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As^{III} oxidation by *Thiomonas arsenivorans* in up-flow fixed-bed reactors coupled to As sequestration onto zero-valent iron-coated sand

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ABSTRACT

The combined processes of biological As^{III} oxidation and removal of As^{III} and As^{V} by zero-valent iron were investigated with synthetic water containing high As^{III} concentration (10 mg L^{-1}). Two up-flow fixed-bed reactors (R1 and R2) were filled with 2 L of sieved sand ($d = 3 \pm 1$ mm) while zero-valent iron powder ($d = 76$ μm; 1% (w/w) of sand) was mixed evenly with sand in R2. *Thiomonas arsenivorans* was inoculated in the two reactors. The pilot unit was studied for 33 days, with HRT of 4 and 1 h. The maximal As^{III} oxidation rate was 8.36 mg h^{-1} L^{-1} in R1 and about 45% of total As was removed in R2 for an HRT of 1 h. A first order model fitted well with the As^{III} concentration evolution at the different levels in R1. At the end of the pilot monitoring, batch tests were conducted with support collected at different levels in R1. They showed that bacterial As^{III} oxidation rate was correlated with the axial length of reactor, which could be explained by biomass distribution in reactor or by bacterial activity. In opposition, As^{III} oxidation rate was not stable in R2 due to the simultaneous bacterial As^{III} oxidation and chemical removal by zero-valent iron and its oxidant products. However, a durable removal of total As was realized and zero-valent iron was not saturated by As over 33 days in R2. Furthermore, the influence of zero-valent iron and its oxidant corrosion products on the evolution of As^{III}-oxidizing bacteria diversity was highlighted by the molecular fingerprinting method of PCR-DGGE using *aoxB* gene as a functional marker of aerobic As^{III} oxidizers.

1. Introduction

The occurrence of arsenic (As) in water resources and thus potentially in drinking water is a public concern. Epidemiological studies demonstrated that there is close link between the chronic exposure to As in drinking water and some medical disorders and cancers (Ng et al., 2003; Vahter et al., 2006). In some countries and regions, groundwater with high As concentration is supplied as drinking water, especially in the developing countries such as Bangladesh and India (Ahmed, 2001). U.S. EPA (2001) reduced the maximum contaminant level for total As in the drinking water from 50 to 10 μg L^{-1}, which was also recommended by WHO (1996). Therefore, removal of As from water is an emergent and important topic for many countries and regions around the world.

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Conventionally, the most commonly used technologies of As treatment were focused on the physico-chemical methods including coagulation (Hering et al., 1997), adsorption (Katsoyiannis and Zouboulis, 2002; Zhang et al., 2003), ion exchange resin (Korngold et al., 2001) and membrane process (Sato et al., 2002; Nguyen et al., 2009). Recently, numerous lab-scale research studies have been conducted to focus on potential application of zero-valent iron (ZVI: Fe^0) in treatment of As contaminated water (Su and Puls, 2001a, b; Sun et al., 2006; Beak and Wilkin, 2009; Ludwig et al., 2009; Wilkin et al., 2009; Biterna et al., 2010). Studies dealing with As removal mechanisms by Fe^0 showed that As was removed due to adsorption/co-precipitation with iron oxides which are formed on the surface of ZVI (Irene et al., 2006). Moreover, Bang et al. (2005) revealed that the removal of As was predominately due to the adsorption on iron oxides formed rapidly on the surface of ZVI in the presence of dissolved oxygen.

In water As is mainly under inorganic forms, the predominant form being arsenite (As^{III}) under reducing conditions and arsenate (As^V) in oxygenated waters. As^{III} is an uncharged molecule ($H_3AsO_3^0$) whereas As^V exists as oxyanions ($H_2AsO_4^-$ and $HAsO_4^{2-}$) (Manning et al., 2002; Villaescusa and Bollinger, 2008). As^{III} is estimated to be about 60 times more toxic than As^V to human being (Neff, 1997). Moreover some studies (Lee et al., 2003; Roberts et al., 2004; Pallier et al., 2010) showed that the adsorption of As^V onto coagulated flocs (or on the surface of minerals) was more efficient than adsorption of As^{III}. Thus As removal process often comprises a preliminary oxidation step.

In drinking water plants, chemical As^{III} oxidation is generally performed: ozone, chlorine, hydrogen peroxide/Fe^{2+}, etc. In comparison with chemical oxidation, biological oxidation of As^{III} could avoid the generation of chemical by-product and reduce the treatment cost (Kim and Nriagu, 2000; Ike et al., 2008). The combination of biological processes with the physico-chemical processes would improve the reliability of the As treatment and maintain As level within the acceptable drinking water standards. The biological oxidation of As^{III} coupled with conventional process could therefore be an effective strategy in the future.

The phenomena of bacterial As^{III} oxidation have already been reported and studied (Turner, 1949; Oremland and Stolz, 2003; Rhine et al., 2005). As^{III}-Oxidizing Bacteria (AOB) could be divided in two principal groups: heterotrophic As^{III} oxidizers (HAOs) (Muller et al., 2003; Kashyap et al., 2006; Ike et al., 2008) and chemolithoautotrophic As^{III} oxidizers (CAOs) (Santini et al., 2000; Battaglia-Brunet et al., 2002; Rhine et al., 2007). CAOs can use inorganic carbon (CO_2 or $NaHCO_3$, etc.) as carbon source and does require less nutrition elements for their metabolism than HAOs. CAOs can get energy from As^{III} oxidation. Considering the low level of organic compounds in waters, CAOs could easier adapt to this environment than HAOs and are thus an attractive application for As^{III} oxidation. The autotrophic consortium CAsO1 (Cheni As Oxidizing culture 1) isolated from the disused Cheni gold mine site (Limousin, France) allowed efficient As^{III} biological oxidation in fixed-bed bioreactors (Battaglia-Brunet et al., 2002; Michon et al., 2010). On the basis of this result, a new species of the genus *Thiomonas* (strain b6^T) named *Thiomonas arsenivorans* (Battaglia-Brunet et al., 2006) was isolated from CAsO1 and was proved to use As^{III} as sole energy source for chemolithoautotrophic growth.

