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Bacterial immobilization and oxidation of arsenic in acid mine drainage (Carnoulès creek, France)

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Abstract

The acid waters (pH = 2.73–3.37) originating from the Carnoulès mine tailings contain high dissolved concentrations of arsenic (1–3.5 mmol l^{-1}) and iron (20–40 mmol l^{-1}). At the outlet, arsenite predominates. During the first 30 m of downflow, 20–60% is removed by coprecipitation with Fe(III). This process results from bacterially mediated As- and Fe-oxidation. The precipitation rates in the creek depend on the oxygen concentration in spring water and are lower during the dry summer period when the anoxic character of the spring water inhibits the activity of oxidizing bacteria. Ex situ experiments show that the presence of bacteria-rich precipitates increases the As- and Fe-removal rates. Three strains of bacteria promoting the oxidation of As have been isolated, and two of them have the characteristics of *Thiomonas ynys1*. The third strain, which is not identified yet, also catalyzes the oxidation of Fe.

Keywords: Arsenic; Oxidation; Bacteria; Acid mine drainage

1. Introduction

Arsenic is omnipresent in the Earth's crust, with a mean concentration of 3 mg kg^{-1} [1]. It is released to the biosphere through a combination of natural processes and human activities, such as the processing of sulfide-rich ores to recover base metals (copper, lead, zinc, gold, etc.) which produces large quantities of As-rich wastes. When exposed to meteoric water, this material generates acid mine drainage characterized by elevated sulfate, arsenic and iron content. Because of the high As toxicity, numerous publications have been dedicated to its chemical behavior in natural systems [2–4] and in acid mine drainage [5–7]. Arsenic mobility is limited by its ability to bind to many solid phases such as Fe oxides and to a lesser extent Al hydrous oxides [8,9]. In addition, Mn oxides are known for their capacity to oxidize As [10]. The affinity of As for these mineral surfaces strongly depends on its oxidation state. At acid pH, arsenate ($H_2AsO_4^-$) adsorbs more strongly than arsenite (H_3AsO_3) on Fe(III)(hydr)oxides [8,11]. Thus, changes in oxidation state strongly influence the mobility of As. The abiotic oxidation of As(III) is relatively slow [12] but it has been demonstrated, as already done for Fe, that bacteria catalyze As oxidation [13,14]. Such bacteria have been isolated in acid hot springs [15,16] and mining effluents [17].

The objective of this paper was to present the chemical and microbial processes that influence As mobilization in the creek of Carnoulès (France), which

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drains pyrite-rich mine tailings. The distribution of redox As species in water has been determined, and the role of bacteria on As distribution and precipitation kinetics was investigated after isolation of some strains living in the local environment.

2. Materials and methods

2.1. Site description

Carnoulès is located in Southern France (Fig. 1) in the drainage basin of the Rhône river. The mining activity, stopped in 1962, has left about 1.5 Mt of sulfidic wastes containing 0.7% Pb, 10% Fe and 0.2% As [18]. These wastes are stored behind a dam, over a segment of the Reigous creek valley. The seepage water drained from the mine percolates through the tailings and is acid. It is collected by a draining pipe which has been buried under the tailings. It emerges at the basis of the dam, forming the spring of the Reigous creek and has a variable flow rate depending on meteoric conditions (see results). The mean annual rainfall is 1100 mm, with a seasonal distribution typical of the Mediterranean climate, including long drought periods and intense precipitation events, especially in spring and autumn. The Reigous creek water contains high (up to 3.3 mmol l^{-1}) and variable As concentrations [18,19]. As-rich (up to 20%) yellow sediments cover the bottom of the creek [18] and agglomerate along a small wall situated at 30 m from the outlet. These sediments have the stoichiometry of ferric arsenate (Fe/As = 1.5–2) [20]. The creek collects downstream seepage waters from the surroundings before joining, at 1.5 km, the relatively pristine Amous river (Fig. 1).

2.2. Sampling and analyses

Water samples were collected during eight surveys carried out in 2001. The sampling stations (Fig. 1) are located over a relatively short distance (40 m), where no addition of seepage water is detected.

