original experimental medium (before accumulation).

Table 5. Arsenic concentrations determined according to Section 2.3.2 (in buffer solution, pH = 4.80) in experiments with bio-accumulation by AN3 *A. niger* strain in experimental media containing As (III).

Arsenic concentration in $\mu g\,L^{-1}$ (mean ± SD); $n = 6$					
As in medium before accumulation	As remaining in medium after accumulation	As accumulated in mycelia	As accumulated in mycelia [%]	Bio-transformed As(III)	Bio-transformed As(III) in [%]
10	4.5 ± 0.3	1.7 ± 0.2	17	3.8	38
50	43.4 ± 4.5	2.7 ± 0.2	5	3.9	8
100	91.2 ± 5.7	5.8 ± 0.2	6	3.0	3
100[a]	56.8 ± 3.8	7.6 ± 0.3	8	35.6	36

Notes: [a]Mycelium removed after 48 h; As remaining in experimental medium after accumulation of As = As(III) determined in media after mycelium removal; As accumulated in mycelia = As(III) determined in mycelium (bio-accumulated As); Biotransformed As(III) = As(III) missing to the total As(III) concentration in original solution (in medium before accumulation).

by several authors [31,32] or were transformed to As compounds which were not identified within the scope of this study. As different strains of the same species of fungi might show differences in their metabolic behaviour when exposed to toxic conditions, and taking into account some findings [33], at the present time it cannot be unequivocally concluded that the bio-transformation resulted in volatilisation of As(III) species. Further research into the process of bio-transformation of *A. niger* strains is needed to exactly answer this question. In general, the ability to bio-transform As(III) was higher using AN1, reaching maxima of 7.3 and 27 $\mu g\,L^{-1}$ As, respectively as compared with 3.8 and 3.9 $\mu g\,L^{-1}$ As released by AN3 strain from the experimental media containing 10 and 50 $\mu g\ L^{-1}$ As(III), respectively. Bio-transformation proceeded to a greater extent when fungi were treated with experimental media containing lower concentrations of As(III) (Table 4).

4. Conclusions

Both strains of the species of *A. niger* examined are able to accumulate As ions from contaminated water. The AN3 strain of *A. niger* isolated from the vicinity of abandoned antimony mines near Pezinok (Slovak Republic) was better adapted to harmful environmental conditions by demonstrating almost two-fold higher capacity to accumulate As(V) from experimental media than the AN1 strain isolated from uncontaminated area near Gabčíkovo (Slovak Republic). It seems that this capacity was enhanced by the presence of As(III) species. The bio-accumulation capacity of the AN3 strain, treated with MW which contained approximately 46 $\mu g\,L^{-1}$ arsenic as As(V), was also higher than the bio-accumulation capacity of the AN1 strain. The AN3 strain also showed a higher capacity (7 *vs.* 4%) to retain As(V) from the MW as compared with the experimental medium of approximately the same concentration (50 $\mu g\,L^{-1}$) of As(V). On the other hand, the AN1 strain demonstrated two-times greater ability to bio-transform As(III) species in the experimental media than the AN3 strain. This could be attributed to an increased enzymic activity of the AN1 strain as its response to the exposure of this toxic element. Using mutant strains of microscopic fungi isolated from polluted areas could be a strategy for remediating the environment by reducing the amount of toxic As ions. Accumulation and transformation of As ions by natural mutant strain can help to solve problems of As contamination of water in the environment.

The optimised reaction conditions for the determination of inorganic As(III) species by continuous HG-AAS provided reliable results which were checked by analyses of various synthetic solutions and natural surface water spiked with As(III). Interference study showed that any of the main ions usually present in surface water did not affect the reliable determination of As(III) by the method applied in this study.

Acknowledgements

The financial support from the Scientific Grant Agency of the Ministry of Education of the Slovak Republic VEGA 1/0430/08 and VEGA 1/0159/08 is gratefully acknowledged.

References

[1] A. Shraim, B. Chiswell, and H. Olsowy, Talanta **50**, 1109 (1999).
[2] M. Kumaresan and P. Riyazuddin, Curr. Sci. **80**, 837 (2001).