In the present study, the As^{III} oxidation capacity of *T. arsenivorans* was investigated in two up-flow fixed-bed reactors filled with sand, as biological support. ZVI was only added in a second fixed-bed reactor (R2). The simultaneous biological oxidation of As^{III} and chemical removal of As^{III} and As^V occurred in R2. Therefore, comparison of the results helped to evaluate the interaction between the chemical removal by ZVI and As^{III} biological oxidation.

2. Materials and methods

2.1. Experimental setup

The experimental setup included two up-flow fixed-bed reactors R1 and R2 with a volume of 2.0 L (Fig. 1). Internal diameter of each reactor was 8.0 cm and height was 40.0 cm.

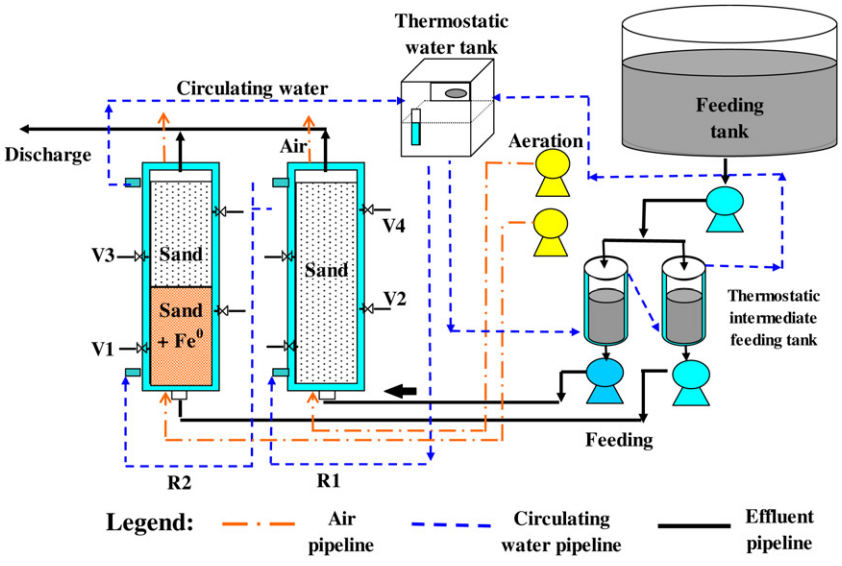

Fig. 1 – Schematic diagram of the experimental setup pilot.

Four valves (V1–V4) were installed at different levels "z" along the reactor, located at 10, 18, 27, 35 cm from the reactor inlet to allow the water collection.

During the whole experiment, both reactors were fed continuously with synthetic groundwater with same flow rate from two intermediate feed tanks (working volume = 12 L) in order to avoid cross-contamination. Operating conditions were chosen to be close to those encountered in groundwater in temperate areas (Martin et al., 2007). The synthetic water composition did correspond to calcareous water, naturally contaminated by As; it was prepared according to the Smith et al. (2002) procedure. Thus reactors and intermediate feeding tanks were maintained at 12 °C thanks to their double thermostatic jacket and a cryothermostat (Polystat cc3, Model UC015-3, Huber Company). These two feeding tanks were fed by a 450 L tank.

Sieved sand ($d = 3 \pm 1$ mm) filled the two reactors as support material. 2 L of sand was directly added in R1, while R2 was filled for half with iron-spiked sand which was a homogenous mixture of 1% (w_{Fe}/w_{sand}) ZVI powder (Jeulin, purity >99.999%, diameter = 76 μm, density = 7.86 g cm^{-3}, 0.010 m^2 g^{-1}) and the upper half with sand (Fig. 1). Considering the filling of the reactors, working volume in R1 and R2 was 1 L.

In order to provide enough oxygen for the bacteria metabolism and to form ZVI oxidation products, a constant air flow (60 NL h^{-1}) was supplied by air diffusers, placed at the bottom of each reactor. The physico-chemical parameters (pH, Eh and temperature) were daily monitored at the outflow of the bioreactor and in the intermediate feeding tanks.

2.2. Hydrodynamic characteristics of fixed-bed reactor

Hydrodynamic characters of the fixed-bed reactors were determined before operating. The volume fraction of fixed-bed (sand), retained liquid and "drained off" liquid were tested in R1 (not in R2 in order to avoid the oxidation of ZVI before the operation). Residence time distribution (RTD) was determined as described by Michon et al. (2010) : NaCl solution (10 g L^{-1}) was used as tracer and detected by conductivity measurements. Mean residence time (t_r) could be calculated according to the first moment of the Eq. (1),

$$E(t) = \frac{C(t)}{\int_0^\infty C(t)dt} \quad (1)$$

where the distribution of residence times $E(t)$ was determined from $C(t)$ (μs cm^{-1}), the measured conductivity at the outlet of reactor and t (min) the corresponding time.

The Hydraulic Residence Time (HRT) was calculated according to Eq. (2). The comparison of these two hydrodynamic values (t_r and HRT) could distinguish the short cut or dead zones in fixed-bed reactor if necessary.

$$HRT = \frac{V_t}{Q} \quad (2)$$

where V_t (mL) was the water retention volume, which was sum of "drained off" water volume and retained volume. Q (mL min^{-1}) was the flow rate.

2.3. T. arsenivorans inoculation

T. arsenivorans was provided by BRGM (Orléans; France) (Battaglia-Brunet et al., 2006). In R1 and R2, 10 mL of inocula were mixed with 1000 mL of enrichment medium as described in Battaglia-Brunet et al. (2002) supplemented with 10 mg L^{-1} of As^{III}. In both reactors, this solution circulated at 12 °C with a flow rate of 30 mL min^{-1} (HRT = 33 min) during 4 h and then circulated for 72 h at a slower flow rate of 5 mL min^{-1} (HRT = 200 min).

2.4. Media and operating conditions strategy

After 3 days of *T. arsenivorans* inoculation, both reactors were fed by synthetic water, whose composition was chosen according to the representative composition of As contaminated groundwater and given in Table 1. In addition, $NaAsO_2$ was added as the unique source of As^{III} (10 mg L^{-1}). Hydrochloric acid (HCl 37%) was added in the main tank to keep under control the pH close to 7.7. During the experiment, HRT was 4 h for 23 days and then it was reduced to 1 h for 10 days by changing the flow rate of peristaltic pump (Masterflex peristaltic pumps L/S Digital).

