Particle size control of biogenic scorodite during the GAC-catalysed As(III) oxidation for efficient arsenic removal in acid wastewaters

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ARTICLE INFO

Keywords: Arsenite oxidation Activated carbon Biological iron oxidation Saturation control Biological crystallization Scorodite

ABSTRACT

The synthesis of biogenic scorodite combined with the oxidation of As(III) catalysed by granular activated carbon (GAC) was previously demonstrated. However, the colloidal size of the formed scorodite particles is still a bottleneck, as it would hinder the easy separation of the precipitates in a full-scale application. Here, we studied the effect of GAC concentration on biological scorodite precipitation at thermoacidophilic conditions in batch experiments. Higher arsenic removal efficiency and precipitation of larger and more stable scorodite particles were found only in biotic tests and at low catalyst concentration of 4 g L\(^{-1}\). Furthermore, with 4 g L\(^{-1}\) GAC, the Fe and As predominantly precipitated in solution while with 20 g L\(^{-1}\) GAC the Fe and As predominantly precipitated on the GAC. For experiments with 4 and 20 g L\(^{-1}\) of GAC, the average particle size was 66 and 2.6 \(\mu\)m, respectively. This could be explained by the lower saturation level of the solution at the lower GAC level. This study shows for the first time that the oxidative catalytic capacity of GAC can be used to influence crystallization of scorodite.

1. Introduction

Arsenic (As) is a toxic metalloid which is widely dispersed throughout the earth’s crust where it is commonly associated with ores of Cu, Zn, Au and Ag [1]. The mining and metallurgical industries exploiting these ores contribute substantially to the economic development of metal-exporting countries [2]. However, it results in the generation of acid effluents with high concentrations of As between 500–10,000 ppm, mainly in the trivalent form, As(III) [3]. The removal and immobilization of arsenic is commonly accomplished by co-precipitation with lime and ferric salts [4]. However, the precipitated arsenic-rich solids are chemically not entirely stable. Therefore, the suitability of such precipitates for long-term storage has been questioned, as uncontrolled emissions of arsenic from stored arsenic-rich solid waste results in unacceptable environmental hazards [5,6]. Due to the increasing worldwide metal demand and the current trend of exploiting low-grade ores, more arsenic-containing waste is generated. Hence, a proper management of these residues for the disposal and storage becomes even more urgent.

The mineral scorodite, crystalline ferric arsenate dihydrate with formula FeAsO\(_4\)\(\cdot\)2H\(_2\)O, has been proposed as a suitable carrier medium for stable immobilization and long-term storage of arsenic, as it combines low solubility with high arsenic content [7,8]. In
previous studies of our group, the biological crystallization of scorodite starting from ferrous iron (Fe(II)) and arsenate (As(V)) containing solutions inoculated with thermoaacidophilic iron-oxidizing microbial cultures was demonstrated [9].

Considering that arsenite (As(III)) is the predominant arsenic species in acidic metallurgical wastewater, the efficient oxidation of arsenite to arsenate is required to achieve removal of arsenic as scorodite. Biological As(III) oxidation under thermoaacidophilic conditions has been scarcely reported [10,11], only Okibe and co-workers have described biological oxidation of As(III) by the archaea Acidianus brierleyi [12–14]. As an alternative, we previously found that granular activated carbon (GAC) was effective as catalyst for As(III) oxidation under thermoaacidophilic conditions in the presence of air. Interestingly, when Fe(II) and the microbial mixed culture were also present, scorodite was ultimately formed [15]. The catalytic As(III) oxidation by granular activated carbon (GAC) in the presence of air or oxygen in acidic solutions was reported previously [16], as well as it application as catalyst for enhanced leaching of sulfide minerals [17,19]. It has been proposed that hydrogen peroxide is formed at the surface of the activated carbon [20], according to:

\[ C_{\text{red}}^\bullet + \frac{1}{2} O_2 + H_2O \rightarrow H_2O_2 + C_{\text{ox}}^\bullet \]  

(1)

Where \( C_{\text{red}}^\bullet \) and \( C_{\text{ox}}^\bullet \) stands for reduced and oxidized functional groups on the carbon surface, respectively. Subsequently, hydrogen peroxide can oxidize As(III) and Fe(II) according to:

\[ H_3AsO_3 + H_2O_2 \rightarrow H_2AsO_4 + H_2O \]  

(2)

\[ Fe^{2+} + H_2O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \]  

(3)

In our preliminary experiments, both As(III) and a fraction of Fe(II) were oxidized by GAC in the absence of an Fe(II)-oxidizing thermoaacidophilic mixed culture [15]. However, the formed As(V) and Fe(III) remained in solution, while under the same conditions but in the presence of a thermoaacidophilic mixed culture, As(V) was depleted from solution and precipitated as scorodite. The scorodite precipitate consisted of colloidal crystallite agglomerates with a size of \(<5 \mu m\), with settling rates below 0.01 m/h. The small particle size was attributed to the relatively high degree of saturation of the solution with respect to scorodite [15]. The poor settling behaviour makes separation of the particles from the process stream in practice difficult and costly. Furthermore, particle size may negatively affect the stability of scorodite [8,21,22].

