Recent advances in the bioremediation of arsenic-contaminated groundwaters

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Abstract

The biological treatment of groundwaters is used primarily to remove electron donors from water sources, providing (biologically) stable drinking water, which preclude bacterial regrowth during subsequent water distribution. To the electron donors belong also the dissolved metal cations of ferrous iron and manganese, which are common contaminants found in most (anaerobic) groundwaters. The removal of iron and manganese is usually accomplished by the application of chemical oxidation and filtration. However, biological oxidation has recently gained increased importance and application due to the existence of certain advantages, over the conventional physicochemical treatment. The oxidation of iron and manganese is accelerated by the presence of certain indigenous bacteria, the so-called iron and manganese oxidizing bacteria. In the present paper, selected long-term experimental results will be presented, regarding the bioremediation of natural groundwaters, containing elevated concentrations of iron and arsenic. Arsenic is considered as a primary pollutant in drinking water due to its high toxicity. Therefore, its efficient removal from natural waters intended for drinking water is considered of great importance.

The application of biological processes for the oxidation and removal of dissolved iron was found to be an efficient treatment technique for the simultaneous removal of arsenic, from initial concentrations between 60 and 80 μg/l to residual (effluent) arsenic concentrations lower than the limit of 10 μg/l. The paper was focused on the removal of As(III) as the most common species in anaerobic groundwaters and generally is removed less efficiently than the oxidized form of As(V). To obtain information for the mechanism of As(III) removal, X-ray photoelectron spectroscopy (XPS) analyses were applied and it was found that As(III) was partially oxidized to As(V), which enabled the high arsenic removal efficiency over a treatment period of 10 months.

Keywords: Groundwater treatment; Bioremediation; Iron; Arsenic; XPS

1. Introduction

The quality of groundwater is of great public interest nowadays. Several years ago, it was believed that incumbent soil layers, acting as natural filters, protected groundwaters. However, groundwater pollution can occur in various ways, in addition to natural (geochemical) contamination, such as by infiltration of polluted surface water, by leaks in pipelines, from landfill leachates, etc. Groundwater pollution can be divided into three main categories (i.e., contamination by organic compounds, by microorganisms, and by inorganic pollutants, such as toxic metals). The contamination of groundwater with metals comprises a severe environmental problem due to the fact that metals are not biodegradable and can cause severe adverse effects on human health.

Several strategies exist for the treatment/decontamination of groundwaters, which can be divided in two main categories: ex situ technologies, such as “pump-and-treat” systems, and in situ technologies, such as “permeable reactive barriers” (PRBs). The application of pump-and-treat systems comprises a more or less conventional strategy for groundwater remediation. In these systems, the contaminated groundwater is extracted from the polluted aquifer by pumping, treated above ground by the application of novel or conventional methods and, finally, discharged or reinjected. On the contrary, PRB systems are considered as a relatively
innovate technology, which enables physical, chemical, or biological in situ treatment of contaminated groundwaters by means of reactive materials (Simon et al., 2002).

The use of biological treatment, either in situ or ex situ, has recently gained increased concern due to certain advantages offered in comparison with conventional physico-chemical treatment methods. Biological treatment does not require the use of chemical reagents, but uses microorganisms to reduce, oxidize, or eliminate groundwater contaminants, either as the sole treatment technique, or combined with other conventional physico-chemical processes, such as sorption, filtration, etc. The basic principle on which its respective biological processes rely is that remediation takes place as the result of oxidation-reduction (usually referred as redox) potential changes. Several metals can be removed from groundwater sources as a result of reduction, mediated by microorganisms (bioreduction). The most common examples are the cases of chromium, uranium, and selenium remediation (Lovley, 1995). The reduced forms of these metals are less mobile than the oxidized ones and, therefore, these microorganism-driven reactions can contribute to their removal. On the contrary, the remediation of other metals, such as iron or manganese, is based on their oxidation and the production of respective insoluble oxide forms, followed by filtration or settling (Mouchet, 1992).

A variety of microorganisms may be involved in the biological oxidation of Fe(II), which may be sheaths (such as Sphaerotilus), Leptothrix group (such as Leptothrix ochracea), or spirally twisted stalks (e.g., Gallionella ferruginea), which are the most common iron bacteria. These bacteria can cause the oxidation of dissolved ferrous cations, resulting in the precipitation of respective iron oxides under specific pH, dissolved oxygen (DO), and redox conditions. The biological removal of iron requires (theoretically) $E_0$ values between 100 and 600 mV, DO between 2 and 4 mg/l, and pH values slightly over 7 (Mouchet, 1992).