2.5. Batch studies

After 33 days of pilot-scale operation, the system was stopped and the effluent was drained off gravitationally; the support media with biofilm was gathered at different levels of each reactor. Samples of support were collected at the inlet (0–2 cm) (S1) and at different levels, situated at 6–8 (S2), 8–11 (S3), 22–24 (S4), and 36–38 cm (S5), respectively. In each selected level, triplicate samples of sand (humid mass = 30 ± 0.2 g) were placed into sterile bottle with 100 mL of synthetic groundwater (2 mg L^{-1} of As^{III}). Finally, samples were fixed on an orbital shaker (Ikalabortechnik, KS 501) at 140 rev min^{-1}, and at room temperature (22 ± 2 °C). Arsenic speciation was analyzed after 30, 60, 120, 150, 210, 270, 330, 400 min of agitation.

2.6. Analytical methods

Samples were collected from the feed tank, reactor outlets and four points along the reactor (V1–V4 on Fig. 1). In order to preserve As speciation until analysis, all samples were filtered (0.2 μm) to which 1% (*m*/*v*) of EDTA added to prevent the interference of iron ions and finally acidified with a 1% (*v*/*v*) HCl solution (Merck, Suprapur, As < 0.0000001%, 2 M) (Bednar et al., 2002).

Monitoring of As speciation was performed by coupling column Liquid Chromatography (LC) with Atomic Fluorescence Spectrometry (AFS) with a quantification limit of 0.1 and 0.3 μg L^{-1} respectively for As^{III} and As^{V}. Soluble Fe analysis was performed by GF-AAS, Varian SpectrAA 220Z, with a quantification limit of 100 μg L^{-1}.

Table 1 – Synthetic water composition.

Component	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}	HCO_3^-	NO_3^-	SiO_2
Concentration (mM)	1.83	0.13	1.44	0.70	0.79	1.25	3.20	0.03	0.14

2.7. Detachment of biofilm

At the end of pilot-scale operation, three moist sand samples (each around 30 g) were collected from three levels (0–2, 8–10 and 36–38 cm) in the two bioreactors. Each sample was added to 100 mL of physiological solution (0.9% NaCl) and placed in ultrasonic bath (30 W) for 5 min. Then the suspended aggregates detached from the surface of sand were collected. The morphology of aggregates was visualized by microscopic observation and 10–12 mL of mixed liquor was used for the DNA extraction.

2.8. Microscopic observation, DNA extraction and PCR-DGGE

The suspended aggregates morphology was observed under light microscopy connected with camera (SONY, Japan) and using charge coupled device image. After centrifugation (10000 × *g*, 4 °C) of suspended samples (10–12 mL) for 1 min, genomic DNA was extracted from the samples by using the Kit PowerSoil™ (MO BIO). The quality of the DNA extract was examined by agarose gel (0.8%) electrophoresis.

The *aoxB* gene, a functional marker to aerobic As^{III} oxidizers, was used to investigate the diversity of As^{III}-oxidizing bacteria in the two reactors (Quéméneur et al., 2008). Here, the primers aoxBM1-2*F*-ND (5′-CCACTTCTGCATCGT-GGGCTGTGGCTA-3′) and aoxBM2-1*R*-ND-GC (5′–GGAGTT-GTAGGCCGGGCGGTTGTGGAT-3′) were used for specific PCR (Polymerase Chain Reaction) amplification (30 cycles, annealing temperature of 55 °C, 10 min elongation time) of a *ca.* 550 bp fragment (Quéméneur et al., 2008).

DGGE (Denaturing Gradient Gel Electrophoresis) analysis was performed using the D-code system (Bio-Rad Laboratories, Hercules, CA, USA). PCR products of each sample were loaded onto 8% (*w*/*v*) polyacrylamide gels (containing 37.5:1 of acrylamide to bis-acrylamide) with a denaturing gradient ranging from 40% to 60%. Electrophoresis was performed at 20 V for 20 min, and then 80 V for 18 h at 60 °C. Finally, the gels were stained for 15 min with 50 mL of a 1:10,000 dilution of GelStar nucleic acid stain, and destained for 15 min in MilliQ water. DGGE profiles were captured with Gel Documentation Systems (Bio-Rad Laboratories, Segrate, Italy) and digitized by the Quality One Quantitation Software (Bio-Rad).

2.9. Mass balance

The fate of incoming As^{III} (mg L^{-1}) was considered to be divided into three fractions: As fixed by the media support in the reactor, soluble oxidized product As^{V} and the residual As^{III} in the outlet. No organic As form was detected in any samples, MMA (monomethylarsonic acid) and DMA (dimethylarsinic acid) limit quantification were respectively: 0.3 μg L^{-1} and 0.4 μg L^{-1}.

$$As^{III}_{inlet} = As_{trapped} + As^{V}_{outlet} + As^{III}_{outlet} \quad (3)$$

As trapped could correspond to As^{III} and As^{V} adsorbed on zero-valentiron corrosion products or co-precipitated in these iron hydroxides (Su and Puls, 2001b). The speciation of As trapped could not be analyzed directly during the experimental operation. In order to evaluate As^{III} oxidation rate, trapped As was considered as non-oxidized and As^{V} in the outlet resulted from oxidation. Therefore, the As^{III} oxidation rate θ (mg h^{-1} L^{-1}) was calculated as follows:

$$\theta_{oxidation} = \left(As^{III}_{inlet} - As^{III}_{outlet} - As^{III+V}_{trapped}\right) Q/V = As^{V}_{outlet} Q/V \quad (4)$$

where Q (L h^{-1}) is the flow rate of effluent and V (L) is the working volume of the reactor.