Therefore, it is desirable to obtain precipitates with a larger particle size in the scorodite biocrystallisation process. To this purpose, we investigated the possible effect of the GAC catalyst concentration in the oxidation process and the biological precipitation of arsenic as scorodite at pH 1.2–1.3 and 70°C.

2. Materials and methods

2.1. Inoculum and medium composition

A thermoaacidophilic iron-oxidizing mixed culture, to which the archaean strain Acidianus brierleyi (DSM 1651) was added, was acclimatized to growth medium containing 6.8 mM (510 mg L\(^{-1}\)) of As(III). The mixed culture has been described elsewhere [15]. The acclimatized culture was inoculated in batch bottles containing the growth medium with GAC and Fe(II) and As(III) at a molar ratio of 1.29. The growth medium for the mixed culture was prepared as described previously [15].

The growth medium was additionally supplied with 8.8 mM (490 mg L\(^{-1}\)) ferrous iron and 6.8 mM (510 mg L\(^{-1}\)) arsenic (As(III)), giving a Fe/As molar ratio of 1.29. Ferrous iron and arsenite stock solutions were prepared as described previously [15] Arsenic stock solutions were prepared from disodium arsenate heptahydrate (Na\(_2\)HAsO\(_4\)7H\(_2\)O) (Fluka, Switzerland). All used chemicals were analytical-reagent grade.

2.2. Batch experiments

Batch experiments were carried out in 250 mL serum bottles (triplicates) closed with a butyl rubber stopper and crimped aluminum seal. The bottles were supplied with 4 or 20 g L\(^{-1}\) ferrous activated carbon (GAC). Bottles for abiotic experiments were filled to a final volume of 100 ml with growth medium containing Fe(II) and As(III). Bottles for biotic tests contained 90 ml growth medium with Fe(II), As(III) and 10 ml of the pre-cultivated thermoaacidophilic mixed culture with a concentration of 1×10\(^7\) cell·ml\(^{-1}\). A summary of the conditions used in the batch experiments is shown in Table 1. The cell concentration in the bottles was determined by direct counting using a Neubauer chamber. The headspace (150 ml) of the bottles consisted of air, implying that at the start, oxygen was present in excess by a factor of 2 with respect to the maximum amount needed for full oxidation of As(III) and Fe(II). The bottles were placed in a thermostat shaker incubator at 150 rpm and 70°C during the experiment and samples were taken regularly for analysis of

| Concentration of GAC g L\(^{-1}\) | As species | Ratio Fe(II):As(III) | Gas |
|---------------------------------|-----------|---------------------|-----|
| 4                               | As(III)   | 1.29                | Air |
| 20                              | As(III)   | 1.29                | Air |
pH, Eh, and dissolved Fe and As species. Since the pH fluctuated between 1.24 and 1.3 during the biotic and abiotic experiments, adjustment of pH of the solution was not necessary.

The precipitates were collected from the bottles at the end of the experiments. First, the carbon granules were manually separated (by sieving) and washed with acid water (50 mM of sulphuric acid) in order to release any solid particle that might be deposited on the GAC. The precipitates were separated from the liquid phase by settling and centrifugation. The collected precipitates were washed with 50 mM sulphuric acid, followed by washing with deionized water and dried in a vacuum oven at 60 °C before characterization. Liquid samples were filtered over a 0.2 μm cellulose acetate membrane filter before analysis. The pH and redox potential of the samples were measured with glass electrodes QP181X and QR480X-Pt triple billed juction (vs. Ag/AgCl) (Prosense, the Netherlands), respectively.

2.3. Pre-treatment of GAC as catalyst for arsenite oxidation

Granular activated carbon NORIT GAC 830 W (Cabot Norit Nederland B.V., Amersfoort, the Netherlands) with a particle size ranging from 0.8 to 2.3 mm was used as catalyst for arsenite oxidation. The GAC was produced from coal followed by thermal activation and possesses a surface area of 885 m² g⁻¹, density of 1.06 g cm⁻³, a pore radius of 8–60 Å, a total pore volume of 0.775 cm³ g⁻¹ (specifications provided by supplier). The GAC was sieved to an average particle size 0.8–1.4 mm before use in the experiments.