[3] A.G. Howard, J, Anal. At. Spectrom. **12**, 267 (1997).
[4] A. López, R. Torralba, M.A. Palacios, and C. Cámara, Talanta **39**, 1342 (1992).
[5] W. Driehaus and M. Jekel, Fresenius J. Anal. Chem. **343**, 352 (1992).
[6] P. Lens and T. Grotenhuis, *Soil and Sediment Remediation: Mechanisms, Technologies and Applications* (IWA Publishing, London, 2005).
[7] B. Daus, R. Wennrich, and H. Weiss, Water Res. **38**, 2948 (2004).
[8] N. Khalid, S. Ahmad, A. Toheed, and J. Ahmed, Adsorpt. Sci. Technol. **16**, 655 (1998).
[9] M.P. Elizalde, J. Mattusch, and R. Wennrich, J. Environ. Monit. **3**, 22 (2001).
[10] G.M. Gadd, J. Microbiol. **39**, 83 (2001).
[11] E. Beceiro-Gonzales, A.T. Calzada, E. Alonso-Rodriguez, P. Lopez-Mahia, S. Miniategui-Lorenzo, and D. Prada-Rodriguez, Trends Analyt. Chem. **19**, 475 (2000).
[12] S. Senthilkumaar, S. Bharathi, D. Nithyanandhi, and V. Subburam, Bioresour. Technol. **75**, 163 (2000).
[13] I.A. Katsoyiannis and A.I. Zouboulis, Water Res. **38**, 17 (2004).
[14] S. Čerňanský, M. Urík, J. Ševc, and M. Khun, Environ. Sci. Pollut. Res. **14**, 31 (2007).
[15] D. Chakraborti, F. Adams, W. Van Mol, and K.J. Irgolic, Anal. Chim. Acta **196**, 23 (1987).
[16] R. Torralba, M. Bonilla, M.A. Palacios, and C. Cámara, Analysis **22**, 478 (1994).
[17] S.P. Quináia and M.C.E. Rollemberg, J. Braz. Chem. Soc. **12**, 37 (2001).
[18] P. Bermejo-Barrera, J. Moreda-Piñeiro, A. Moreda-Piñeiro, and A. Bermejo-Barrera, Anal. Chim. Acta **374**, 231 (1998).
[19] J.Y. Cabon and N. Cabon, Fresenius J. Anal. Chem. **368**, 484 (2000).
[20] A.R. Kumar and P. Riyazuddin, Int. J. Environ. Anal. Chem. **87**, 469 (2007).
[21] A.R. Kumar and P. Riyazuddin, Int. J. Environ. Anal. Chem. **88**, 255 (2008).
[22] J. Aggett and A.C. Aspell, Analyst **101**, 341 (1976).
[23] M. Bučková, J. Godočíková, A. Šimonovičová, and B. Polek, Curr. Microbiol. **50**, 175 (2005).
[24] P. Visoottiviseth and N. Panviroj, Sci. Asia **21**, 83 (2001).
[25] B.B. Edvantoro, R. Naidu, M. Megharai, G. Merrington, and I. Singleton, Appl. Soil Ecol. **25**, 207 (2004).
[26] D. Cánovac, R. Mukhopadhyay, B.P. Rosen, and V. de Lorenzo, Environ. Microbiol. **5**, 1087 (2003).
[27] M. Urík, S. Čerňanský, J. Ševc, A. Šimonovičová, and P. Littera, Water Air Soil Polut. **186**, 337 (2007).
[28] M. Bučková, J. Godočíková, and B. Polek, J. Basic Microbiol. **47**, 295 (2007).
[29] G. Vizárová, K. Bacigálová, and L. Tamás, Czech Mycol. **54**, 261 (2003).
[30] A. Šimonovičová, P. Ferianc, E. Franková, K. Pavličková, and E. Piecková, *Microbiology for Environmentalists (in Slovak)* (UK Publisher Comenius University, Bratislava, 2008), p. 156.
[31] D.P. Cox, in *Arsenic Pesticides*, edited by E.A. Woolson (American Chemical Society, Washington, DC, 1975), pp. 81–95.
[32] W.R. Cullen and K.J. Reimer, Chem. Rev. **89**, 713 (1989).
[33] R. Bentley and T.G. Chasteen, Microbiology and Molecular Biol. Rev. **66**, 250 (2002).