The As^{III} oxidation and total As removal efficiency was calculated as following:

$$\eta_{oxidation}(\%) = 100 \times \left(As^{III}_{inlet} - As^{III}_{outlet} - As^{III+V}_{trapped}\right) \Big/ As^{III}_{inlet} = 100 \times \left(As^{V}_{outlet}\right) / As^{III}_{inlet} \quad (5)$$

$$\eta_{removal}(\%) = 100 \times \left(As^{III}_{inlet} - As^{III}_{outlet} - As^{V}_{outlet}\right) / As^{III}_{inlet} \quad (6)$$

3. Results

3.1. Hydrodynamic characteristics of up-flow fixed-bed reactors

As shown in Fig. 2A, half volume of the reactor was dominated by the support media sand and 11% of total volume was occupied by the retained liquid due to the physical functions. Retained liquid could be assumed to generate a water film on the surface of sand (Kokusho, 1999), which was probably a favorable environment for the growth of autotrophic bacteria and their attachment on the surface of sand, especially at high flow rate.

The mean residence time (t_r) fit well with the HRT (Fig. 2B), which proved that almost no dead zones or short cuts existed in the reactor. Flow pattern was close to the plug flow reactor, which would help to simplify the biological kinetics model in 1D mode between the axial length of reactor and the biological activity.

3.2. Evolution of As speciation in fixed-bed reactors

After 76 h of inoculation, the two reactors worked for 23 days under an HRT of 4 h, which was then decreased to 1 h for 10 days (Fig. 3). The influent As^{III} was kept around 10 mg L^{-1} for both reactors during the whole period. After the initial 3 days of slow As^{III} oxidation, the effluent As^{III} concentration swift decreased and was completely oxidized in As^{V} on day-14 in R1. When reducing HRT from 4 to 1 h, the effluent As^{III} concentration increased momentarily and stayed close to 2 mg L^{-1} on day-30 due to the higher As^{III} loading in R1.

In R2, the As^{III} concentration at the outlet decreased regularly during the first 14 days (Fig. 3). The As removal increased also regularly, due to progressive iron hydroxides formation. From day 7 to 10, As removal decreased whereas As oxidation increased (increase of As^{V} concentration); this period corresponded to the one where biological oxidation increased in R1. From day 10 to 15, As removal increased again with decrease of As^{III} concentration. After day 15, As removal was stable and no As^{III} was detected at the outlet. After HRT

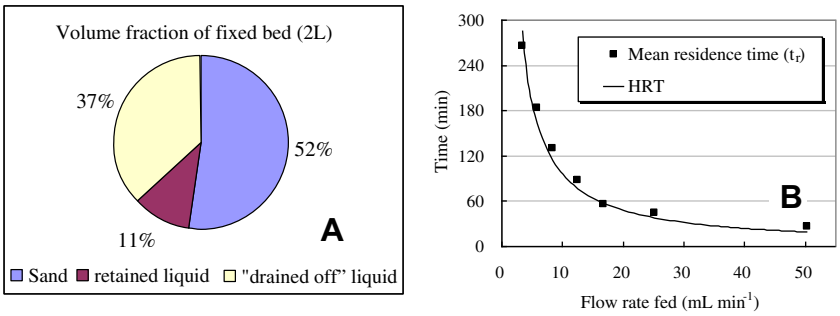

Fig. 2 – Characterization of hydrodynamic conditions in bed-fixed reactor. (A) Volume fraction of fixed-bed; (B) Comparison of mean residence time (t_r) with HRT.

was decreased to 1 h on day-24, the effluent As^{III} concentration increased to 3 mg L^{-1} and As^{V} concentration slowly increased to 5 mg L^{-1}, respectively.

The different results obtained in R1 and R2 show that ZVI played a significant role in As oxidation and removal.

3.3. *Batch tests*

After 33 days' operation, the two reactors were taken off and media support samples (sand) were carefully collected at five different levels in the two bioreactors. These samples were used to investigate kinetics parameters of As^{III} oxidation and removal in batch tests. The monitoring of As^{III} concentration (Fig. 4: R1–A and R2–A) showed that the As^{III} oxidation varied according to the level in both bioreactors. The layer closest to the bioreactor inlet showed high As^{III} oxidation rate, whereas very low oxidation rate was observed on the topmost layer. The As^{III} oxidation was confirmed by the concomitant As^{v} production (Fig. 4: R1–B and R1–C) and the stability of total As concentration over time. Therefore, for continuous working conditions, only oxidation occurred in R1.

In opposition, the batch test conducted with R2 support samples showed that total As concentration (Fig. 4. R2–C) decreased for the layers 0–2, 6–8 and 8–11 cm. This As removal resulted from As adsorption or co-precipitation by the ZVI oxidant products.

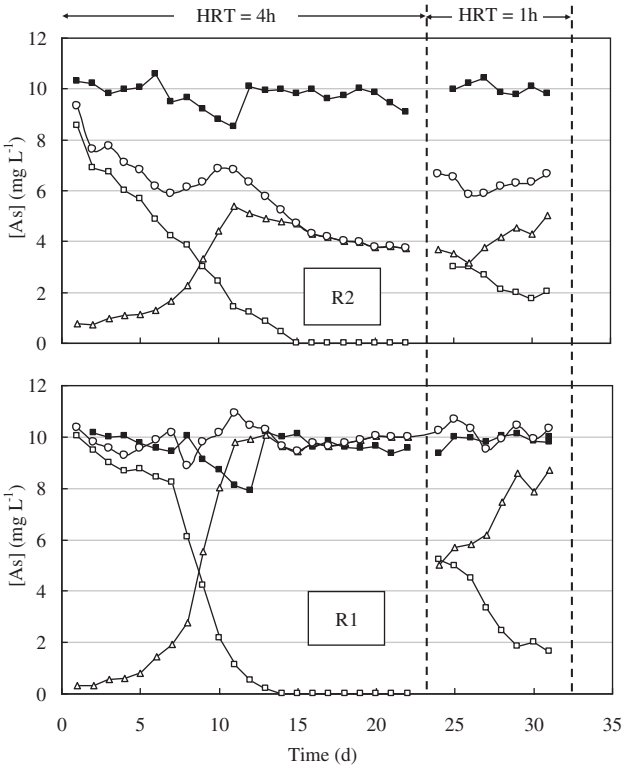

Fig. 3 – Evolution of As speciation and removal in bioreactors R1 and R2: As^{III} in the inlet (■) and outlet (□); As^{v} in outlet (△) and As^{III+V} in outlet (○).