2.4. Chemical analysis

Fe(II) and Fe(III) concentrations in solution were measured using Dr. Lange Cuvette test LCK 320 and a Xion 500 spectrophotometer (Hach-Lange, Germany). As(III) and As(V) concentrations in solution were measured with an HPLC connected to a UV photomultiplier spectrometer. The HPLC was an ultimate VWD 3000 RS (Dionex, the Netherlands) equipped with an ion exclusion column using 10 mM sulphuric acid as the mobile phase. The concentration of H₂O₂ was measured by a semi-quantitative measurement using reagent strips (QuanTox).

The Fe and As content of the activated carbon and of precipitates was determined after microwave digestion with aqua regia with inductively coupled plasma-optical emission spectrometry (ICP-OES) equipped with a megapixel (MPX) CCD detector (VISTA-MPX CCD Simultaneous, VARIAN Inc.).

2.5. Characterization of the solids

The method for identification of crystalline phases with X-Ray Diffraction (XRD) is described elsewhere [15]. Phase identification was assessed with the software DIFFRAC.EVA V4.1.1 (Bruker Axs) and crystallography open database. The same software was used to calculate crystallinity of solid samples based on peak to noise ratio’s. Fourier transform infrared (FT-IR) spectra of the samples were obtained with a Varian Scimitar 1000 FT-IR spectrometer equipped with a deuterated triglycine sulfate (DTSG) detector. The measurement resolution was set at 4 cm⁻¹, and the spectra were collected in the ATR (Attenuated Total Reflection) mode in the range 4000–650 cm⁻¹ with 128 co-added scans. ATR was performed on a PIKE MIRacle ATR with a diamond w/ZnSe lens single reflection plate. The sample chamber was purged with N₂ during 10 min before the scanning. The structural H₂O content of the solids was determined with a Thermogravimetric Analyser (Perkin-Elmer TGA7 equipped with Pyris software). The thermal gravimetric analysis was performed with 10 mg of air-dried powdered material at a heating rate of 10 °C min⁻¹ from 20 °C to 600 °C under an air atmosphere. Particle size distribution was measured with a Shimadzu Particle Size Analyzer SALD-2300.

The morphology of the precipitates was investigated with scanning electron microscopy (SEM). The samples were fixed on sample holders by carbon adhesive tabs and subsequently coated with about 10 nm of carbon (K950X, Quorum Technologies). Samples were analysed at SE detection 2 kV, 50 pA, WD 5 mm at room temperature, in a field emission scanning electron microscope (Magellan 400, FEI, Eindhoven, the Netherlands). The arsenic leachability of the precipitates was evaluated with the standard toxicity characterization leaching procedure (TCLP) of US Environmental Protection Agency (USEPA) [23]. The test was conducted at 30 °C and an acetate buffer at pH 4.98 was used as extraction solution at a solid to liquid mass ratio of 20. Samples were withdrawn after 24 h and after 30 days. The sampling volume was replaced by fresh leaching solution.

2.6. Calculation of ion activity product

The ion activity product (IAP), defined as the product of the ferric and arsenate ion concentration in solution was calculated considering the congruent dissolution of mineral scorodite defined as:

\[
\text{FeAsO}_4 \cdot 2 \text{H}_2\text{O} \text{ (scorodite)} \leftrightarrow \text{Fe}^{3+} + \text{AsO}_4^{3−} + 2 \text{H}_2\text{O}
\]  

(4)

The saturation index of the solution is defined as the ratio between the IAP of ferric arsenate in the solution the solubility product (Ksp) of scorodite.

\[
\text{IAP}_{\text{scorodite}} = (a\text{Fe}^{3+})(a\text{AsO}_4^{3−})
\]  

(5)

\[
\text{Ksp}_{\text{scorodite}} = (a\text{Fe}^{3+})(a\text{AsO}_4^{3−}) \text{ at equilibrium}
\]  

(6)

\[
\text{SI} = \frac{\text{IAP}_{\text{scorodite}}}{\text{Ksp}_{\text{scorodite}}}
\]  

(7)
Equations (8) and (9) were used to calculate the activity of arsenate and ferric ions in solution respectively. Values for dissociation constants are shown in Table S1.