The main physicochemical parameters (pH, O_2) were measured in the field. pH was measured with an Ultrameter™ Model 6P (Myron L Company, Camlab, Cambridge) equipped with a pH sensor. Dissolved O_2 was measured with CHEMets® tests (CHEMetrics, Calverton, USA) based on colorimetric detection after reaction of O_2 with indigo carmine for the range of 0–375 μM and with rhodazine D for 0–30 μM. The detection limit is 3 μM.

Water samples were filtered immediately through 0.45 μm Millipore membranes fitted on Sartorius polycarbonate filterholders. Samples for total Fe and As determination were acidified to pH = 1 with HNO_3 (14.5 M), and stored at 4°C in polyethylene bottles until

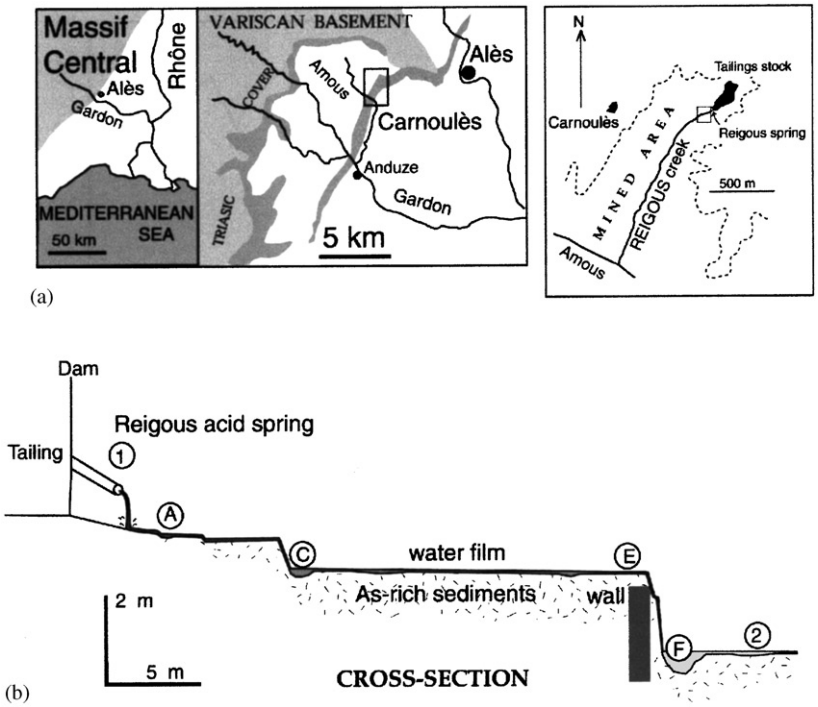

Fig. 1. (a) Location and geology of the studied area and (b) cross-section showing the location of the sampling stations (1, A, C, E, F, 2). The transit time of water between stations 1 and 2 is about 1 h.

analysis. The samples for Fe and As speciation and sulfate determination were stored in the dark and analyzed within 24 h.

The analysis of total dissolved As was performed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The elevated As concentrations necessitated a dilution factor of 1000, and no interference due to ArCl was detected. Calibration of the ICP-MS was done by calibrating peak intensity, acquired in peak jump mode, with standard solutions. ^{115}In was used as internal standard to correct for changes in peak intensities due to instrumental drift.

For the determination of dissolved As(III), the pH was adjusted to 4.8 using an acetic acid–sodium acetate buffer. Arsenite was determined using a hydride generation system coupled to the ICP-MS after reaction of As(III) with sodium borohydride. This method is similar to that described by Seyler and Martin [21], but has been modified according to Branch et al. [22]. The detection limit is 1 nM and the precision better than 5%. As(V) was calculated as the difference between total dissolved As and As(III).

For Fe(II) determinations, filtered samples were buffered to pH 4.5 with an ammonium acetate/acetic acid buffer in the field, and Fe(II) was complexed by adding 1 ml of a 0.5% (w/w) 1,10-phenanthrolinium chloride solution to 10 ml of sample [23]. Analyses were made by colorimetry at 510 nm. The detection limit is 0.2 mM and the precision better than 5%. Total Fe (dissolved and colloidal) was determined by Flame Atomic Absorption Spectrometry.