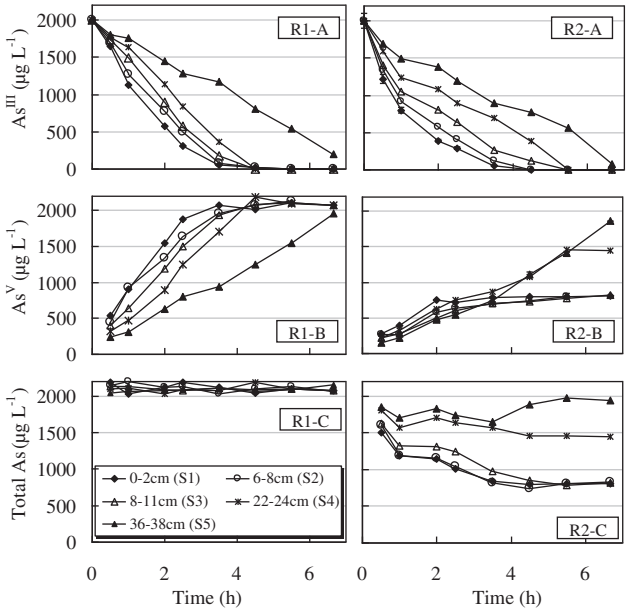

Fig. 4 – Arsenic monitoring (A for As^{III}; B for As^{V} and C for total As) in batch tests conducted with sand support collected at different levels in R1 and R2 at room temperature. Batch tests performed with synthetic water, [As] = 2 mg L^{-1}, solid liquid ratio 1/10.

3.4. Microscopic observations

Fig. 5 shows that the aggregates of R1 were like conventional flocs and highly agglomerated even after ultrasonication. However, the aggregates collected from R2 were more disperse and less dense, which can be the result of a lower level biomass in R2 than in R1 or due to the influence of the existence of zero-valent iron and its oxidant products.

3.5. PCR-DGGE of aoxB genes

The DGGE profiles (Fig. 6) showed that the autotrophic *T. arsenivorans* (indicator: B2 and B5) was present in both reactors after 33 days' operation. Others species carrying the *aoxB* gene (B3 and B4) were detected in both reactors due to non-sterilized conditions. The closer to bioreactor inlet the layer was, the more quantity of the PCR products were found for both reactors through the DGGE analysis, which was probably the result of relatively more abundant As^{III}-oxidizing bacteria developed in the lower layer of reactor. Another species carrying an *aoxB* gene (B1) was only found in R2. The presence of iron can explain the occurrence of different microorganisms in R2 bioreactor. Many species of bacteria are generally observed in groundwater containing Fe, including sulfate-reducing bacteria, denitrifying bacteria, etc. (Gu et al., 2002). Furthermore, it was reported that some bacteria could use Fe for growth from insoluble Fe minerals and oxidize As^{III} in the aerobic condition (Hersman et al., 2001; Shrestha et al., 2008).

4. Discussion

4.1. Comparison of As oxidation in both bioreactors

Fig. 7 shows that the As^{III} oxidation rate of both reactors increased rapidly and in R1 100% of As^{III} was oxidized after 15 days of running under the As^{III} volume loading of 2.5 mg h^{-1} L^{-1} (considering the working volume of 1 L). Since no As removal was found in R1, the biological As^{III} oxidation was the only occurring phenomena. After a short adaption time (about one week) oxidation rate reached 100%. When As^{III} volume loading increased to 10 mg h^{-1} L^{-1}, the final oxidation rate reached 80%, that corresponded to a maximum oxidation rate of 8 mg h^{-1} L^{-1} in R1 after 33 days of operating.

From day 0 to 7, the As^{III} oxidation rate in R2 was slightly faster than that in R1, due to biological combined to chemical oxidation by ZVI (Lien and Wilkin, 2005). Since no As^{III} and relative stable As^{V} concentrations were observed at the outlet of R2 after 15 days' operation, about 60% of total As was removed and 40% of As^{III} oxidated rate, according to the Eq. (5) and (6). When the As^{III} volume loading increased to 10 mg h^{-1} L^{-1}, the final oxidation rate was close to 50% and the maximal removal rate was 4 mg h^{-1} L^{-1} in R2. Under applied experimental conditions, many As^{III} chemical oxidation reactions can occur: oxidation by (i) soluble iron (Fe^{3+} and Fe^{2+}) with dissolved oxygen (Hug et al., 2001), (ii) particular iron hydroxides (Melitas et al., 2002; Su and Puls, 2004), (iii) reactive molecules formed by the reaction of ZVI with dissolved oxygen (e.g., $HO_2/{\bullet}O_2^-$, ·OH) (Katsoyiannis et al., 2008).