\[
a_{\text{AsO}_4^{3-}} = \frac{[\text{Arsenate}_{\text{total}}]}{1 + \frac{\text{Arsenate}_{\text{total}}}{K_{\text{As(III)}}}} + \frac{[\text{As(III)}]{\text{Fe(III)}}}{K_{\text{As(III)}K_{\text{Fe(III)}}}}
\]

\[
a_{\text{Fe}^{3+}} = \frac{[\text{Ferric}_{\text{total}}]}{1 + \frac{\text{Ferric}_{\text{total}}}{K_{\text{Fe(III)}}}} + \frac{[\text{As(III)}]{\text{Fe(III)}}}{K_{\text{As(III)}K_{\text{Fe(III)}}}}
\]

3. Results and discussion

3.1. As(III) and Fe(II) oxidation without GAC

Arsenite oxidation was evaluated in batch tests containing 0, 4 and 20 g L\(^{-1}\) GAC with initial concentrations of 510 mg L\(^{-1}\) As(III) and 490 mg L\(^{-1}\) Fe(II) (Fig. 1A). Without GAC and without the culture, As(III) was not oxidized (Fig. 1A.), while only 7% of the Fe(II) was oxidized in 16 days. Without GAC but with the culture, 228 mg L\(^{-1}\) As(III) was removed from solution. The As(V) concentration increased, reaching a maximum of 79 mg L\(^{-1}\) on day 7 and subsequently decreased to 17 mg L\(^{-1}\) on day 16 (data not shown). The formed As(V) in solution, did not match the depleted As(III), implying that the total As concentration in solution decreased as shown in Fig. 1B. In the same experiment, 275 mg L\(^{-1}\) Fe(II) was removed, while only 11 mg L\(^{-1}\) Fe(III) accumulated from start to end. These results indicate that the mixed culture could oxidize As(III) to some extent when grown on Fe(II) as energy source. Without Fe(II), the culture did not oxidize As(III) (data not shown). Hardly any As(III) and Fe(II) was oxidized after day 9, revealing the inability of the culture to oxidize As(III) to low concentrations under the applied conditions. The results furthermore reveal that Fe and As precipitated to some extent, with a molar ratio of 1.0–1.4 of Fe-precipitated: As-precipitated.

3.2. As(III) and Fe(II) oxidation in the presence of 4 and 20 g L\(^{-1}\) GAC

With 4 g L\(^{-1}\) GAC, a zero order oxidation rate of 160 and 125 mg L\(^{-1}\) d\(^{-1}\) As(III) was estimated in biotic and abiotic experiments, respectively, until an As(III) concentration of 100 mg L\(^{-1}\) was reached (Fig. 1A). The difference in abiotic and biotic rate can be explained by the contribution of microbially induced As(III) oxidation, because in the absence of GAC an initial As(III) oxidation rate of 27 mg L\(^{-1}\) d\(^{-1}\) was found (Fig. 1A). With 20 g L\(^{-1}\) GAC, As(III) oxidation was almost complete within 2 days with no difference in the depletion curve between the biotic and abiotic experiments (Fig. 1A). Around 1 mg L\(^{-1}\) of hydrogen peroxide was detected in the bottles with 20 g L\(^{-1}\) of GAC immediately after the addition of the granules to the solution (Fig. S1), confirming that equations (1) and (2) play a role in the oxidation of As(III) [6,15].

With 4 g L\(^{-1}\) GAC in the biotic experiment, a specific oxidation rate of 40 ± 1 mgAs gGAC\(^{-1}\) d\(^{-1}\) was calculated, which decreased with almost a factor of 4 in the bottles with 20 g L\(^{-1}\) GAC (Table 2). Thus, in the experiment with 20 g L\(^{-1}\) GAC, the catalyst did not exert its maximum oxidation capacity, indicating a limitation. Possibly the oxidation was hampered by the presence of ferric precipitates on the GAC surface, as with 20 g L\(^{-1}\) GAC, 7 times more ferric was associated to the GAC at the end of the experiment (day 16), compared to the experiment with 4 g L\(^{-1}\) (Table 3).

It is noted that some adsorption of arsenic on GAC was observed at the beginning of the experiments as the As(III) concentration (520 ± 10 mg L\(^{-1}\)) decreased immediately after the addition of the GAC. The adsorption amounted to 1.5 ± 0.5 mg arsenic per gram of GAC, which lies in the range (0.16–3.5 mgAs gGAC\(^{-1}\)) reported in literature [24–26]. Although at lower GAC concentration the amount of adsorbed arsenic is negligible, it becomes more substantial with increasing concentration of the catalyst, such as in the bottles with 20 g L\(^{-1}\) of GAC, where only 470 mg L\(^{-1}\) As(III) was measured at the beginning of the experiment.