During previous investigations, it was found that biological oxidation of iron and manganese provided very efficient removal of these contaminants. In addition, when arsenic was simultaneously present in the groundwater, it was found to be removed efficiently from initial concentrations in the range between 50 and 200 μg/l (Katsoyiannis et al., 2002). As(III) was found to be partially oxidized to As(V) and the oxidized form was removed from the water by sorption on biogenic iron oxides (Katsoyiannis and Zouboulis, 2004).

The problem of arsenic contamination of groundwaters has been under extensive discussion especially during the recent years because of its severe adverse effects on human health. The European Commission has recently revised the maximum contaminant level (MCL) of arsenic in drinking water. According to the new directive, by 2003, all drinking water supply systems within the European Union would have to comply with the new concentration limit, which has been reduced from the previous value of 50 μg/l down to 10 μg/l (EC, 1998). Recently, EPA (2002) also decided to implement the same standard for arsenic in drinking water. As a result, several groundwaters containing 20–50 μg/l now require additional treatment to achieve the new concentration limit.

The distribution of arsenic species $[\text{As(III)}, \text{As(V)}]$ in natural waters is mainly dependent on redox potential and pH conditions (Tallman and Shaikh, 1980). Under oxidizing conditions, such as those prevailing in surface waters, the predominant species is pentavalent arsenic, which is mainly present with the respective oxy-anionic forms $(\text{H}_2\text{AsO}_4^-, \text{HAsO}_4^{2-})$ having $pK_f=2.19$ and $pK_s=6.94$, respectively. On the other hand, under mildly reducing conditions, such as those prevailing in most groundwaters, As(III) is the thermodynamically stable form, which at common pH values of most groundwaters or natural waters is present as the nonionic form of arsenic acid $(\text{H}_3\text{AsO}_3, pK=9.22)$ (Cullen and Reimer, 1989). As a result, As(III) may interact in smaller extent with most solid surfaces; therefore, it is more difficult to remove by the application of conventional treatment methods, such as adsorption, precipitation, etc.

Several treatment technologies have been applied for the removal of arsenic from contaminated waters, such as coagulation/filtration, ion exchange, lime softening, adsorption on iron oxides or activated alumina, and reverse osmosis (Jekel, 1994; Zouboulis and Katsoyiannis, 2002). Most of these technologies are not efficient enough for the removal of As(III); hence, they are mainly applied for the removal of As(V). Therefore, a preoxidation step is usually required to transform the trivalent form to the pentavalent one. The oxidation procedure is mainly performed by the addition of chemical reagents, such as potassium permanganate, chlorine, ozone, hydrogen peroxide, or manganese oxide (Jekel, 1994; Kim and Nriangu, 2000). Although these reagents are effective for the oxidation of trivalent arsenic, they may also cause several secondary problems, arising mainly by the presence of residuals or from by-products formation, inducing simultaneously a significant increase to the operational costs of respective methods.

The main objective of this work was to apply the biological arsenic removal technology under different conditions from those already published (Katsoyiannis and Zouboulis, 2004; Katsoyiannis et al., 2004). The specific treatment technique was examined for arsenic removal only for one groundwater source, from the wells of Marienfelde in Berlin and not for long-term viability. Therefore, in the present study, the application of biological processes for the oxidation and removal of dissolved ferrous cations and arsenic oxyanions has been tested during long-term experiments (of several months duration) and for the treatment of different groundwaters from the well of Evangelistria in Thessaloniki. Furthermore, arsenic speciation on solid surfaces was examined using X-ray photoelectron spectroscopy (XPS) studies, in order to confirm the previously published results regarding the hypothesis that As(III) was primarily oxidized to As(V) before sorption on the biogenic iron oxides.
2. Materials and methods

2.1. Materials

The experiments were performed using groundwater from the well of Evangelistria area in Thessaloniki, Greece. This well was located next to the Chemistry Department (Aristotle University of Thessaloniki) and was preferred for convenience (i.e., the treatment unit could be installed in the laboratory, so that it could be continuously supervised). The main characteristics of the examined groundwater are shown in Table 1. It is worth noting that due to its relatively low quality, this groundwater is not used for drinking purposes.

In order to investigate the removal of iron and arsenic, stock solutions containing these elements were spiked in the influent. Stock solutions of Fe(II) were prepared from FeSO₄·2H₂O (1500 mg/l) in acidified deionised water. As(III) stock solutions (100 mg/l) were prepared by dissolving arsenic oxide (As₂O₃; AnalaR) in deionised water using 10 ml/l HCl (37% proanalysis; Merck) and mixing the solution under heating.