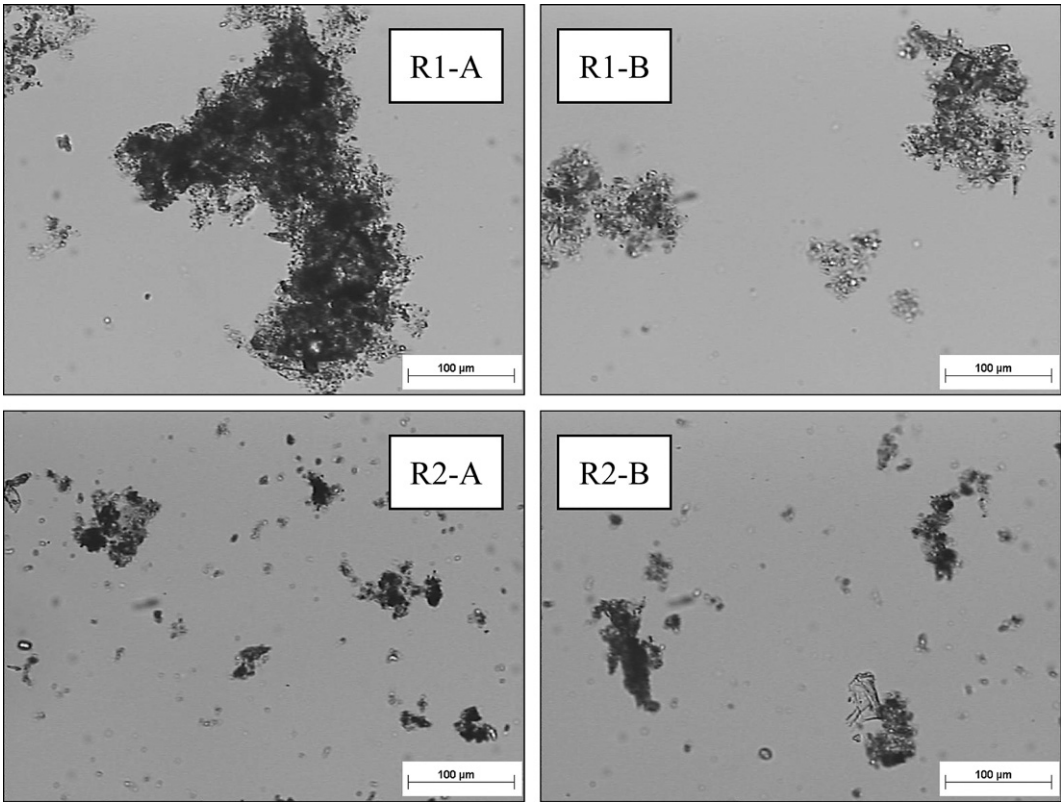

Fig. 5 – Microscopic observations of bio-aggregates collected from R1 and R2 (bar: 100 μm).

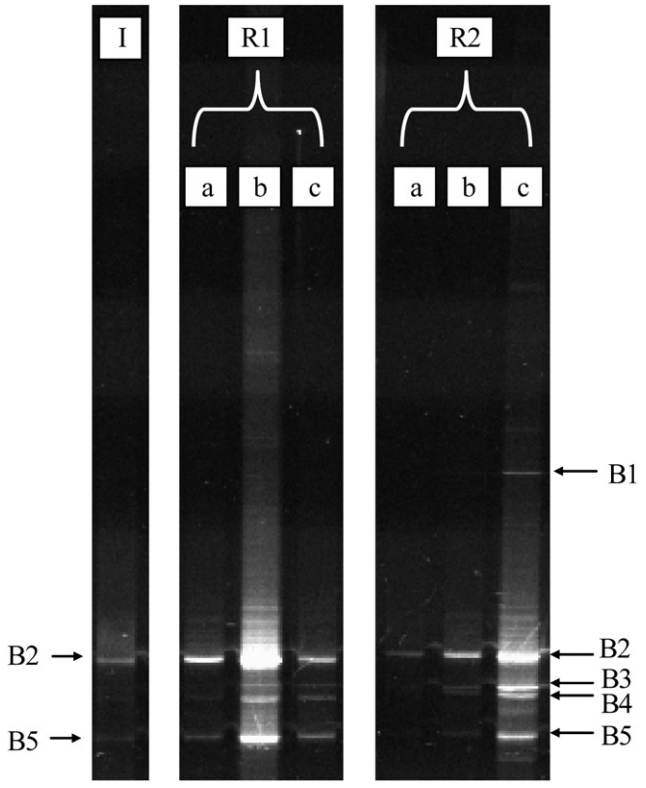

Fig. 6 – DGGE profiles of *aoxB* gene diversity at different levels (a: 36–38, b: 8–11; c: 0–6 cm) in R1 and R2 on day-33 and from the *Thiomonas arsenivorans* inoculum (I).

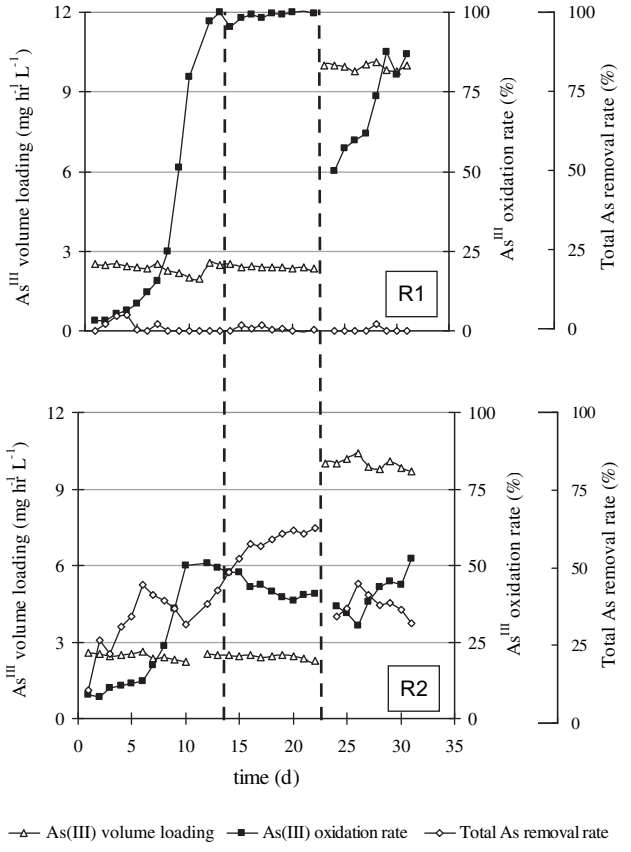

Fig. 7 – Comparison of As oxidation in bioreactors R1 and R2 at 12 °C.

Since low dissolved iron concentrations (<100 μg L^{-1} of soluble Fe^{3+} and Fe^{2+}) were found in the outlet of R2, oxidation with dissolved iron was not considered as predominant.