In the biotic experiment with 4 g L\(^{-1}\) of GAC, 453 mg L\(^{-1}\) (96%) of Fe(II) was oxidized in 16 days while in the abiotic experiment this was only 123 mg L\(^{-1}\) (25%) (Fig. 2C). Thus, the contribution of the microbial culture to Fe(II) oxidation was dominant compared to...
Table 2
Rates of As(III) oxidation obtained as a function of the catalyst (GAC) concentrations in 2 days of batch experiment, with the mixed culture (biotic) and the abiotic control (C).

| unit | 4 g L\(^{-1}\) GAC | 4 g L\(^{-1}\) GAC | 20 g L\(^{-1}\) GAC | 20 g L\(^{-1}\) GAC |
|------|------------------|------------------|-----------------|------------------|
|      | Biotic | Abiotic | Biotic | Abiotic |
| Max. volumetric As(III) oxidation rate day 0-2 | mg L\(^{-1}\) d\(^{-1}\) | 160 | 125 | 236 | 232 |
| As(III) oxidized after 2 days | % | 64 | 50 | 98 | 96 |
| Specific As(III) oxidation rate day 0-2 | mgAs gGAC\(^{-1}\) d\(^{-1}\) | 40 | 31 | 11 | 11 |

Table 3
Fe mass balance in biotic and abiotic experiments containing 4 g L\(^{-1}\) and 20 g L\(^{-1}\) of GAC, respectively with 510 mg L\(^{-1}\) As(III) and 490 mg L\(^{-1}\) Fe(II) at 70\(^\circ\)C and pH 1.3.

|          | Biotic 4 g L\(^{-1}\) GAC | Abiotic 4 g L\(^{-1}\) GAC | Biotic 20 g L\(^{-1}\) GAC | Abiotic 20 g L\(^{-1}\) GAC |
|----------|----------------|----------------|----------------|----------------|
| Fe in solution t\(_0\) | mg/bottle | 50.5 | 49.6 | 48.9 | 49.3 |
| Fe in solution t\(_{16}\) | mg/bottle (%) | 8.2 (16) | 45.9 (90) | 6.4 (13) | 27.1 (53) |
| Fe in precipitates t\(_{16}\) | mg/bottle (%) | 39.2 (76) | 0.0 (0) | 17.2 (34) | 0.0 |
| Fe associated with GAC t\(_{16}\) | mg/bottle (%) | 3.9 (8) | 5.2 (10) | 26.8 (54) | 23.7 (47) |
| Recovery\(^b\) | % | 102 | 103 | 103 | 103 |

\(^a\) 100% of the precipitates assumed as scorodite as indicated by XRD analysis.
\(^b\) Recovery = 100\% * (Fe in solution t\(_{16}\) + Fe in precipitates t\(_{16}\) + Fe associated with GAC t\(_{16}\))/ (Fe in solution t\(_0\)).

Fig. 2. Ferrous iron oxidation and Fe precipitation (solid and dashed line respectively) containing 0 g L\(^{-1}\) GAC (A and B), 4 g L\(^{-1}\) of GAC (C and D) and 20 g L\(^{-1}\) GAC (E and F). Fe(II): □ abiotic, ■ biotic, Fe(III): ○ abiotic, ● biotic, Total Fe: Δ abiotic, ▲ biotic. Solid and dashed lines correspond to biotic and abiotic experiments, respectively. Error bars indicate standard deviation of the mean.
the contribution of GAC. With 20 g L\(^{-1}\) of GAC, the difference was less pronounced with 64% and 93% Fe(II) oxidized in the abiotic and biotic experiment, respectively (Fig. 2E). Still, this reveals that Fe(II) can also be oxidized with GAC as catalyst, even though oxidation of As(III) was much faster in our experiments when both ions were present (Figs. 1A, 2C and 2E).

3.3. Effect of GAC on Fe and as precipitation

In the biotic experiment with 4 g L\(^{-1}\) GAC, the solution became opaque and the first greenish precipitates, resembling the colour of scorodite, were visible with the naked eye after 4 days. In the biotic experiment with 20 g L\(^{-1}\) GAC, removal of As was evident already after day 1 (Fig. 1B), and colloidal-like precipitates were visible after 2 days (Fig. 3B). In abiotic experiments, the solution remained transparent (Fig. 3), which confirms previous results [15].