2.3. Kinetic studies

Two kinds of ex situ experiments have been carried out. For each experiment, 100 ml of freshly sampled raw spring water from the Reigous creek (containing free bacteria cells) were incubated for 150 h at room temperature. The agitation was maintained during the experiment in order to favor oxygenation. Control samples with spring water sterilized by filtration through 0.45 μm Millipore filters and addition of formaldehyde (0.5% v/v), were run in the same conditions. Subsamples (1 ml) were taken at regular time intervals (approximately 12 h) and filtered using disposable in-line 0.45 μm pore size filters and analyzed for As(III), total As, Fe(II) and total Fe as described above. The raw spring water and the control were incubated both in the dark and under a UV-lamp.

The second experiment was carried out in the presence of a bacteria-rich precipitate which forms spontaneously on the walls of a container filled with Reigous spring water. The precipitate was formed from the spring water incubated in flasks during 5 days. After this time, the water used for the formation of the precipitate was discarded and replaced by freshly sampled spring water. It was then incubated in the dark and under a UV lamp in order to ensure that As oxidation is not related to the photochemical oxidation of As(III) by Fe described by Emett and Khoe [24].

2.4. Bioassay to test arsenite and iron oxidation

The bacteria present in the Reigous spring water were cultured in two different media. The first, an R2A medium (Difco laboratory) was used for organo-heterotrophic bacteria [25]. The second, a 100:10 medium devoid of organic matter for the culture of lithotrophic bacteria. This medium, which is acid (pH 3.5), contains thiosulfate and iron and is recommended for the culture of acidophilic bacteria such as *Acidithiobacillus ferrooxidans* [26].

Three morphologically different colonies corresponding to bacterial strains B1, B2, B3 were isolated by three successive streaking on the R2A medium. Strains B4, B5 and B6 were isolated in the same way from the 100:10 thiosulfate medium.

For the oxidation assay, filter-sterilized spring water (100 ml) was inoculated with 0.1 ml of a pure strain of bacterial suspension (10^6 cells ml^{-1}). After an incubation period of 8 days at 30°C under continuous shaking, the samples were filtered on 0.45 μm pore size filters. The speciation of As and Fe and their total concentrations in the aqueous phase were determined.

3. Results

3.1. Aqueous chemistry

The discharge of the Reigous spring varies between 0.2 and 1.3 l/s, reflecting the wet and dry periods (Fig. 2a). The temperature varies over a 1-year period from 13.2°C to 16.5°C and the pH, from 2.73 to 3.37 (Fig. 2b). The O_2 content is $\geqslant 6\,\mu M$ during the wet season and $\leqslant 3\,\mu M$ during the dry season (Fig. 2b), depending on the variable rainwater input. Dissolved As and Fe concentrations (Fig. 2c) also vary in a large range with higher values during the dry season. The main species are As(III) and Fe(II), corroborating the previous findings of Michard and Faucherre [19]. Whereas Fe(III) is totally absent, variable amounts of As(V), between 0% and 40% of total As, have been recorded during three surveys (Table 1).

Whatever the initial O_2 content of the spring water, O_2 concentrations increase systematically abruptly between locations 1 and A (Fig. 3) and reach values higher than 125 μmol l^{-1} over a distance of 10 m.

Fe(II) and As(III) concentrations decrease downstream (Fig. 3) and are strongly correlated ($0.87 \leqslant r^2 \leqslant 0.99$). The rate of Fe(II) and As(III) removal from the spring down to the wall (location E) located at