4.2. *Kinetic studies of As^{III} oxidation in fixed-bed bioreactors*

The first order rate equation ($d[As^{III}]/dt = k_{obs}[As^{III}]$) was applied to simulate the oxidation of As^{III} in the fixed-bed reactors. The observed As^{III} oxidation rate constant k_{obs} was evaluated by calculating the slope of "$Ln([As^{III}]_0/[As^{III}]_z)$" versus HRT (samples extracted from the inlet, V1–V3 of reactor, for 1 h, Q = 16 mL min^{-1}) (Fig. 8). The observed oxidation kinetic constant k_{obs} on day-31 was 0.051 min^{-1} ($r^2 = 0.989$) in R1 and 0.046 min^{-1} ($r^2 = 0.876$) for R2. Furthermore, the half-time of As^{III} oxidation in R1, calculated from the relationship $t_{1/2} = (\ln 2)/k_{obs}$ was 13.6 min. This value was similar to the result obtained with the autotrophic consortium CAsO1 in fixed-bed reactors (Michon et al., 2010) ($k_{obs} = 0.042\ min^{-1}$, $t_{1/2} = 16.5$ min). DGGE fingerprints confirmed that *T. arsenivorans* existed and developed in the different layers of the both reactors. This specie probably plays an important role on the biological As^{III} oxidation in comparison with others species from the consortium CAsO1 (Battaglia-Brunet et al., 2002, 2006).

4.3. *As^{III} oxidation in batch tests*

T. arsenivorans can use As^{III} as an electron donor to supply energy for the bacteria growth (Battaglia-Brunet et al., 2006). Bryan et al. (2009) showed that a linear relationship exists between As^{III} oxidized and carbon fixed, corresponding to 1 g of As^{III} oxidized for 3.9 mg C fixed. Therefore, the rate of substrate utilization (biological As^{III} oxidation rate *n*) could be considered to be proportional to the rate of bacterial growth. The Monod's Model is widely accepted by the majority of the scientific community to represent the bacterial growth and, subsequent, oxidation of substrates. In experiments with high substrate concentrations, this formalism is modified in the literature to account potential inhibition effect. For example, Dastidar and Wang (2009) applied the Haldane substrate inhibition model to characterise As^{III} oxidation by *T. arsenivorans* with high As^{III} concentration range, from 10 to 1000 mg L^{-1}. Inhibition coefficient was determined as kinetic parameter, $K_i = 602.4 \pm 33.6$ mg L^{-1}. In our case, considering the low tested concentration (2 mg L^{-1} see 2.5), the influence of the K_i parameter could be neglected. The classic Monod kinetic could thus be used according to Eq. (7),

$$v = \frac{ds}{dt} = -\frac{\mu}{Y}X = -\frac{\mu_{max}}{Y}.\frac{S}{S + K_s}X \qquad (7)$$

where *S* is the substrate (As^{III}) concentration (mg L^{-1}); μ is the specific growth rate (min^{-1}); *Y* is yield coefficient; the expression μ/Y is referred to as the specific substrate utilization rate (min^{-1}); *X* is the concentration of biomass (mg L^{-1}) and K_s is the half-saturation coefficient (mg L^{-1}).

In batch tests, As^{III} concentrations decreased linearly as a function of time for each support sample collected in the

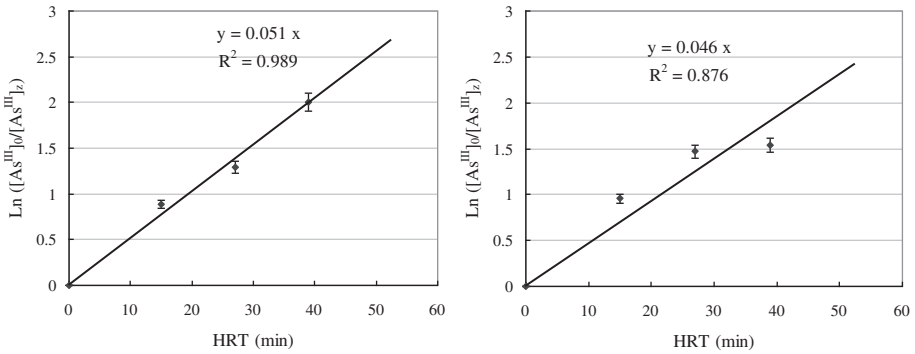

Fig. 8 – Evolution of kinetic parameter (k_{obs}): As^{III} oxidation rate (Ln ($[As^{III}]_0/[As^{III}]_z$) versus HRT in the two reactors with a flow of 16 mL min^{-1} on day-31.

bioreactors (Fig. 9: R1–A and R2–A), that show a stable As^{III} oxidation rate during the first 3 h. This result can be explained by the high As^{III} concentration ([S] > > K_s) according to Eq.(7). Therefore, Monod kinetics approaches zero order and the different maximal As^{III} oxidation rates could be evaluated according to the samples taken from different levels in reactor R1 (Fig. 9: R1–A and R1–B). Furthermore, the difference of maximal oxidation rates (v_i) among the different levels could be seen as the result of the distribution of biomass or the bacterial activity in the reactor. When the biological As^{III} oxidation rate of the inlet level (S1: 0–2 cm) (black line in Fig. 9: R1–A and R2–A) was considered as the maximal oxidation rate (v_{max}), the nearly proportional relationship was obtained between the ratio (v_i/v_{max}) and the axial distance (z) of the reactor (Fig. 9: R1–B and R2–B). In other words, the distribution of biomass (or the bacteria activity) was closely correlated with the structure of the reactor due to the various As^{III} concentrations applied at the different levels in the bioreactors.

4.4. *As removal via zero-valent iron*

Table 2 summarizes results from present work and previous studies dealing with As removal by ZVI. The total As removal capacity by ZVI was a key parameter and reported by many studies (Su and Puls, 2001b; Nikolaidis et al., 2003; Lien and Wilkin, 2005).