2.2. Methods

The treatment method was based on an upflow fixed-bed filtration unit (Fig. 1). The experimental set-up consisted of a PVC column, which was filled with polystyrene beads (mean diameter 3 mm), used as filtration media. The indigenous microorganisms have been immobilized and accumulated onto the filter media; this method does not require the maintenance of pure cultures, whereas the oxidation of iron resulted in the formation of hydrous iron oxide sludge, in which both main “iron-oxidizing bacteria” (i.e., Gallionella and Leptothrix) were entrapped (Katsoyiannis and Zouboulis, 2004).

The groundwater flow was pumped continuously from point (1). At point (2), Fe(II) and As(III) stock solutions were spiked in the groundwater stream through the use of a peristaltic pump (point (3)). The two streams, groundwater and metal/metalloid solutions, were mixed at point (4) in a plastic vessel. From point (5), air was injected, regulating the dissolved oxygen content and enabling the bacteria to grow and oxidize mainly Fe(II) and/or As(III). The aeration was performed in a separate column (point (6)) before filtration in order to avoid the collision of bubbles with the deposited sludge, which may cause disturbance of the system and increased concentrations of metals in the effluent. The column was backwashed every 3 days, by using a limited amount of treated water (3 l), based on previous practical experience. During the backwashing stage, the deposited quantities of iron oxides were removed and thus the clogging of the column was avoided. Samples of the backwashing sludge were used for the spectroscopic characterization of the oxidation products. Samples were collected from five backwashing actions, homogenised, and then dried and used for XPS analyses. The water samples, which were collected to determine the removal of iron and arsenic, were 20 ml and were acidified with dilute HCl acid. The sampling frequency was two times per day for the influent and effluent, and one sample every 3 days for the backwashing action. The experiments were performed between May 2001 and March 2002.

Experiments were also carried out by disinfecting the filters in order to study the removal of arsenic by dead microorganisms and compare the results with those obtained with active microorganisms. The disinfection of the filters was performed by the use of NaClO (5%) according to the method described by Dimitrakos et al. (1992).

2.3. Analytical determinations

The determination of total arsenic, as well as of As(III) species, was performed by hydride generation (Perkin Elmer-MHS 10) atomic absorption spectrometry (Perkin Elmer 2380), according to the Driehaus and Jekel (1992) modified method. The selective determination of As(III) (i.e., arsenic speciation) was performed by the pretreatment of samples with acetic acid and subsequent hydride generation. This method allows the rapid determination of inorganic arsenic species at concentrations down to 1 µg/l. Iron determination and speciation (between Fe(II) and Fe(III)) were performed spectrophotometrically, according to standard methods (APHA-AWWA-WPCF, 1989).

The redox meter (E396 B; Metrohm) using two electrodes (Ag/AgCl and Pt) was employed for the measurement of redox potential. The dissolved oxygen content was measured by the Dissolved Oxygen Meter (Z 521; Consort). The pH, which was measured before and after the treatment, was found to be constant. The redox was continuously measured at the outlet of the treatment column and periodically from samples of the inlet. The dissolved oxygen was measured after the aeration of the inlet water.

The characterization of biological oxidation products was performed by XPS (LHS-10 SPECS). The XPS spectra were recorded using AlKα X-ray source (1486.6 eV). The

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Table 1
Physico-chemical characteristics of the groundwater from the well of Evangelistria area (Thessaloniki, Greece)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values/concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₃</td>
<td>0.6 mg/l</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>152 mg/l</td>
</tr>
<tr>
<td>Nitrates</td>
<td>45 mg/l</td>
</tr>
<tr>
<td>Hardness</td>
<td>380 mg/l (CaCO₃)</td>
</tr>
<tr>
<td>Turbidity</td>
<td>1.3 NTU</td>
</tr>
<tr>
<td>Conductivity</td>
<td>1250 µS/cm</td>
</tr>
<tr>
<td>pH</td>
<td>6.6</td>
</tr>
<tr>
<td>[As]₃⁻</td>
<td>&lt;1 µg/l</td>
</tr>
<tr>
<td>[Fe]²⁺</td>
<td>&lt;1 µg/l</td>
</tr>
<tr>
<td>[Mn]⁺</td>
<td>&lt;1 µg/l</td>
</tr>
</tbody>
</table>
samples were collected from the sludge obtained from five different backwashing actions. These samples were homogenized and dried before XPS analyses.