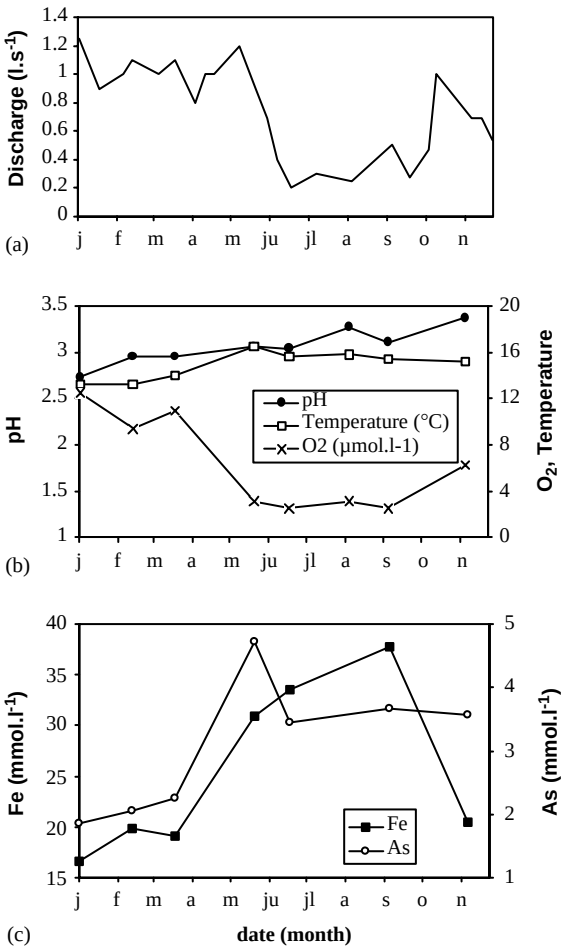

Fig. 2. Variations of discharge (a), pH, O_2 and temperature (b), dissolved As and Fe (c) of the Reigous spring (station 1) over the year 2001.

30 m varies seasonally (Fig. 3). During the wet season, Fe and As concentrations decrease immediately at the water outlet. During the dry season, the removal of the two elements is minor until the wall. Downstream from the wall, the Fe and As concentrations drop systematically.

3.2. Role of bacteria in As and Fe removal and oxidation

In the absence of bacteria, the amount of As and Fe removed from the solution is small (22% and 3%) after 8 days (sample SA, Fig. 4).

More interestingly, the results indicate that three bacterial strains B2, B3, B6, play a significant role in As- or Fe-oxidation. Based on 16S rDNA sequence, B2 and B3 have the characteristics of the *Thiomonas ynys1* ([27], pers. comm.). These strains favor largely As oxidation and within the duration of the experiment, all the As in the dissolved phase has been oxidized (Fig. 4). B1, B2, B3, B4 and B5 have little or no influence on Fe, only B6 favors Fe-oxidation and thus its precipitation and decreasing Fe(II) values in solution. However, B1, B4, B5 and B6 are not identified yet. The occurrence of As(V) in the aqueous phase suggests that B6 also catalyzes the oxidation of As whose major part coprecipitates with Fe(III).

3.3. Kinetic studies

When bacteria are present as free living cells, 75% of As and 13% of Fe are removed within 150 h while no removal occurred in the absence of bacteria. The removal rates increase 2-fold for As and 10-fold for Fe in the presence of bacteria-rich precipitates (Fig. 5). Unlike Emett and Khoe [24] and Hug et al. [28], the UV experiments do not reveal an influence of photochemistry on As oxidation rate and its subsequent precipitation with Fe. In fact, the photochemical influence is negligible compared to that of bacteria.

4. Discussion

The removal of Fe in the upper part of the Reigous creek varies seasonally. The highest removal rate ($0.26\,mmol\,l^{-1}\,min^{-1}$) is reached during winter. It results from the oxidation of Fe(II) by iron oxidizing bacteria such as the strain B6. Such a process had been known for a long time in acid Fe^{2+} and SO_4^{2-}-rich waters and was related to the activity of *Acidithiobacillus ferrooxidans* which are common in acid mine drainage [29]. In the first 12 m of the creek, the Fe removal is correlated ($r = 0.85$) with the O_2 content in spring water (Fig. 6) which depends on the meteoric conditions. During the wet season, the oxygen content of spring water at the outlet of the draining pipe is high enough to promote immediately the activity of iron oxidizing bacteria and the rate of Fe removal in the creek increases. During the dry season, the spring water at the drain outlet is nearly anoxic. The activity of aerobic Fe oxidizing bacteria seems to be inhibited in the first 12 m. The rate of oxidation becomes slower and the Fe removal is negligible (Fig. 3).