70.4 mg g^{-1} of total As was removed by ZVI for 33 days, which was 9 times more than the removed capacity obtained in previous study (Lien and Wilkin, 2005). Moreover, the following batch tests showed that the ZVI or its oxide products were not saturated and continued to remove As in certain

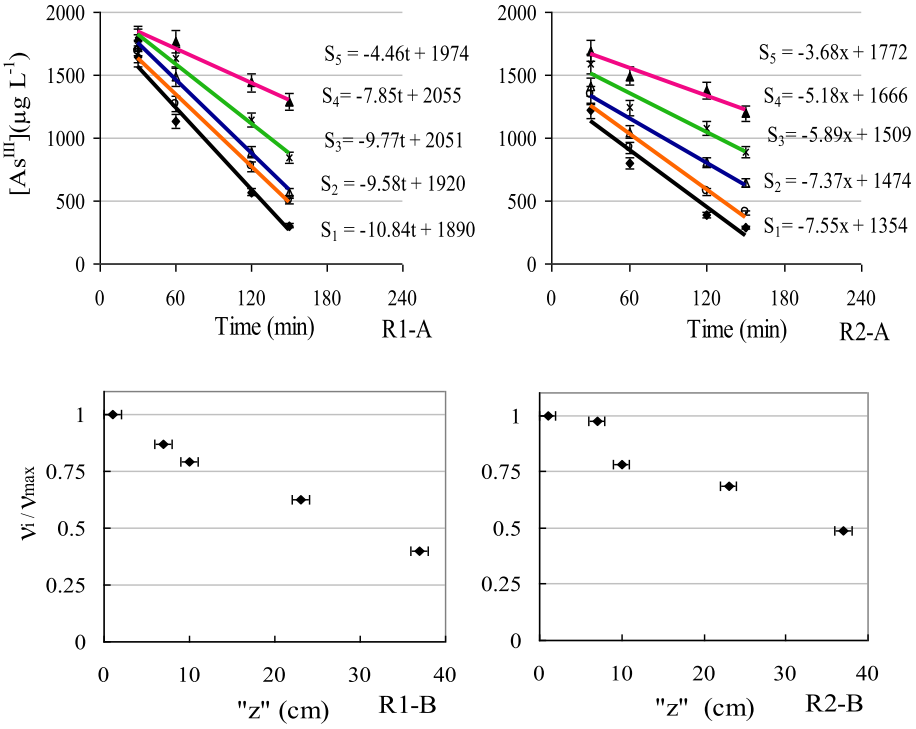

Fig. 9 – Kinetic study from batch tests conducted at room temperature with synthetic water, [As] = 2 mg L^{-1}, solid liquid ratio 1/10; R1–A and R2–A: As^{III} monitoring in S1–S5 levels from bioreactors R1 and R2; R1–B and R2–B: relation between oxidation rate ratio (v_i/v_{max}) and axial distance (z) of the reactor.

Table 2 – Comparison of As^{III} removal with ZVI under different conditions.

Research	Removal capacity (mg g^{-1})	Size (mm)	Aeration	Reactor	Detected As forms in the solid phase	Possible mechanisms of As reaction with ZVI
Present work	≥70.4	0.076	Yes	Column	–	Oxidation/adsorption /surface precipitation
Ramos et al. (2009)	–	6×10^{-5}	No	–	As^0:As^{III}:As^V = 14:51:35	Oxidation, reduction and adsorption
Sasaki et al. (2009)	i) 1.92 ii) 2.28	0.25–2	No	i) Batch ii) Column	As^{III}; As^V (dominant)	Oxidation; adsorption; co-precipitation with Fe(III)-bearing minerals
Katsoyiannis et al. (2008)	–	0.045–0.15	Yes	–	As^{III}; As^V	Oxidation by reactive intermediates; adsorption by hydrous ferric oxides
Leupin and Hug (2005)	–	–	Yes	Column	As^{III}; As^V (dominant)	Oxidation by reactive intermediates; adsorption by hydrous ferric oxides
Bang et al. (2005)	–	0.125–0.8	i) No ii) Yes	Batch	i) As^0; As^{III} ii) As^{III}	i) reduction; adsorption ii) adsorption by iron hydroxide,
Lien and Wilkin, (2005)	7.5	0.3–2.4	No	Column	As^{III}; As^V	Oxidation, adsorption
Nikolaidis et al. (2003)	4.4[a]	0.25–2	No	Column	–	–
Manning et al. (2002b)	–	0.16; 0.47	Yes	Batch	As^{III}; As^V	Oxidation, adsorption
Su and Puls (2001b)	1.77	Peerless	Stirring 50 rpm	Batch	As^{III}; As^V	Oxidation, adsorption by iron oxides
Lackovic et al. (2000)	1.15	Connelly iron	–	Column	As^{III}; As^V	Adsorption/surface precipitation (dominant)

[a] Maximal mg As per g of media containing both iron and sand (initial 500 mg Fe/g of media mass and final 137 mg Fe/g of media in the column).

layers of R2 (Fig. 4: R2–C). ZVI used in this study was smaller than the one used in previous studies (Nikolaidis et al., 2003; Lien and Wilkin, 2005). This higher ZVI specific surface improved the formation of iron hydroxides and thus the As removal capacity. Furthermore, the continuous aeration in the bioreactor could accelerate the ZVI oxidation and thus the formation of reactive iron hydroxide products. Arsenic removal with ZVI corrosion products can be due to adsorption and/or co-precipitation (Irene et al., 2006). In the present study, adsorption of As^{III} and As^V by iron hydroxide products with crystalline or amorphous forms should be the dominant As removal mechanism (Bang et al., 2005; Irene et al., 2006).

Total As removal stayed constant and represented about 60% after 14 days of running. The incomplete As removal was not associated to saturation of the whole support, as shown by batch tests conducted at the end of the experiment. This result can probably be explained (i) by kinetic limitation of As removal (adsorption or precipitation kinetics) or (ii) by a continuous formation of iron corrosion products, enable to trap As. From day 5 to 10, a dramatically increase of As oxidation was observed in R1 and R2 due to biological oxidation (Fig. 7). At the same time As removal decreased in R2. The development of As^{III}-oxidizing bacteria could probably change physico-chemical parameters and influence the chemical As removal pathway as a result of the accumulation of biofilm attached on the ZVI surface.

5. Conclusions

This work investigated the coupled process: biological As^{III} oxidation and physico-chemical removal by ZVI. Small quantity of ZVI mixed with sand (1% w/w) allowed the simultaneous As^{III} biological oxidation and chemical removal in the bioreactor. The very high As removal capacity (more than 70 mg As g^{-1} Fe), could be explained both ZVI handling and redox conditions in the reactor due to aeration. The kinetic parameter determined from continuous system and batch tests showed that the distribution of bacterial population or bacterial activity was correlated with the axial distance of the fixed-bed reactor. The evidence of a divergent *aoxB* gene (B1) in bioreactor containing ZVI, shows that after 33 days of running, the biodiversity of As^{III}-oxidizing bacteria was influenced by the addition of ZVI.

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