3. Results and discussion

3.1. Biological oxidation of dissolved Fe(II) during groundwater treatment

The biological removal of iron requires a start-up period of almost 1 month. During this period, the removal of iron increases gradually and reaches over 90% removal, after operation for 1 month (data not shown). Once the experimental conditions are optimised and kept constant, the filters can run for long time periods without presenting operational problems, such as those commonly encountered in sorption processes (i.e., breakthrough) (Fig. 2). The breakthrough concept has been defined elsewhere (Katsoyiannis and Zouboulis, 2002). Even when the operation was discontinued for a short period (around 1 month, for example due to a technical problem), the efficient restart of treatment was achieved within the following 1—2 days.

3.2. Arsenic(III) removal during biological oxidation of Fe(II)—comparison between biotic and abiotic experiments

Biological oxidation can be alternatively applied, in order to avoid the use of chemical oxidants, based on the presence of iron-oxidizing bacteria. Results from previous publications (Katsoyiannis et al., 2002; Katsoyiannis and Zouboulis, 2004) have indicated that the removal of arsenic(III) during Fe(II) oxidation was very efficient, reaching percentage efficiencies over 90%. In order to obtain data about the significance of bacterial presence in the successful operation of the treatment system, the filters were disinfected, resulting in dead biomass, and the results were compared with those obtained with the alive bacteria (Fig. 3). When the bacteria were alive and active, the removal of As(III) accounted for more than 90%, whereas after filter disinfection, causing the

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**Fig. 1.** Schematic representation of the experimental set-up based on an upflow fixed-bed filtration unit.

**Fig. 2.** Biological oxidation of iron and removal from groundwaters by the filtration unit over 10 months. Conditions: pH 6.6, linear velocity 10 m/h, DO 4 mg/l, $E_h$ 320–340 mV.

**Fig. 3.** Effect of redox potential on the removal of As(III) during the biological oxidation of Fe(II). Experimental conditions: [Fe]$_j$, 1–1.5 mg/l, DO 4 mg/l, [As]$_i$, 35–40 µg/l, pH 6.6.
inactivation of microorganisms, the removal of As(III) was substantially reduced, therefore indicating the possible oxidizing activity of these bacteria.

Fig. 3 illustrates that the removal of trivalent arsenic follows closely the redox potential variation, which was provoked by the application of chlorination to the filters. When the redox potential was around 320–340 mV, the removal of As(III) was increased up to 95%, whereas it was reduced substantially during the reduction of redox potential. It was also worth mentioning that for these redox values, the residual arsenic was always below 5 μg/l, considering the initial concentration of 35–40 μg/l. For redox values below 300 mV, the residual arsenic concentration was close to the limit of arsenic in drinking water (10 μg/l), corresponding to 75% arsenic removal. These results indicated that during the biological removal of iron, a possible oxidative action was also involved in the case of As(III) removal because, by increasing again the redox potential, a significant simultaneous increase of arsenic removal was also observed.

3.3. Long-term As(III) removal during biological oxidation of iron

Long-term experiments, regarding the removal of arsenic during the biological oxidation of iron, have been performed in order to evaluate the effectiveness and durability of this method and to conclude on its applicability and efficiency towards the removal of low levels of arsenic concentrations from contaminated groundwaters. The experiments have been performed over a period of 10 months. During this period, the groundwater was continuously spiked with arsenic(III). The respective results are presented in Fig. 4. The removal of trivalent arsenic was efficient during the whole operational period, which was cut off for 1 month due to a technical problem, although the efficient restart of the treatment process and the effective removal of arsenic was achieved within 4 days.

Fig. 4. Long-term As(III) removal during the biological oxidation of iron. Experimental conditions: [Fe], 1–1.5 mg/l, pH 6.6, redox 320–340 mV, linear velocity 10 m/h.

Blank experiments (i.e., without Fe(II) spiking) have been performed during previous publications (Katsoyiannis and Zouboulis, 2002) and it was found that arsenic removal was very low and reached a breakthrough point after the saturation of the deposited iron oxides.

3.4. Surface characterization of the product of biological Fe(II) and As(III) removal using XPS

In order to investigate further the mechanism of arsenic removal during the biological oxidation of iron, XPS studies have been performed, aiming at the detection of arsenic speciation on insoluble products, since the different arsenic species have characteristic binding energies (BEs), which are associated with the As 3d photoelectron (Nesbitt et al., 1998).

The detailed spectra of major peaks C1s, O1s, Fe2p3/2, and As3d photoelectrons, representing the major components of the studied system (i.e., C, O, Fe, and As), are shown in Fig. 5. The spectrum of carbon (Fig. 5a) was analyzed into three subcomponents. The binding energy at 284.6 eV can be attributed to C–H or C–C groups. The peak at binding energy 286.4 eV can be attributed to the alcoholic groups, whereas the peak at 288.5 eV is characteristic of esteric groups (Burg et al., 2002).