The rate of Fe removal in the kinetic experiments is increased by the presence of a bacteria-rich precipitate, indicating that fixed bacteria are more efficient than free bacteria. However, the ex situ rates are always slower ($0.004\,mmol\,l^{-1}\,min^{-1}$) than the rates observed in the field, where Fe removal occurs in 30 m or after about 1 h of water flushing time. This suggests that the surface of the bacteria-rich precipitates relative to the surface of water, higher in the field, increases the rate of Fe-oxidation reaction.

The removal of As is associated with that of Fe, as indicated by the correlation between the two elements in

Table 1
Chemical analysis of the aqueous phase

Station	Date	*d*	*T*	pH	O_2	SO_4^{2-}	As III	As V	As T	Fe II[a]
	10/01/01									
1		0	13.2	2.73	13	nd	1.10	0.76	1.86	16.6
A		2	13.2	2.86	94	nd	0.89	0.20	1.09	14.1
C		12	12.7	2.97	125	nd	0.71	0.03	0.75	11.6
E		30	12.5	3.04	156	nd	0.66	0.00	0.66	10.9
F		32	12.3	3.03	156	nd	0.64	0.17	0.81	10.1
2		38	12.3	3	156	nd	0.69	0.27	0.96	10.0
	21/02/01									
1		0	13.2	2.95	9	39	2.06	nd	2.06	19.9
A		2	12.8	3.1	63	nd	1.78	nd	1.78	18.1
C		12	12	3.05	125	nd	1.72	nd	1.72	15.8
E		30	11.4	3.1	172	nd	1.57	nd	1.57	14.9
F		32	11	3.1	188	nd	1.47	nd	1.47	13.7
2		38	11.1	3.11	188	30	1.53	nd	1.53	15.2
	27/03/01									
1		0	14	2.95	11	31	2.14	0.12	2.26	19.1
A		2	13.7	3.15	47	26	1.78	0.29	2.08	15.6
C		12	13.9	3.18	125	27	nd	nd	nd	14.0
E		30	13.8	3.24	141	26	1.22	0.49	1.70	12.8
F		32	13.7	3.22	141	22	0.99	0.34	1.32	11.7
2		38	14	3.3	156	25	1.09	0.57	1.66	12.5
	29/05/01									
1		0	16.5	3.06	DL	36	3.49	1.23	4.72	31.0
A		2	17	3.03	63	37	3.48	0.69	4.16	30.6
C		12	16.7	3.03	125	34	3.47	0.58	4.05	30.5
E		30	20	3.07	172	31	3.25	0.58	3.83	30.2
F		32	20.2	3.05	109	39	2.92	0.49	3.41	27.5
2		38	21.5	3.04	156	34	2.97	1.41	4.39	27.2
	26/06/01									
1		0	15.6	3.04	DL	30	3.45	nd	3.45	33.5
A		2	16.3	3	109	27	3.55	nd	3.55	33.2
C		12	17	2.99	156	29	3.45	nd	3.45	31.0
E		30	20.6	3.04	172	28	3.20	nd	3.20	31.1
F		32	21.2	3.05	188	30	2.70	nd	2.70	27.1
2		38	22.3	3.02	172	29	2.85	nd	2.85	26.7
	13/08/01									
1		0	15.8	3.27	DL	nd	3.67	nd	3.67	nd
A		2	17.2	3.18	125	nd	3.74	nd	3.74	nd
C		12	17.4	3.17	156	nd	3.57	nd	3.57	nd
E		30	19.7	3.18	172	nd	3.22	nd	3.22	nd
F		32	19.5	3.13	156	nd	2.72	nd	2.72	nd
2		38	20.8	3.14	188	nd	2.70	nd	2.70	nd
	13/09/01									
1		0	15.4	3.11	DL	57	3.39	0.19	3.57	37.8
A		2	15.3	3.12	94	56	3.39	nd	3.27	35.2
C		12	15.3	3.04	147	56	3.31	nd	3.20	34.9
E		30	14.6	3.06	141	54	3.03	nd	3.00	34.2
F		32	14.9	3.06	109	51	2.83	nd	2.77	32.3
2		38	15.3	3.05	125	51	2.61	nd	2.59	30.5