Tables 3 and 4 show the mass balance of As and Fe in the bottles, and their distribution in the solution (dissolved Fe and As species), in the precipitates, and associated with the GAC (predominantly as precipitate, and adsorbed, as explained in section 3.2). After 16 days in the biotic experiment with 4 g L\(^{-1}\) GAC, 76% of the Fe and 78% of the As was present in the precipitate and only 8% of the Fe and 10% of the As was found in the GAC. The remainder of Fe (16%) and As (12%) remained in solution. Thus, precipitation in the solution was predominant with only little precipitation on the GAC. With 20 g L\(^{-1}\) of GAC, 34% of the Fe and 34% of the As was present in the precipitate while 54% of the Fe and 49% of the As was found in the GAC, revealing that with the higher GAC concentration, precipitation on the GAC was predominant over precipitation in solution.

With 4 g L\(^{-1}\) of GAC in the abiotic experiment, only 10% of Fe and As was associated with the GAC after 16 days, with the remainder still in solution mainly as Fe(II) and As(V). With 20 g L\(^{-1}\) of GAC, 47% of Fe and 34% of As was associated with the GAC. Interestingly, the amount of Fe associated with the GAC is similar for both conditions with 13 and 12 mg Fe g\(^{-1}\) GAC for 4 and 20 g L\(^{-1}\) of GAC respectively. For As, these values are 12 and 8 mg As g\(^{-1}\) GAC, respectively. In both abiotic experiments, arsenite and ferrous oxidation stopped around day 7, and the concentrations of Fe(II), Fe(III), As(III) and As(V) did not change after this day, revealing that precipitation had also stopped.

The above results suggest that in the abiotic experiments the Fe–As precipitates covered oxidation sites on the GAC surface, thereby preventing further oxidation and subsequent precipitation. In the biotic experiments, the Fe(II)-oxidizing microorganisms ‘compete’ with the GAC for Fe(II). Clearly, at the higher GAC concentration of 20 g L\(^{-1}\), more Fe(II) is oxidized by the GAC and more Fe precipitates on the GAC rather than in solution. With 4 g L\(^{-1}\) of GAC, the oxidation and precipitation of Fe on GAC does not appear to be affected by the microorganisms as the biotic and abiotic show similar amounts of Fe and As on the GAC (Tables 3 and 4). This is also true for Fe with 20 g L\(^{-1}\) of GAC, and to a lesser extent for As. The Fe–As precipitates on the GAC had a molar ratio of 1.0–1.9 (Table 4).

From the above the following scheme emerges; in the presence of GAC and the iron-oxidizing culture, the GAC and the microorganisms compete for Fe(II). Following microbial oxidation, the Fe(III) precipitates with As(V) in the solution or on the cell surface, while Fe(II) oxidized by the GAC, precipitates with As(V) on the surface of the GAC, thereby inactivating the GAC.

![Fig. 3. Photo of batch bottles with 4 g L\(^{-1}\) (A) and 20 g L\(^{-1}\) (B) of GAC at day 10. Duplicates of the biotic test are in the middle and left, to right the chemical control.](image-url)
Table 4

| As in solution t(0) | Biotic 4 g L\(^{-1}\) GAC | Abiotic 4 g L\(^{-1}\) GAC | Biotic 20 g L\(^{-1}\) GAC | Abiotic 20 g L\(^{-1}\) GAC |
|---------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| mg/bottle           | 49.9                      | 49.8                      | 47.2                      | 47.0                      |
| As in solution t(10) | mg/bottle (%)             | 6.1 (12)                  | 46.6 (90)                 | 8.6 (17)                  |
| As in precipitates t(10) | mg/bottle (%)             | 40.5 (78)                 | 0.0 (0)                   | 17.2 (34)                 |
| As associated with GAC t(16) | mg/bottle (%)             | 5.2 (10)                  | 4.9 (10)                  | 24.9 (49)                 |
| As Recovery\(^a\)   | %                         | 103                       | 103                       | 107                       |
| Fe/As ratio of precipitate | mol/mol                  | 1.2                       | –                         | 1.4                       |
| Fe/As ratio of GAC   | mol/mol                   | 1.0                       | 1.4                       | 1.9                       |

\(^a\) 100% of the precipitates assumed as scorodite as indicated by XRD analysis.

\(^b\) Recovery = 100%*(As in solution t(16) + As in precipitates t(16) + As associated with GAC t(16))/ (As in solution t(0)).