Two subpeaks can be assigned to O1s (Fig. 5b)—a low binding energy at 530.6 eV, which is generally accepted as lattice oxygen in the form of O2– (metal oxygen bond). This peak is characteristic of the oxygen in iron and manganese oxides (Harvey and Linton, 1981). The second peak at 532.8 eV can be assigned to the oxygen adsorbed on the surface in the form of OH–.

The peak of iron (Fe2p3/2) was observed at 711.6 eV (Fig. 5c). According to previously published work, this is the characteristic energy of iron oxy-hydroxides, probably goethite or ferrihydrite (Harvey and Linton, 1981). Goethite is expected to show the peak of Fe2p3/2 at 711.9 eV, whereas ferrihydrite shows the peak at 711.6 eV. Therefore, the most probable form of iron precipitates after the application of biological treatment was amorphous ferrihydrite, which is in agreement with previous studies of biological iron removal (Sogaard et al., 2001).

In Fig. 5d, the detailed spectra of As3d are presented. It can be noticed that two characteristic peaks of As3d were observed: first, at 44.5 eV, which is a characteristic binding energy of trivalent arsenic, and, second, at 46.5 eV, which is the characteristic binding energy of pentavalent arsenic (Nesbitt et al., 1998; Dambies et al., 2002). The two peaks are of around the same intensity, meaning that As(III) and As(V) participate with almost the same quantity on the resulting solids, as the intensity is analogous to the quantity of the species on the solids’ surface. It is worth noting also that these experiments of arsenic removal were performed by spiking only the trivalent arsenic (As(III)) in the influent. Therefore, if the oxidation of As(III) has not taken place during the biological treatment, then only the peak at 44.5 eV would be expected. Therefore, the results obtained by
XPS show a strong indication that the removal of trivalent arsenic may occur through a two-step mechanism. Firstly, As(III) is sorbed onto the biogenic iron oxides. However, as the removal of trivalent arsenic on iron oxides is not efficient enough, the nonreacted As(III) content is biologically oxidized by the same bacteria and the As(V), which is produced, can be subsequently sorbed onto iron oxides, promoting the efficient overall removal of arsenic.

Literature findings show that the oxidation of As(III) by dissolved oxygen is a very slow procedure, which may take days or even months (Kim and Nriangu, 2000). Therefore, it is not possible to occur in such a short period of experimental time (as the empty bed contact time was in the range of 7 min only); as a result, the reaction of As(III) oxidation should be catalyzed, in order to proceed within a time scale of a few minutes. In the present treatment system, the bacteria can catalyze this reaction, supporting also the previously presented results of Fig. 3.

It has been proposed that during the biological oxidation of iron, the iron-oxidizing bacteria may release a specific enzyme (catalase), which acts as a peroxidase, catalyzing the formation of hydrogen peroxide (Mouchet, 1992). Hydrogen peroxide is a strong oxidant and could contribute to the oxidation of As(III); however, Pettine et al. (1999) have shown that at the pH value of 7.5, the kinetics of As(III) oxidation by H\textsubscript{2}O\textsubscript{2} is in the order of hours. Therefore, the reaction of As(III) oxidation should be catalyzed in order to proceed within the time scale of a few minutes. Voegelin and Hug (2003) have also recently studied the oxidation of As(III) by H\textsubscript{2}O\textsubscript{2} in the presence of
ferrihydrite; they have found that this reaction can be catalyzed on the surface of ferrihydrite, whereas no oxidation was observed within minutes and up to several hours prior to H₂O₂ addition.

4. Conclusions

The biological oxidation of iron can serve as an efficient treatment method for the simultaneous removal of arsenic from contaminated groundwaters. Under specified experimental conditions, the trivalent form of arsenic was found to be oxidized and the respective pentavalent form was efficiently sorbed onto the deposited (biogenic) iron oxides. The results regarding the formation of insoluble products were confirmed using XPS studies. The treatment method was evaluated for long-term operation (10 months). It was found that during the whole period of operation, the removal of trivalent arsenic was efficient and that the residual arsenic concentration was below the MCL of 10 μg/l. The method offers several advantages towards the application of conventional oxidation (physico-chemical) treatment. The use of chemical reagents for the oxidation of As(III) to As(V) can be avoided, which renders the methods more economical and safer for the environment. It can also provide the simultaneous removal of major groundwater contaminants, such as iron and arsenic; whereas when manganese would be also present, it can be coremoved after the completion of biological oxidation of iron, applying a secondary filtration stage.

References