Table 1 (*continued*)

Station	Date	*d*	*T*	pH	O_2	SO_4^{2-}	As III	As V	As T	Fe II[a]
	14/11/01									
1		0	15.2	3.37	6	24	2.15	0.20	2.34	20.5
A		2	15	3.38	94	15	2.04	0.32	2.37	21.3
C		12	14.6	3.33	156	30	1.97	0.36	2.32	20.7
E		30	13.6	3.3	188	27	1.77	0.34	2.12	19.4
F		32	13.3	3.23	172	25	1.59	0.28	1.87	18.1
2		38	13	3.25	188	20	1.60	0.28	1.88	18.2

Note: All concentrations are in mmol l^{-1}, except O_2 in μmol l^{-1}. *d*, distance from the spring (meters); *T*, water temperature (degree Celsius); nd, not determined; DL, detection limit.

[a]Total Fe concentrations were identical to Fe II.

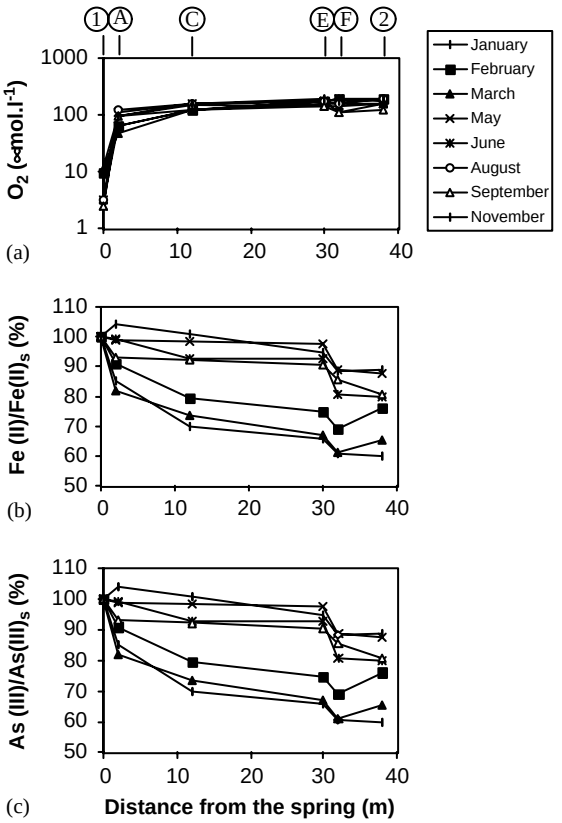

Fig. 3. Variation of O_2 (a), Fe(II) (b) and dissolved As(III) (c) along the Reigous creek. Fe and As concentrations are expressed as a percentage of their concentration in spring water (suffix s). Total dissolved As varies as As(III). 1, A, C, E, F, 2: Sample locations (cf. Fig. 1b).

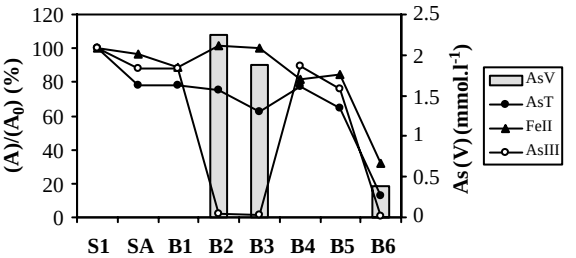

Fig. 4. Bioassay experiments: Oxidation of As and removal of Fe and As. The removal is expressed as the ratio A/A_0 where A is the concentration of Fe(II) or total dissolved As in the aqueous phase and A_0 is the concentration in the Carnoulès spring water before incubation (S1) with the following values in nM: 3.09 for total dissolved As and As(III), 31 for Fe(II). SA is sterile spring water without any inoculation incubated 8 days. B1, B2, B3, B4, B5 and B6 are pure bacterial strains isolated from the Reigous spring water.