3.4. Characterization of the precipitates

The concentration of GAC influenced the As(III) and Fe(II) oxidation rates, and consequently, also the saturation state of the solution is affected (Fig. 4A). The saturation index (SI) of the solution, calculated from ratio of IAP and the \(K_{sp}\) of scorodite (10\(^{-22}\)) [27], ranged between 1-1.4, reaching the maximum value on day 2 in the biotic experiments with 4 g L\(^{-1}\) GAC, which coincided with the onset of depletion of As and Fe from solution (Fig. 4B). Furthermore, the IAP in solution reached values close to -22 observed between day 1-7 (Fig. 4A) which are in the range of reported \(K_{sp}\) values for scorodite (10\(^{-22}\)) [9,28-30], indicating that the solution was only slightly oversaturated in that period.

The particle size distribution (PSD) of precipitates collected at the end of the biotic experiments is shown in Fig. 5. With 4 g L\(^{-1}\) GAC the average particle size was 66 \(\mu\)m, while with 20 g L\(^{-1}\) GAC it was only 2.6 \(\mu\)m, with a substantial colloidal fraction with particle sizes under 1 \(\mu\)m. Apparently, as the higher saturation index with 20 g L\(^{-1}\) GAC, nucleation was favoured over crystal growth, thereby yielding particles with small crystal size [31].

The XRD analysis of the precipitates confirmed that scorodite was formed in all biotic experiments (Fig. 6). However, sharper peaks were found in the diffractogram of solids from the experiment with 4 g L\(^{-1}\) GAC compared to experiments with 20 g L\(^{-1}\), indicating a higher crystallinity. The computed crystallinity of the samples by crystallography open database (COD) indicated 83% and 54% of crystallinity in the precipitates collected from experiments with 4 and 20 g L\(^{-1}\) of GAC, respectively.

The higher background or “hump” observed in the diffractogram of precipitates with 20 g L\(^{-1}\) GAC is indicative either of poorly crystalline material, or of fine carbon particles present in the sample since a similar pattern was detected in the XRD of the raw GAC (data not shown). Besides, in the diffractogram a broad peak in the region 2\(\theta\) 10-17 could not be identified in the database, but this peak was also observed in the pattern of carbon granules washed with sulphuric acid (data not shown).

The Fe/As molar ratio of the solids measured by ICP-OES was 1.2 and 1.35 respectively (Table 1, supplementary information). The structural water content of the precipitates was also determined by thermogravimetric analysis (TGA) (Fig. 7). The TGA curve of the precipitates collected from bottles with 4 g L\(^{-1}\) GAC showed the inflection point between 160 and 240 °C with a calculated weight loss of 15.4% (Fig. 7A), this value is close to the theoretical value of 15.6% corresponding to 2 molecules of water in mineral scorodite. Between 245 and 500 °C the water loss was 2.3%. This was also observed in a previous study of our group [9] in which we suggested that this was due to the presence of organic matter. The water content of the precipitates collected from experiments supplied with 20 g L\(^{-1}\) GAC was around 18.5% with the inflection point between 130-230 °C. This higher value implies the formation of poorly crystalline phases rather than fully crystallized scorodite [32].

The FT-IR analysis of the precipitates displayed peaks at 819 and 795 cm\(^{-1}\) (Fig. 7B and D), characteristic for arsenate stretching and bending bands (\(\nu_3\)AsO\(_4^3\)) in agreement with the reported bands for biogenic and mineral scorodite [9,33,34]. Another vibration band observed at 1054 cm\(^{-1}\) in Fig. 7B was related to phosphate or organic material (Ondrus, Skála [35]. The vibration bands for
Fig. 5. Particle size distribution volume based of the precipitates collected at day 16 from biotic experiments containing 4 (■) and 20 g L⁻¹ (▲) of GAC with As(III).

Fig. 6. XRD diffractogram of solids collected from biotic tests containing 4 and 20 g L⁻¹ GAC (6A and 6B, respectively) with 510 mg L⁻¹ As(III) and 490 mg L⁻¹ Fe(II).

Fig. 7. Characterization of the scorodite precipitates obtained in batch experiments: TGA and FT-IR analysis of solids collected from biotic tests with 4 g L⁻¹ (A and B) and 20 g L⁻¹ of GAC (C and D) respectively. The structural water content measured by TGA was calculated from the mass loss between 150 and 250 °C.
sulfate were absent in the spectra of the biogenic precipitates with 4 g L\(^{-1}\) GAC, indicating that the sample was free of sulfate. Contrarily, a strong band occurring at 983 cm\(^{-1}\) in the spectra of solids collected from 20 g L\(^{-1}\) of GAC in Fig. 7D indicated the presence of sulfate in the precipitates. Presumably, this was due to the presence of basic ferric arsenate sulfate [36].