each survey (Fig. 6), as well as by the ex situ experiments. The amount of As removed from the Reigous creek reaches up to 0.06 mmol l^{-1} min^{-1} in the first 12 m and the As/Fe ratio calculated from the amount of As and Fe removed from the aqueous phase ranges between 0.13 and 1.3. Such ratio reflects the formation of mixed As(V)–Fe(III) precipitates which occurs in some As-rich AMD [7] rather than adsorption of As on Fe-oxyhydroxide. The scavenging of As by Fe is efficient because of the acid character of the water. Indeed, when the waters are alkaline [16], Fe precipitates without any effect on As distribution. But in some acid waters, where the removal of Fe is incipient, As is not scavenged and there is a build-up of As(V) in the aqueous phase [15]. When present in Reigous water, As(V) has been generated inside the tailings as indicated by its content at the outlet. Otherwise it is not evidenced from the analysis of creek water, but the bioassays clearly indicate the formation of As(V) in the presence of certain type of bacteria. The results suggest that the As(V) formed in the creek water is immediately scavenged by Fe(III). The decoupling of the decrease of As(III) and Fe(II) in the bioassays seems to indicate the existence of two separate As-oxidizing and Fe-oxidizing strains. The presence or absence of these strains may influence the mobility of arsenic. As-oxidizing strains such as *Thiomonas sp.* isolated from the Reigous Creek may be significantly involved in the natural attenuation of As; however, the efficiency of the

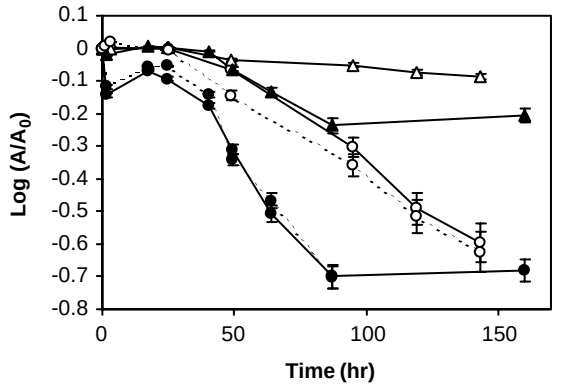

Fig. 5. Kinetics of Fe (triangles) and As removal (circles). Open symbol with free bacteria cells. Close symbols with a bacterial precipitate present (fixed bacteria). Continuous line for UV-light, dotted line for dark experiments. A and A_0 same as in Fig. 4.

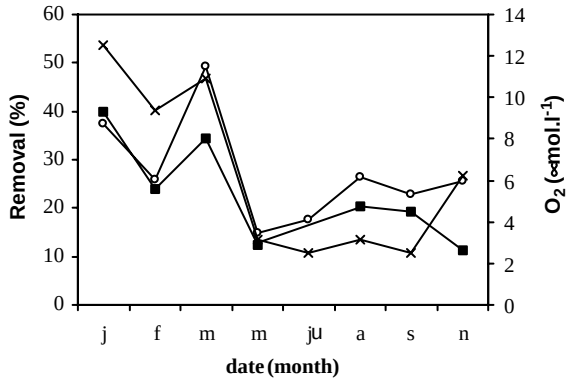

Fig. 6. Removal of Fe(II) (squares) and As(III) (circles) in the Reigous creek and O_2 content (crosses) in spring water during a year (2001).

removal process seems to be related to the activity of Fe-oxidizing bacteria such as B6.

Further experiments are needed to know if As oxidation is the result of a detoxification pathway, or if it is used, instead of iron oxidation, for the fixation of inorganic carbon (chemolithoautotrophic growth).

5. Conclusion

Arsenic is precipitated very rapidly with Fe(III) during the flow of the Reigous acid creek. The rate of precipitation is seasonally variable, in relation to the oxygen content at the spring of the Reigous creek. The precipitation rate is related to iron oxidation and is therefore increased with iron-oxidizing bacteria such as B6, isolated from the creek. The presence of fixed bacteria in the precipitates which cover the bottom of the Reigous creek increases As- and Fe-removal rates compared to free bacterial cells. Rapid arsenic oxidation also occurs in the creek water due to the activity of As-oxidizing bacteria. Two strains isolated from the creek water and identified as *Thomonas sp*. have the ability to oxidize As(III).

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