Bending vibration of the water molecule in the solids were also found at 1619 cm\(^{-1}\) and 1587 cm\(^{-1}\) (Fig. 7B and D, respectively). Both values are in agreement with those reported previously [35,37]. Furthermore, the bands displayed at 2964 cm\(^{-1}\) and 2997 cm\(^{-1}\) correlate to the O–H bond between crystalline water groups and oxygen of the arsenate molecules that occurs in the region 2900–3080, as well the similar stretch bands observed at 3518–3520 cm\(^{-1}\) coincide with weaker O–H bond between oxygen atoms in crystalline water (Fig. 7B and D) [38].

Scanning electron microscopy of the precipitates collected at day 16, revealed the presence of solids with the typical dipyramidal habit of scorodite (Fig. 8A and B). Also, rod-shaped microorganisms associated with the scorodite precipitates and the GAC surface were found (Fig. S2). This observation supports the hypothesis that the microbial surface served as heterogeneous nucleation, perhaps after adsorption of ferric and arsenate ions.

The results of the arsenic leaching test revealed that the scorodite precipitates collected from the experiment with 4 g L\(^{-1}\) GAC leached around 0.87 ± 0.2 mg L\(^{-1}\) of As after 24 h. Similarly, a concentration of 0.91 ± 0.07 mg L\(^{-1}\) As was measured in leachate samples after 30 days. These results revealed that the produced scorodite was stable under the studied conditions. In contrast, the leaching of the solids produced in experiments with 20 g L\(^{-1}\) GAC showed an increase from 3.63 mg L\(^{-1}\) As after 24 h to 5.11 ± 0.15 mg L\(^{-1}\) As at the end of the leaching tests. The leached concentration of arsenic with these precipitates was above the permissible US EPA level of 5 mg L\(^{-1}\) As.

Although the difference in the leaching behaviour of scorodite (produced under atmospheric or hydrothermal conditions) has been attributed to different factors such as particle size and the molar Fe/As, the crystallinity of the precipitates seem to be an important parameter determining leaching characteristics of scorodite [7,28]. The formation of poorly crystalline phases has been explained by the fast precipitation rate caused by the rapid Fe(II) oxidation which consequently affects the saturation of the solution allowing nucleus formation over the growth of the crystal [39,40]. The uncontrolled precipitation as observed in biotic tests with 20 g L\(^{-1}\) GAC has led to the formation of fine precipitates, identified mainly as scorodite by powder diffraction analysis. Due to the low computed crystallinity of these solids (54%), it is possible that non-crystalline phases have developed along with scorodite, triggering the fast leaching of arsenic [41].

4. Conclusions

The results presented here demonstrate the impact of the concentration of GAC on the precipitation of scorodite starting from As (III) and Fe(II) containing medium, inoculated with thermoacidophilic iron-oxidizing microorganisms. With higher GAC concentrations, the contribution of GAC-catalysed iron oxidation increases relative to microbial oxidation. Fe(II) oxidized by GAC has a tendency to precipitate with As(V), formed through GAC-catalysed oxidation of As(III), on the surface of the GAC. This results in inactivation of the oxidative capacity of the GAC. With higher GAC concentrations, relatively more Fe and As precipitate on the catalyst.

Furthermore, higher GAC concentrations result in higher Fe(II) and As(III) oxidation rates and higher concentrations of Fe(III) and As(V), resulting in a higher saturation state of the solution with respect to scorodite. In turn, this results in the formation of smaller scorodite particles which are less stable. The controlled biological oxidation of Fe(II) achieved in the experiments with 4 g L\(^{-1}\) GAC allowed to keep the saturation index below 1.5, leading to the formation of settleable particles which As leaching behaviour that comply with the USEPA limit value, even after 30 days.

The proposed mechanism is a potential option for the treatment of diluted As(III) acid streams. Therefore, future studies aim to reproduce and scale-up the process for the continuous treatment of As(III)-containing acid streams.

Declaration of competing interest

The authors declare no competing financial interests.

Fig. 8. Scanning electron microscopy of the scorodite precipitates (manually colored in green) collected from biotic experiments containing 4 g L\(^{-1}\) GAC with As(III). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
CRediT authorship contribution statement
Silvia Vega-Hernandez: Methodology, Data curation, Writing - original draft. Jan Weijma: Conceptualization, Supervision, Writing - review & editing. Cees J.N. Buisman: Conceptualization, Supervision.

Acknowledgements
This work was supported by the Netherlands Enterprise Agency’s TKI program and Paques BV. The authors acknowledge the personal scholarship from CONICYT-Chile to S. Vega. We thank Å. Sandström of Luleå University (Sweden) for providing the mixed thermoacidophilic culture used in this study.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.wri.2020.100128.

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